HANDBOOK ON THE TOXICOLOGY OF METALS

3rd Edition

Gunnar F. Nordberg • Bruce A. Fowler
Monica Nordberg • Lars Friberg

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Third Edition

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Preface

The *Handbook on the Toxicology of Metals* is a comprehensive review dealing with the effects of metallic elements and their compounds on biological systems. Special emphasis has been laid on the toxic effects in humans, although toxic effects in animals and biological systems *in vitro* are also discussed whenever relevant. As a basis for a better understanding of the potential for adverse effects on human health, information is also given on sources, transport, and transformation of metals in the environment and on certain aspects of the ecological effects of metals.

The first edition of the handbook appeared in 1979, and was followed by a second edition in 1986. The work rapidly fulfilled the aims of the editors and became a standard reference work for physicians, toxicologists, and engineers in the fields of environmental and occupational health. There has been a long interval between the 2nd edition and the present one, but the aims of this third edition are basically the same as those of the previous editions, i. e., to provide easy access to basic toxicological data and also give more in-depth treatment of some information, including a general introduction to the toxicology and risk assessment of metals and their compounds.

As with the previous editions, writing the 3rd edition of this book has been a part of the activities within the Scientific Committee on the Toxicology of Metals under the International Commission on Occupational Health, and the editors are happy that the work to make a third edition has been given a high priority among members. In some cases, we have been honored to include authors from outside of this committee. The chapter authors have, as far as possible, been the same as those who wrote the second edition, but in many cases, we were happy to introduce new colleagues.

Since the publication of the 2nd edition, a wealth of data has appeared, and several of the chapters dealing with specific metals have been completely rewritten; others have undergone a comprehensive updating. In order to not expand the present book and make it much larger than the second edition, which was published in two volumes, some of the general chapters have been merged and shortened, and the present book is published in one volume in a larger format. For the interested reader who searches more detailed information on specific topics, each chapter contains a large number of relevant references also to recent reviews whenever these are available.

The development of modern devices in society demand new chapters which reflect the present concerns of the use of new materials, such as semiconductors in electronic devices, metallic nanotechnology devices, and platinum- and palladium-based catalytic converters.

The increasing use of biomarkers in occupational and environmental health has made it necessary to add a new chapter on biological monitoring and biomarkers. Immunotoxicology is an expanding field, and considerable achievements have been made in recent years. A chapter on "immunotoxicology of metals" has therefore been included. Immunological and genetic findings provide, in some cases, good explanations for the differences in susceptibility to development of disease from exposure to metals. Principles for prevention of the toxic effects of metals and risk assessment are important chapters, somewhat expanded, and a new chapter on a related topic "Essential Metals: Assessing Risks from Deficiency and Toxicity" brings up-to-date knowledge into this 3rd edition.

Before the manuscript of this 3rd edition could be finalized, our co-editor and friend Professor Lars Friberg died. He was the main editor of the two first editions of this handbook, and his ideas constituted the basis for the present edition. His stringent analytical views were invaluable, and his expertise and knowledge are greatly missed. We will also remember him as a loyal, generous, and warm friend, and hope that this book will be a lasting tribute to his memory.

The editors acknowledge each contributor to this book for their devotion and enthusiasm and for having prioritized the work to make the 3rd edition of *Handbook on Toxicology of Metals* available to the reader.

Gunnar F. Nordberg
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Foreword: Outlook

Metals – a new old environmental problem

"Toxic metals" are one of the oldest environmental problems. Today, there are new dimensions of the problem, such as the production of metals in developing countries, leading to occupational exposure and exposure to the general public through the ambient air, drinking water, food, and consumer products. High technology development has also resulted in new products that need more metals in, for example, electronics, fuel cells and car exhaust technology. E-waste, together with drug waste, are new waste problems. The use of metals like gallium, indium, and germanium, which are used in semiconductors has increased steadily over the last 25 years. The e-Waste problem is further augmented by the export of electronic waste from developed to developing countries. Nanotechnology can also lead to unforeseen problems caused by consumer products and combustion of material based on nanoparticles.

Arsenic is a common toxic element which produces clinical disease in India and Southeast Asia from drinking water. This region is also experiencing growing use in semiconductor production. High concentrations of arsenic in drinking water also occur in South America and the U.S. Arsenic is a good example showing how old knowledge is forgotten or ignored, creating new problems. In a number of developing countries, this problem is further exacerbated by expanding human populations and the overexploitation of ground water.

Environmental health problems—and successes stories from the toxicology of metals—have been highlighted in the EEA report on Environment and Health 2005. Some of the key conclusions concerning toxic metals are:

- A number of chemicals are potentially carcinogenic. Approximately 500 metals are classified as carcinogens and are not legally allowed to reach the consumer. They may, however, reach the environment via diffuse sources, for example, in accidental cases. Arsenic in drinking water and cadmium from diffuse sources are environmental contaminants of special concern, because of increasing environmental exposure and their established carcinogenicity.
- Radon exposure is the best documented environmentally related cause of cancer, but is localized in geographical areas where radon precursors (uranium) occur naturally in the ground. Uranium can also contaminate drinking water, leading to kidney injuries.
- Mercury at concentrations that are sometimes observed in the environment is well known to have neurodevelopmental effects, for example, attention problems, reduced learning ability, and slightly reduced IQ in children. Measures are now being taken in Europe to reduce, *inter alia*, prenatal mercury exposure in order to ensure that tolerable daily intakes for pregnant women are not exceeded.
- Lead is an established neurodevelopmental toxicant to humans. Mild mental retardation of children 0-4 years of age in the WHO-Europe region resulting from lead exposure accounts for 4.4% of DALYs (disability adjusted life years). Recent studies on the effects of lead in humans suggest that a "safe" exposure level currently cannot be established. More data on lead exposure of European citizens are necessary and are currently being collected. A ban on leaded petrol has been very successful in lowering blood lead levels in children, which clearly indicates a reduced exposure.
- The global distribution of "new" metals used in automobile catalytic converters to reduce hydrocarbon pollution is clearly shown in the Arctic. Concentrations of platinum, palladium, and rhodium in ice and snow in Greenland have increased rapidly since the 1970; the same trend has been observed in Germany.
- The cadmium contamination of agricultural land has increased during the 20th Century in Europe, leading to exposure from vegetables. Suspicions of kidney and skeleton injuries exist in Europe.

- The effects of combined, long-term and cumulative exposures to *Mixtures of Metals* from diffuse sources might be under-estimated (sometimes called the "*cocktail effect*"). Research in this area needs to develop methods and models to analyze exposures and pathways to disease from *Combinations of Toxic Metals*.

21st Century approaches for 21st Century problems

The shift in focus from legislation for stationary sources to diffuse sources is clearly demonstrated in European Union (EU) legislation, exemplified with directives on integrated pollution prevention and control, Eco-Management and Audit Scheme, European Eco-label, and Integrated Product Policy looking at all phases of a products' life-cycle and taking action where it is most effective. The precautionary principle is also an important starting point.

However, the problems of the 21st century need tools developed in the 21st century. The issue of environment and health is characterized by multicausality with different strengths of association. This means that the links between exposures and their health consequences depend on the environmental pollutants and diseases being considered, but are also influenced by factors such as genetic constitution, age, nutrition and lifestyle, and socio-economic factors, such as poverty and level of education. Important elements of exposure and risk assessment are the estimation of the body burden of chemicals, combined exposures from multiple sources (food, air and water) and the timing of exposures. Preventive measures require the development of proactive risk assessment and management replies that can contribute to the formulation of adequate responses, not at least consider the costs of action and non-action.

Given the complexities and uncertainties relating to environmental health issues, a new participatory framework for risk assessment and risk management is developing, involving a broader framing of scientific assessment of risks, uncertainties, and ignorance, and options for action, communication, monitoring, and evaluation of the effectiveness of actions. Approaches, systems, and services are required to support many different types of actors and other afflicted individuals, not just the policymakers. At a symposium organized by the Scientific Committee on the Toxicology of Metals, International Commission on Occupational Health, a number of these approaches for toxic metals and metalloids were discussed, along with the ongoing need for international collaboration. This "Symposium on Risk Assessment of Metals," hosted by the European Environment Agency in Copenhagen, Denmark, June 13-14, 2005 was sponsored by FORMAS, (Sweden) and co-sponsored by the Agency for Toxic Substances and Disease Registry (USA). This conference brought together a number of international experts on the toxicology of metals to review, discuss, and critique chapters for the *Handbook on the Toxicology of Metals* so that the most relevant and up-to-date information will be available for the production of this important reference work.

Research, knowledge, assessments, and monitoring

Environmental research is a prerequisite for evidence-based policymaking, assessing new knowledge and early warnings. The production of the handbook is one assessment example. The project has also highlighted biomarkers and biological monitoring as an important tool to identify and quantify the exposure, predict health effects, sensitive populations, and perhaps also diagnose a disease. Bio-monitoring is also an effective mean to evaluate the target setting and other policy interventions. However, it raises ethical questions that must be addressed.

Copenhagen 13th June 2005

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Table of Contents

CHAPTER

3

Routes of Exposure, Dose, and Metabolism of Metals

WILLIAM S. BECKETT, GUNNAR F. NORDBERG, AND THOMAS W. CLARKSON

CHAPTER

4

Biological Monitoring and Biomarkers

ANTERO AITIO, ALFRED BERNARD, BRUCE A. FOWLER, AND GUNNAR F. NORDBERG

CHAPTER

5

Selected Molecular Mechanisms of Metal Toxicity and Carcinogenicity

TODD DAVIDSON, QINGDONG KE, AND MAX COSTA

CHAPTER

6

General Considerations of Dose-Effect and Dose-Response Relationships

DAPHNE B. MOFFETT, HISHAM A. EL-MASRI, AND BRUCE A. FOWLER

CHAPTER

7

Interactions in Metal Toxicology

GUNNAR F. NORDBERG, LARS GERHARDSSON, KARIN BROBERG, MOIZ MUMTAZ, PATRICIA RUIZ, AND BRUCE A. FOWLER

CHAPTER

8

Epidemiological Methods for Assessing Dose-Response and Dose-Effect Relationships

TORD KJELLSTROM AND PHILIPPE GRANDJEAN

CHAPTER

9

Essential Metals: Assessing Risks from Deficiency and Toxicity

GEORGE C. BECKING, MONICA NORDBERG, AND GUNNAR F. NORDBERG

CHAPTER

10

Carcinogenicity of Metal Compounds

QINGDONG KE, MAX COSTA, AND GEORGE KAZANTZIS

CHAPTER

11

Immunotoxicology of Metals

PER HULTMAN

CHAPTER

12

Reproductive and Developmental Toxicity of Metals

PIETRO APOSTOLI, SPOMENKA TELIŠMAN, AND POLLY R. SAGER

CHAPTER

13

Ecotoxicology of Metals—Sources, Transport, and Effects in the Ecosystem

POUL BJERREGAARD AND OLE ANDERSEN

CHAPTER

14

Risk Assessment

GUNNAR F. NORDBERG AND BRUCE A. FOWLER

CHAPTER

15

Diagnosis and Treatment of Metal Poisoning—General Aspects

GEORGE KAZANTZIS

CHAPTER

16

Principles for Prevention of the Toxic Effects of Metals

PHILIP J. LANDRIGAN, DAVID KOTELCHUCK, AND PHILIPPE GRANDJEAN

CHAPTER

17

Aluminum

BENGT SJÖGREN, ANDERS IREGREN, CARL-GUSTAF ELINDER, AND ROBERT A. YOKEL

CHAPTER

18

Antimony

CAROLYN A. TYLENDA AND BRUCE A. FOWLER

CHAPTER

19

Arsenic

BRUCE A. FOWLER, C.-H. SELENE J. CHOU, ROBERT L. JONES, AND C.-J. CHEN

CHAPTER

20

Barium

AGNETA OSKARSSON AND ANDREW L. REEVES

CHAPTER

21

Beryllium

MAREK JAKUBOWSKI AND CEZARY PALCZYNSKI

CHAPTER

22

Bismuth

BRUCE A. FOWLER AND MARY J. SEXTON

CHAPTER

23

Cadmium

GUNNAR F. NORDBERG, KOJI NOGAWA, MONICA NORDBERG, LARS T. FRIBERG

CHAPTER

24

Chromium

SVERRE LANGÅRD AND MAX COSTA

CHAPTER

25

Cobalt

DOMINIQUE LISON

CHAPTER

26

Copper

DAG G. ELLINGSEN, NINA HORN, AND JAN AASETH

CHAPTER

27

Gallium and Semiconductor Compounds

BRUCE A. FOWLER AND MARY J. SEXTON

CHAPTER

28

Germanium

OBAID M. FAROON, L. SAMUEL KEITH, HUGH HANSEN, AND BRUCE A. FOWLER

CHAPTER

29

Indium

BRUCE A. FOWLER

CHAPTER

30

Iron

PREM PONKA, MILTON TENENBEIN, AND JOHN W. EATON

CHAPTER

31

Lead

STAFFAN SKERFVING AND INGVAR A. BERGDAHL

CHAPTER

32

Manganese

MARKO ŠARIĆ AND ROBERTO LUCCHINI

CHAPTER

33

Mercury

MATHS BERLIN, RUDOLFS K. ZALUPS, AND BRUCE A. FOWLER

CHAPTER

34

Molybdenum

JUDITH R. TURNLUND AND LARS T. FRIBERG

CHAPTER

35

Nickel

CATHERINE KLEIN AND MAX COSTA

CHAPTER

36

Palladium

HIROSHI SATOH

CHAPTER

37

Platinum

MIRJA KIILUNEN AND ANTERO AITIO

CHAPTER

38

Selenium

JOHAN HÖGBERG AND JAN ALEXANDER

CHAPTER

39

Silver

JAMES S. HOLLER, GUNNAR F. NORDBERG, AND BRUCE A. FOWLER

CHAPTER

40

Tellurium

LARS GERHARDSSON

CHAPTER

44

Tungsten

GEORGE KAZANTZIS AND PER LEFFLER

CHAPTER

45

Uranium

L. SAMUEL KEITH, OBAID M. FAROON, AND BRUCE A. FOWLER

CHAPTER

46

Vanadium

BIRGITTA J-SON LAGERKVIST AND AGNETA OSKARSSON

CHAPTER

47

Zinc

HAROLD H. SANDSTEAD AND WILLIAM AU

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CHAPTER

1

Introduction—General Considerations and International Perspectives

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ABSTRACT

This introductory chapter is composed of two parts. The first section is a brief history of the science of the toxicology of metals by the late Dr. Lars Friberg. He delineates the early realization of the need for international cooperation and consensus that have guided seminal studies related to environmental and occupational toxicology. In this spirit, he initiated work on the first edition of the *Handbook of Toxicology of Metals* that included contributors from around the world.

The second section takes up some current concerns related to the toxicology of metals. It highlights such concerns in relation to the current status of the scientific understanding to date of the metals included and discussed fully in the chapters of the Handbook. Furthermore, it draws attention to future directions in generating new knowledge to fill gaps in the continued quest to assemble the knowledge base necessary for the protection of human health from adverse consequences related to exposure to metals.

1 METALS AND HEALTH—AN INTERNATIONAL PERSPECTIVE*

Lars Friberg

In the years after the Second World War, lists of MAC concentrations (maximum allowable concentrations) for chemicals were constructed for use primarily in industry. There were often substantial differences between the values in different lists (e.g., from the United States and Russia) for the same substance. Reliable background information on methods used was also often lacking or for other reasons difficult to interpret.

At an International symposium on MAC values in 1963, it was recommended to have expert groups convene to establish international MAC concentrations of a number of the more important compounds. In 1966, the Subcommittee for MAC values (chairman, R. Truhaut) under the auspices of the Permanent Commission and International Association on Occupational Health met in Vienna, and responsibility was assigned for formation of groups to evaluate the scientific basis of MAC values for certain specific substances. As a consequence of decisions made at these meetings, an evaluation of MAC values for mercury and its compounds was made at a symposium at the Karolinska Institutet in Stockholm, 1968.

One of the recommendations from the Stockholm symposium was to form a group experienced in the toxicology of metals. The Scientific Committee on the Toxicology of Metals (SCTM) was established during the 16th congress of the International Commission on Occupational Health (ICOH) in Tokyo in 1969. Lars Friberg was elected chairman of the Committee and held this position until 1990. Gunnar Nordberg (1990) and later Bruce Fowler (1997) and Monica Nordberg (2003) took over the responsibility of leading the committee. At present, it has 60 members from

*Presentation at the International Symposium on Risk Assessment of Metals—with special reference to occupational exposures and human environmental exposures in contaminated areas, Copenhagen, June 13–14, 2005.

countries from all over the world. The first meeting was held in Stockholm in 1970.

The serious problems with the development of reliable MAC values were also acknowledged by international agencies like the World Health Organization (WHO), the United Nations Environment Programme (UNEP), and the International Labour Organization (ILO). In a joint venture, they initiated the International Programme on Chemical Safety (IPCS; see preamble to Environmental Health Criteria WHO/IPCS 2006).

The main objective of IPCS has been to carry out and disseminate evaluations of the effects of chemicals on human health and the quality of the environment. Supporting activities have included risk assessment methods that could produce internationally comparable results. The first Criteria Document was the one for mercury 1978. One reason why mercury had a high priority was probably the alarming situation worldwide due to the effects on humans from consumption of mercury in fish.

The Health Criteria Documents often constitute the best available information on the toxicology of chemicals. As a rule, they are very valuable, and it is hoped that the production of documents will continue as planned. There are, however, examples of intensive lobbying by states and industrial groups before, during, and after the preparation of the reports. Because practice has been to require consensus reports, some Criteria Documents were very difficult to finish. The Criteria Document for cadmium is an example in which such problems were formidable. The work on this Criteria Document took approximately 15 years to complete.

The Scientific Committee started a series of workshops and meetings dealing with basic aspects of factors related to metabolism, dose effects, dose response, and critical organs for metals. The first of these meetings was held in Slanchev Briag, Bulgaria, in 1971. It was followed by meetings in Buenos Aires, Argentina in 1972 (TGMA, 1973), Tokyo, Japan in 1974, and Stockholm, Sweden in 1977. Results of the consensus reached were published ("Effects and Dose Response Relationships of Toxic Metals" edited by G. F. Nordberg and published by Elsevier 1976 and the "Handbook on the Toxicology of Metals," 1st ed. 1979, 2nd ed. 1986). Several meetings dealt with more specific topics such as developmental and reproductive toxicity of metals (Clarkson *et al*., 1983) and the role of carcinogenesis. A workshop on neurotoxic metals was organized in Brescia in 2006. When there was a need for information on specific metals, we arranged meetings focusing on single metals, such as arsenic, mercury, cadmium, and lead.

As a result of ongoing research and the need for accurate information the interest in metal toxicology at the Karolinska Institutet increased. We prepared independent publications with an international perspective for evaluating the health risks of certain metals. At that time, we already had a close contact with the US Environmental Protection Agency, which wanted background information for mercury and cadmium for future Criteria Documents. This resulted in formal contracts, and the resulting reviews were submitted to the US Environmental Protection Agency and also published by CRC Press in a number of monographs: *Mercury in the Environment* (Friberg and Vostal, 1972), *Cadmium in the Environment*, and *Cadmium and Health* (Friberg *et al.*, 1985). It was also the start of the work for the *Handbook on the Toxicology of Metals*.

We had been aware for several years of the possible dangers from the use of methyl mercury. In 1958, Swedish researchers already demonstrated that seed-eating birds had high Hg levels in their feathers. Some years later, it was found that fish from several lakes and rivers had high levels of methyl mercury in their organs. One major source for the mercury exposure could be industrial discharge of mercury, but methyl mercury had also been used extensively over many years for the dressing of seed.

In Minamata and Niigata in Japan, there had been two outbreaks of severe poisoning with many fatal cases and prenatal poisonings. Reliable information on exposure and dose effect and dose response was lacking. It became obvious, however, that the source of the poisoning was fish and shellfish heavily contaminated by industrial discharge of mercury into the local waters.

We found increased concentrations of mercury in the blood of Swedish fishermen, who had extensively consumed fish. The mercury levels were alarmingly high, even if no signs of mercury poisoning were found. Some values later turned out to be as high as or even higher than the lowest that could be estimated to have caused symptoms in Japan. It points out that to get signs of intoxication when exposure is fairly low, you must belong to a sensitive part of the population. Based on an extensive research program with considerable international aspects, we were able to produce data that were of value for interpreting the serious effects in Japan. The report from a Swedish Expert Group ("Methyl Mercury in Fish," Berglund *et al.*, 1971) has been used to also evaluate health risks of methyl mercury in other countries.

In Iraq, several hundred farmers died after eating grain dressed with methyl mercury. The disaster has been studied in detail by a group from Rochester, New York. Their research program on mercury is still ongoing, and, in collaboration with Swedish

researchers, includes studies on the effect of exposure to MeHg through consumption of contaminated fish among inhabitants of the Seychelle islands. A Danish research group is carrying out a similar project at Färö Islands.

Inorganic arsenic occurs in high concentrations in bedrocks and groundwater in several parts of the world (e.g., in Taiwan, India, Bangladesh, Argentina, and Chile). Large populations have been exposed, and in some places mass outbreaks of severe poisonings have occurred. Skin cancer and cardiovascular disorders are common. I had the opportunity, as a WHO consultant, to observe the disastrous effect arsenic had had in some places in Taiwan.

In 1976, the Scientific Committee was one of the sponsors of an International Conference on Environmental Arsenic at Fort Lauderdale, Florida. In 1980, a WHO Task Group Meeting on Environmental Health Criteria for Arsenic was held in Stockholm, Sweden. It was pointed out that there was a risk for skin disease and certain forms of cancer from drinking water contaminated with arsenic.

The consequences apparently have not been taken seriously. Through the use of international foreign aid money, including Swedish, British, and the World Bank, a massive assistance program was launched in Bangladesh for several years with the aim to drill deep into the bedrock to get ground water. This was badly needed because contaminated surface water was the main source of water. No one was aware of the risk for arsenic poisoning. A detailed survey of the consequences of the Swedish assistance project, sponsored by SIDA (Swedish International Development Cooperation Agency), was recently published in a Swedish newspaper (DN April 19, 2005). Sweden was financing the drilling of approximately 12,000 wells between 1990 and 2000. In total, the drilling of approximately a million wells was sponsored by international organizations. Now the drilling has been stopped, and emphasis is on analytical and technical programs.

One meeting of particular importance for the Handbook and referred to previously (TGMT, 1979) concerned the concept of a critical concentration of a metal in a cell or in an organ. The metal concentration at which adverse functional changes occur in the cell was defined as the critical concentration for the cell. The critical organ concentration was defined as the mean concentration in an organ that would be necessary for a number of the most sensitive cells in the organ to be affected. The term "critical organ" was used to identify that particular organ that first attains the critical concentration of a metal under specified circumstances of exposure and for a given population. This definition differs from other ones used in radiation protection, in which the term "critical organ" refers to that organ of the body whose damage results in the greatest injury to the individual. The critical effect as defined by the Scientific Committee gives possibilities of preventing more serious effects. It is also important to recognize that the critical concentration varies between individuals and that it is, therefore, not possible to talk about one single value only.

With some modifications, which have been included in the glossary (Nordberg *et al.*, 2004a), we have, when drafting the different chapters in the new edition of the Handbook, tried to use the aforementioned concepts.

Another topic of great importance for evaluating exposure and risks relates to biological monitoring of metals in, for example, blood and urine. The quality of published data is often very poor, even if the situation has improved. The assessment of metals in trace concentrations in biological media is fraught with difficulties from the collection, handling, and storage of samples to chemical analyses.

A systematic approach to quality assurance aspects related to biological monitoring was taken in a 3-year global project by WHO/UNEP. Quality control exercises were carried out with 10 participating laboratories in different parts of the world. The Scientific Committee sponsored a meeting where the different aspects of biological monitoring were discussed in detail (Clarkson *et al.*, 1988).

Metals do not break down. As a consequence, a metal stays in the body until it is excreted. During this time, the metal may be transformed into another more toxic or less toxic species. Inhalation of mercury vapor may be used as an example. Metallic mercury vapor is released from both old and new amalgam fillings. It is taken up in the blood during inhalation and is oxidized within minutes to divalent mercury. Parts of the mercury vapor penetrate the blood–brain barrier and in pregnant women also the placental barrier. In the brain, it is oxidized into divalent inorganic mercury and is excreted only very slowly with a biological halftime of years. This explains why even minor inhalation of mercury vapor from amalgam fillings may create health problems.

Some reports indicate a potential danger of using dental amalgam, whereas others deny it. In some countries, the use of amalgam has been banned. The situation is to some extent similar to the banning of tetraethyl lead in gasoline. Even so there are problems. Old fillings will continue to release mercury for many years. The symptoms many patients complain about are nonspecific and are seen in many other diseases. It would be useful to collect as much information as possible on the health effects of amalgam in different countries (Chapter 33, Bellinger *et al.*, 2006).

Cadmium occurs naturally in the environment and some places, around, for example, mines may be heavily contaminated. Nowadays humans are exposed

primarily through contaminated food. Chronic occupational cadmium poisoning was observed in the late 1940s in Sweden in the production of alkaline batteries. It was shown that many of the symptoms were similar to what was seen in some areas of Japan and called Itai-Itai disease, where inhabitants were eating rice contaminated by cadmium from a river from a nearby mining industry. Japanese and Swedish researchers began long-term collaborative research projects. The research clearly showed that exposure to cadmium was a necessary factor for the development of Itai-Itai disease. Chronic cadmium poisoning has now been observed in several areas of the world, including Belgium, The Netherlands, and China. In many more countries, increased risks are seen as indicated by excessive concentrations in certain foodstuffs (e.g., rice).

Cadmium is a good example of the need and value of international collaboration. Questions relating to cadmium poisoning have been on the agenda at several meetings arranged by national and international bodies. IPCS published a Health Criteria Document in 1992 (WHO/IPCS, 1992) based on several earlier drafts during the entire 1980s. The Scientific Committee held a meeting in Bethesda, Maryland, in 1978 and one in Shanghai, China, in 2003. The last meeting focused on health impacts of cadmium in China and its prevention (Nordberg *et al.,* 2004b).

I hope this has given some information on the need to have international perspectives when evaluating and preventing health effects of metals. The responsibility for this is partly up to governments and international and national organizations. The Scientific Committee has an important role as an organization with high competence and with no formal strings to governments, communities, or industries. It is important that in the future IPCS also gives a high priority to metal toxicity.

It is important to disseminate information on metals and their occurrence and toxicity not only to active scientists and administrators but also to local doctors and engineers. Here the concept of a Handbook is extremely important. The earlier editions of the *Handbook on the Toxicology of Metals* have served this purpose. We expect that the third edition will continue to have such a tutorial task. A Handbook also gives the easiest access to the entire spectrum of metal toxicology.

2 CURRENT CONCERNS RELATED TO THE TOXICOLOGY OF METALS

In addition to the considerations in the first part of this chapter, including the need for international joint action to control identified risks from exposures to metals in humans, the following considerations are of importance with regard to current and future areas of research. This section of the chapter will summarize a number of cross-cutting research areas for metals that are discussed in much greater detail in subsequent individual chapters.

2.1 Expanding Current Industrial New Technological Uses of Metals

The toxicology of metals is concerned with some 80 elements and related species, ranging from comparatively simple ionic salts to complicated structures, such as complexes consisting of a metal atom and a set of ligands and organometallic compounds. Pollution of the environment and human exposure to metallic elements may occur naturally, for example, by erosion of surface deposits of metal minerals, as well as from human activities, such as mining, smelting, fossil fuel combustion, and industrial application of metals. The modern chemical industry is based largely on catalysts, many of which are metals or metal compounds. Production of plastics, such as polyvinyl chloride, involves the use of metal compounds, particularly as heat stabilizers. Plating and the manufacture of lubricants are still other examples of industrial uses of metals. The industrial and commercial uses of metals are continuously increasing. In the development of advanced technology materials, new applications have been found for the most familiar and for the somewhat less familiar metallic elements. Most notable are their uses in the development and production of semiconductors, superconductors, metallic glasses, magnetic alloys, high-strength, low-alloy steel, and most recently in nanotechnology (IARC, 2006).

Some of the new uses like nanotechnology have the potential of causing direct exposure of humans. Many other uses, and also discharges from nonmetal-producing activities such as electricity production from coal combustion, may increase both the amount of and the distance over which metals are discharged into the human environment. The distance from source of emissions of metals to their sinks can sometimes be more than 1000 km for airborne transport. When metals are transported along aquatic and terrestrial routes, they often enter into the food chain. Furthermore, the use and disposition of the new technological equipment increase E-waste. If not recycled in appropriate waste-handling systems, metals/metalloids used in semiconductors will enter the ecological pathways.

2.2 Ecological and Natural Environmental Mobilization Processes

The acidification of soil and lakes by sulfur and nitrogen oxides has increased the possibility for adverse

effects of metals in the environment. The long-range transport of air pollutants not only contributes to the increasing metal load to ecosystems but also alters the mobility of metals. An increasing acidity of surface waters, including lakes, which is caused by acid precipitation, may increase the mobility of metallic compounds, thus increasing human exposures (Nordberg *et al.*, 1985). Natural events, like hurricanes and flooding, possibly amplified by global warming, will increase the mobility of metals, as seen, for example, for mercury in forestry in Sweden and of lead in flooding in New Orleans. Increased exposure may occur by means of increased concentrations of metallic compounds in drinking water and/or food. In addition, increased exposure to lead, cadmium, mercury, and aluminium is well recognized to be the result of long-range transport of air pollutants and the occurrence of acid rain (see Chapter 13).

The worldwide production of the two metals mercury and lead is several million tons per year. Their widespread use has added to natural cycles of these metals, and there is increasing epidemiological evidence that even background exposures may affect human health.

It has been known for a long time that anemia and effects on the brain in the form of cognitive deficits are common findings in children who eat lead paint (Lin-Fu, 1977). Exposure from lead paint in houses or soil near industrial stack gases has been a problem worldwide. In Texas, for example, of the young children living near a smelter and for whom paint was not an important source of exposure, 53% were found to have blood lead values of >400 μg/L—a level high enough to expect adverse effects (Landrigan *et al.*, 1975). Lead from automobile exhaust increases the body's burden of lead in areas where organolead is still used as an additive to gasoline and where there is heavy traffic. The important impact of this source of lead has been established in several countries by the fall in blood levels after the ban of lead in gasoline. Because of the possibility of adverse effects on the central nervous system, the US Centers for Disease Control and Prevention (CDC) guidelines identifies a blood level >0.48 μmol Pb/L (100 μg/L) to be of concern in children (see Chapters 12 and 31), and it was recently recommended by a group of scientists at a meeting in Brescia (see Chapter 16) that this level be lowered to 0.24 μmol Pb/L (50 μg/L). These are levels that sometimes occur as background levels in some countries.

Concern for metal exposure in developing countries was highlighted by WHO in South-East Asia (WHO, 2005). An example is the transference of the manufacture of lead products to developing countries, where child labor increases the exposure of children to lead.

Neurotoxic effects from exposure to lead, mercury, and manganese are well established, and increasing recognition of adverse health effects from Pb and Hg has initiated preventive action by banning or limiting certain uses of these metals. It is necessary to highlight that it is important to avoid using Mn and other neurotoxic metals in a way that causes widespread dispersion in ambient air. As stated in the declaration of Brescia, the avoidance of such new applications of the metals is of fundamental importance in protecting humans from potential adverse health effects (Landrigan *et al.*, 2006). It is also obvious that there is a need to find out more about the toxicology of other metals now suggested as replacements for the known toxic metals being phased out.

What is it that makes exposure to metals a specific problem? Metals are elements and have been an intrinsic component of the environment to which humans and animals have adapted. A "natural exposure" to any metal may thus be harmless to human beings and other species. However, geological factors and widespread contamination from industrialization, forming the general background exposure in some countries, give rise to adverse health effects among sensitive sections of the general human population. Some metals are required for human life (essential metals) because they have a biological function, for example, in many enzymes. In areas where food consumption is based on local produce, and where the levels of some minerals are low for geological reasons, deficiencies may occur. The impact of geological factors on human health is, considered by the science of "medical geology," recently attracting increasing attention (Selinus, 2005).

2.3 Routes of Exposure

Exposure to metals may take place by inhalation, ingestion, or skin penetration. For organometallic compounds, dermal uptake can cause substantial, sometimes lethal, doses (Nierenberg *et al.*, 1998). Inhalation is usually the most important occupational exposure route. Ambient air, except in the vicinity of an emission source, does not usually contribute significantly to the total exposure. Contaminated air may pollute soil and water secondarily, resulting in contaminated crops and vegetables. In countries where people eat food that originates from several or many different areas, the health significance of such contamination may be rather minor. In other countries, such as Japan and other rice-growing countries in Asia, as mentioned earlier, it has been the custom for farmers to depend, to a great extent, on locally grown products. Drinking water is sometimes a significant route of exposure. The extensive exposure to arsenic through ground water contaminated by geological occurrence of arsenic in Bangladesh and the West Bengal has already been mentioned. Other metals causing exposure through drinking water are aluminium,

iron, uranium, and sometimes manganese, cadmium, and lead. Whether local contamination exists, ingestion of metals via food and drinking water is ordinarily the main pathway of exposure for the general population.

A route of exposure that must not be forgotten is the inhalation of tobacco smoke, which contains a number of metals, including cadmium, nickel, arsenic, and lead. It has been shown in a number of studies that cadmium in cigarette smoke may have contributed a third of the total body burden found at age 50 (Friberg *et al.*, 1985; WHO, 1992). The relative contribution of cadmium made by tobacco smoke will, of course, be considerably less when the intake of cadmium via food or air is large. Some data clearly show that external metal contamination of cigarettes in industries where an exposure to metals through air already occurs may increase the workers' inhalation dose of metals several fold. In areas where tobacco is grown and soils are contaminated by cadmium, substantial exposure may occur through smoking (Cai *et al.*, 1995).

2.4 Essentiality of Metals

Metallic elements are found in all living organisms and play a variety of roles. They may be structural elements, stabilizers of biological structures, components of control mechanisms (e.g., in nerves and muscles), and, in particular, are activators or components of redox systems. Thus, some metals are essential elements, and their deficiency results in impairment of biological functions. Essential metals, when present in excess, may even be toxic (Chapter 9). Although this Handbook is concerned primarily with human health effects resulting from excessive exposure to metals and their compounds, it should be recognized that metals might also have deleterious effects on other animal species and plants. Such effects may lead to modification of an entire population or species assembly in an ecosystem. Such effects of metals may be of great significance to human life and should be considered in the total evaluation of environmental pollution by metals and their compounds (see Chapter 13).

It is difficult to rid the environment of a metal with which it has been contaminated. Two striking examples are mercury in the bottom sludge of lakes and cadmium in soil. In addition, several metals and metalloids may undergo methylation during their environmental and biological cycles (e.g., tin, palladium, platinum, gold, thallium, arsenic, selenium, and tellurium), but mercury is the only metal presently known to undergo biomagnification in food chains. The potential influence of acid precipitation on the methylation process for mercury has been considered as one possible explanation for the fact that concentrations of methyl mercury found in fish from acid lakes were higher than in lakes with waters with neutral pH (Wood, 1985; Chapter 13). It is obviously of great importance to understand the ecological cycles of metals to evaluate potential threats to human health from consumption of food and drinking water. There are still considerable gaps in our knowledge in this scientific field.

Essential metals may be toxic if exposure is excessive (e.g., molybdenum). Other metals are not known to have an essential function, and they may give rise to toxic manifestations even when intakes are only moderately in excess of the "natural" intake. Metal toxicity is explainable on the basis of the interference with cellular biochemical systems. Metals often interact at important sites such as the SH groups of enzyme systems. They may also compete with other essential metals as enzyme cofactors (Chapters 3, 5, 7, and 9). Thus the effects of a toxic metal may mimic the deficiency of an essential metal. For example, cadmium does not penetrate into the fetus to any considerable degree but causes an effect on the fetus most likely as a result of a secondary zinc deficiency. Cadmium has been found to be high in the placenta and most likely blocks the zinc uptake of the placenta. Several of the toxic metals have a long biological half-time and tend to accumulate in the body. For cadmium, the biological half-time in man is estimated to be 15–20 years or more. This long half-time may be related to the fact that cadmium is the most potent inducer of the synthesis of metallothionein, a high-affinity metal-binding protein (Kägi *et al.*, 1984; Nordberg, 1984; 1998; Nordberg and Kojima, 1979). With constant exposure, the long biological half-times imply that accumulation will sometimes take place over an entire lifetime.

2.5 Human Health Effects

Our information on metal toxicity, as far as humans are concerned, is derived from industrial health, large-scale, severe episodes of poisoning via contaminated drinking water and food (MethylHg, Cd, Pb) and recently from recognition of widespread occurrence of less obvious but adverse effects on large population groups. There are at least 20 metals or metal-like elements that can give rise to rather well-defined toxic effects in man. These are dealt with in the specific chapters of this Handbook. Arsenic, cadmium, lead, manganese, and mercury have been studied most thoroughly, but other metals are also of concern. Molybdenum brings about goutlike signs, and aluminum has been shown to have serious effects on the central nervous system (CNS) under certain circumstances. Antimony and cobalt may have effects on the cardiovascular system. Some organometallic tin compounds give rise to effects

in the CNS and may also affect the immune system. The latter type of effects are also known to be a result of exposure to platinum, palladium, and beryllium, in the latter case constituting the mechanism for development of chronic beryllium disease, a form of pneumoconiosis (Chapters 11 and 21). Effects on the lungs in the form of pneumoconiosis can also arise after exposure to aluminum, antimony, barium, cobalt, iron, tin, and tungsten or their compounds.

2.6 Metal Carcinogenesis and Reproductive Toxicology

Recognition of the carcinogenicity of metals is of ever-increasing public health importance. Conclusive evidence based on epidemiological, experimental, and mechanistic data has existed since the 1980s, with an increasing number of metals evaluated by International Agency for Research on Cancer (IARC). Chromium and nickel were the first metals classified as carcinogens by IARC. During the 1980s, animal studies with inorganic arsenic confirmed existing epidemiological evidence. On the basis of the convincing evidence for carcinogenicity of arsenic in humans and the fact that arsenic is released from gallium arsenide *in vivo* in animals, this compound has been classified as a human carcinogen. For beryllium the situation is the reverse: results of epidemiological studies have confirmed earlier experimental evidence. Cadmium can contribute to the development of lung cancer and possibly to cancer of the prostate. During the 1980s long-term studies on rats inhaling cadmium chloride showed remarkable dose-related incidence of lung cancer at low exposure levels. Cadmium and its compounds have been classified as carcinogenic to humans by IARC. Lead and its inorganic compounds have recently been evaluated by IARC and are classified as probable human carcinogens based on a combination of human and animal data. The experience with these metals emphasizes the importance of carefully evaluating the consequences in humans of exposures to metals proven to be carcinogenic in animals. Cobalt compounds and antimony trioxide are examples of a metal compounds considered to be possible human carcinogens mainly on the basis of animal data. A few metal and metalloid derivatives have shown chromosomal effects *in vitro* and *in vivo*, as well as point mutations in, for example, *Salmonella*, *Escherichia coli*, and *Drosophila*. However, for most of the carcinogenic metals and their compounds, epigenetic mechanisms are considered of importance in expressing their carcinogenicity. Carcinogenicity of metal compounds is further discussed in Chapters 5, 10, and 14.

Prenatal effects are known to take place in poisoning with lead, methyl mercury, and arsenic. Reproductive and developmental effects are not well documented in human exposures to other metals, but such effects have been observed in animals after exposure to large doses of cadmium, indium, lithium, nickel, selenium, and tellurium (Clarkson *et al.*, 1985; Chapter 12). The difficulties that have occurred in the past when performing epidemiological studies regarding these effects in humans are pointed out in Chapter 12. Further studies with improved methods, taking into consideration present knowledge about human reproductive endocrinology, developmental biology, and metal toxicology, are urgently needed.

2.7 Toxicokinetics and Metabolism

Once reliable data on critical organs, critical concentrations, and critical effects have been established, it may be possible to estimate the exposure required to give rise to such concentrations, provided enough information is available on the metabolism and kinetics of the metal. A toxicokinetic model may then be set up with data on the absorption, distribution, biotransformation, and excretion of a given metal. In only a few cases have adequate models been established, one example being methyl mercury (Berglund *et al.*, 1971; Chapter 23). Almost 100% of ingested methyl mercury is taken up. The accumulation may be described by a one-compartment model, indicating that the exchange between the different organs is considerably faster than the excretion of the metal. The biological half-time is on average approximately 70 days. This means that approximately 1% of the total body burden is excreted daily, primarily through the bile.

The metabolism of cadmium is more complicated, and an appropriate model is more difficult to establish. Some facts are well recognized, however. The absorption of cadmium from food will average around 5% in men and 10% in women, but under certain nutritional conditions, such as a low intake of calcium or iron, absorption levels as high as 20% may be reached (Flanagan *et al.*, 1978; Chapters 3 and 23). Absorption of cadmium after inhalation may vary between 10 and 50%, depending on particle size distribution. In long-term, low-level exposures, several series of autopsy data have shown that approximately one third of the total body burden could be in the kidneys and about one half in the kidneys and liver together. With higher exposure, proportionally more cadmium would be found in the liver. A physiologically based multicompartment model has been developed describing the behavior of cadmium. In long-term, low-level exposure, the biological half-time is in the order of one to two decades (Friberg *et al.*, 1985; Nordberg and Nordberg, 2002; Nordberg and Kjellstrom, 1979; WHO, 1992; Chapters 3 and 23). The usefulness and validity of this

model has been shown using NHANES data (Choudhury *et al.*, 2001). It has been possible to estimate the daily intake required to reach the critical concentration of cadmium in the kidney and of methyl mercury in the CNS, these being the two critical organs, respectively (Berglund *et al.*, 1971; Diamond *et al.*, 2003; Friberg et al., 1985; Kjellstrom and Nordberg, 1978; Nordberg and Strangert, 1985; Task Group on Metal Toxicity, 1976; WHO, 1990; 1991; 1992). These estimates are in fairly good agreement with directly observed associations between exposure, concentrations in organs, and effects. Toxicokinetic models have also been published for inorganic lead and for chromium compounds (see Chapter 3). There is a great need to set up appropriate toxicokinetic models for other metals, but in most cases no adequate data are available. Clarkson *et al.* (1988) (see also Chapter 3) advanced this issue and presented metabolic models for the metals seen as most important at that time. The concept is important and should be applicable for further improvement of existing models and for metals of interest in the future.

2.8 Biological Monitoring

Exposure biomonitoring using concentrations of metals in urine, blood, or hair has been increasingly used in recent years (see Chapter 4). When interpreting such data, an appropriate toxicokinetic model is of great value, because it will depict the relationships between concentrations in indicator media and concentrations in critical organs and in the body as a whole.

Blood levels, particularly red cell levels, of methyl mercury are valuable for assessing the concentration of methyl mercury in the CNS, as well as in the body as a whole. As a rule, urinary levels cannot be used, because the excretion of methyl mercury in urine is extremely small, the main excretion route being the bile (Chapter 33). For cadmium the situation is more complicated. In long-term, low-level exposures, urinary levels on a group basis give an indication of the concentration in the kidneys and the total body burden. In individual cases, caution has to be exercised because of wide individual variations.

When the critical level has been reached in the kidneys, cadmium excretion through urine increases simultaneously with the occurrence of tubular proteinuria. The cadmium concentration in urine under such conditions no longer reflects the total body burden. Blood levels may be useful for evaluating recent exposure, and in long-term, low-level blood exposures can also be used for estimating the body burden. The biological half-time of cadmium in blood is considerably shorter than that in the kidneys (Friberg *et al.*, 1985; Chapter 23).

2.9 Risk Assessment

It is obvious that exposure to metals in industrial and general environments may be related to health risks. The crucial questions are, of course, which safety margins are needed and which ones are available. These questions are not easy to answer because of a considerable lack of adequate data (Chapter 14). If we consider methyl mercury, we know that early developmental effects are caused by methyl mercury. Risks of such effects may result from regular consumption of fish like pike, tuna, shark, and swordfish by pregnant women. Risk assessments have resulted in restrictions of methyl mercury in food both in the United States and in the European Union. Some countries (e.g., Sweden) have high concentrations of methyl mercury in lake fish because of acid rain, and thus a government-directed program of information aimed at women of child-bearing age to avoid fish high in methyl mercury has been established. Grandjean and Landrigan (2006), on the basis of the slow development of knowledge about neurodevelopmental toxicity of lead and methyl mercury in humans, pointed out the lack of adequate data for a risk assessment of neurodevelopmental effects of a number of neurotoxic metal compounds like aluminum, bismuth, ethyl-mercury, inorganic mercury, tellurium, thallium, and tin compounds. Considerable efforts are thus required to fill the gaps in knowledge.

The process of establishing health criteria for exposure standards is a difficult, but important, task, as is being recognized increasingly on national and international levels. Discussions have been devoted to the question of whether it would be advisable to apply the philosophy of collective dose to some chemical substances, including metals. According to this concept, which is used in radiation protection, any emission, from a power plant, for example, is considered to involve a certain degree of risk even if the effects may be remote both geographically and time wise, and may occur only when a simultaneous exposure from a number of other sources takes place. These and other aspects on risk assessment are discussed further in Chapter 14.

Risk assessment in human health has been refined and improved by introducing new methods such as assessing benchmark dose, identifying new biomarkers, and differentiating assessments by speciation of metals (Chapter 14; WHO/IPCS, 2007).

2.10 Interactions Among Metals

Interactions among different metals and interactions among metals, other substances, and different host factors constitute a subject on which more and more

attention is being focused. This matter has been discussed at several international symposia (Fowler, 2005; Nordberg, 1978; Nordberg and Andersen, 1981; Nordberg and Pershagen, 1985). By means of recent methods for identification of genetic variation in populations, the role of genetic polymorphisms is presently being examined in relation to metal toxicology (Chapter 7). It is obvious that such interactions occur frequently and can both increase and decrease the toxicity of metals. For example, data support the conclusion that the risk of chronic beryllium disease developing is considerably increased among persons belonging to a specific HLA group. Another example is that selenium can decrease the toxicity of methylmercury. Age seems to be an interaction factor of particular importance. Several data indicate that the absorption of both cadmium and lead is substantially higher in young age groups than in other age groups. Children are more susceptible to development of neurotoxic effect of lead than adults (see Chapters 6, 7, and 31).

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CHAPTER

2

General Chemistry, Sampling, Analytical Methods, and Speciation*

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ABSTRACT

This chapter provides an introduction to the general chemistry of metals with particular emphasis on the biological and toxicological characteristics. This is followed by an elaborate description of analytical chemistry aspects relevant to trace element analysis. Because total element analysis is giving way to elemental speciation and fractionation, detailed information is given about useful strategies in sampling and sample preparation, followed by separation techniques and detection methods for the elemental species. In addition, the major issues of calibration, reference materials, and quality assurance are dealt with.

Of 111 identified elements—90 occurring naturally on earth—most (67) form metals, and all but one of these (Hg) are solid. Eleven elements make up atomic or molecular gases, and 12 more elements form solid or liquid nonmetals. The chemistry of metals represents a major part of inorganic chemistry. Understanding the toxicology of metal species has advanced substantially during the past decades, thanks to the considerable contributions of bioinorganic chemistry (e.g., the discovery that metal species undergo biomethylation and thus can form organometallic compounds). This discipline goes hand in hand with chemical speciation, which is instrumental in understanding metal toxicology and related adverse health effects.

The commonly used term "heavy metals" to describe metals or metalloids that can give rise to toxicity was brought to the attention of the participants of workshops held by the Scientific Committee on Toxicology of Metals (SCTM) under the International Commission on Occupational Health (ICOH) in the 1970s. It was concluded by the Task Groups that the term should not be used (Task Group on Metal Accumulation, 1973; Nordberg, 1976). Recently, the improper use of the term "heavy metals" to design a group of metals and semimetals (metalloids) associated with contamination and potential toxicity or ecotoxicity was brought to further attention, and the misuse of the term was critically commented upon (Duffus, 2002). Nevertheless, the term continues to be commonly (mis)used in toxicology and legislation to encompass the pure metal and all its chemical species. This meaningless terminology totally ignores the fact that pure metals and their compounds do not have the same physiochemical, biological, and toxicological properties. It is obvious that metal species need to be addressed in each case.

Considering the persistence of the misuse of the term "heavy metals," it is interesting to learn something more about the origin of the term. An excellent historic overview of the terminology "heavy metals" has been published by J. Duffus (2002), to which the reader is referred to find the historic references. A brief summary might be helpful to illustrate the confusion that surrounds the term and avoid its further

*Partly based on Chapter 2: General chemistry of metals by V. Vouk and Chapter 3: Sampling and analytical methods by T. J. Kneip and L. Friberg in Friberg *et al.* (1986).

use in matters concerning chemistry, policy, and regulations. Before 1936, the term was used with the meanings "guns or shot of large size" or "great ability." The oldest scientific use of the term to be found in the English literature is cited in Bjerrum's *Inorganic Chemistry* published in 1936. The Bjerrum definition is based on density of the elemental form of the metal, and he classifies heavy metals as those metals with elemental densities >7 g/cm^3. Over the years, this definition has been modified by various authors, and there is no consistency. There were various classifications according to the specific gravity either >4, a little >5, then 4.5, also 6, and even 3.5. It is evident that it is impossible to come up with a consensus on the basis of specific gravity, because it yields nothing but confusion. At some point in the history of the term, it has been realized that specific gravity is not of great significance in relation to the reactivity of the metal. Accordingly, definitions have been formulated in terms of atomic mass. The criterion was still unclear as some scientists opted for atomic mass greater than 23 (sodium), this means magnesium. Others take 40 as a criterion, thus starting with Sc. Another suggestion is the ability of the element to form soaps with fatty acids as a criterion of "heaviness." Still another group of definitions is based on atomic number, suggesting citing metals above sodium (11) as heavy. One more group of definitions is based on chemical properties, with little in common: density for radiation screening, density of crystals, and reaction with dithizone. With the preceding in mind, the rationale is that there is no basis for deciding which metals should be included in this "category" of "heavy metals." The term is hopelessly imprecise, leads to confusion, and is useless to describe toxic properties.

Detailed information about the chemistry of metals is given in a number of textbooks (Cotton *et al.*, 1999; Heslop and Jones, 1976; Parish, 1977), and the introductory text by Pauling (1970). A good source of information on the properties of ions in solution, an important field for understanding metal toxicology, is the introductory monograph by Pass (1973). The principles of bioinorganic chemistry were presented in the 1970s by Hughes (1972), Phipps (1976), Williams (1976), and Fiabane and Williams (1977). More advanced aspects about bioinorganic chemistry can be found in Dessy *et al.* (1971), Addison *et al.* (1977), Fraústo da Silva and Williams (1991), and Williams and Fraústo da Silva (1996). Theoretical and chemical aspects on the toxicology of metals can be read in Hill and Matrone (1970), Brakhnova (1975), and Goyer *et al.* (1995). Two handbooks on elemental speciation covering analytical techniques, methodology, and element-by-element review were recently published by Cornelis *et al.* (2003; 2005).

1 DEFINITION OF METALS

Metals are usually defined on the basis of their physical properties in the solid state. The physical properties of great technological significance are:

1. High reflectivity that is responsible for the characteristic metallic luster
2. High electrical conductivity, decreasing with increasing temperature
3. High thermal conductivity
4. Mechanical properties such as strength and ductility

Metals in the solid state are also characterized by their crystal structure, by the specific chemical bond in which electrons are delocalized and mobile, and by the magnetic properties. These physical properties have only limited value for understanding the systemic toxic effects, although some may be important in understanding the local effects of metal aerosols.

A more useful definition of metals to make it possible to explain the toxic effects is based on their properties in aqueous solutions. This definition is: "a metal is an element which under biological conditions may react by losing one or more electrons to form a cation." In the following text, the discussions will be based on the behavior of metals/metal ions in solution and, where applicable, in biological media.

The distinction in metal toxicology between metals and nonmetals, whether on the basis of their physical or on their chemical properties, is not sharp. In metal toxicology, some strictly defined metalloids are included because they produce adverse health effects in humans, either by themselves or by interaction. They exhibit certain properties that are typical of metals, whereas other properties make them similar to nonmetals. In general, in some groups of the periodic system, a gradual transition of properties occurs from nonmetals to metals when descending from the lighter to the heavier elements (e.g., C, Si, Ge, Sn, and Pb in group 14). Borderline elements such as As, Ge, Sb, Se, and Te are sometimes called metalloids.

2 THE PERIODIC TABLE

The periodic table consists of seven horizontal rows called periods and 18 vertical columns, as presented in Table 1 *(http://www.iupac.org/reports/periodic_table/index.html).* The element with the lowest number of protons is H with one proton. Increasing the number of protons increases the atomic number and yields a different element. With an equal number of protons, the number of neutrons for each element determines the

TABLE 1 The Periodic Table

Group / Period	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	1 H																	2 He
2	3 Li	4 Be											5 B	6 C	7 N	8 O	9 F	10 Ne
3	11 Na	12 Mg											13 Al	14 Si	15 P	16 S	17 Cl	18 Ar
4	19 K	20 Ca	21 Sc	22 Ti	23 V	24 Cr	25 Mn	26 Fe	27 Co	28 Ni	29 Cu	30 Zn	31 Ga	32 Ge	33 As	34 Se	35 Br	36 Kr
5	37 Rb	38 Sr	39 Y	40 Zr	41 Nb	42 Mo	43 Tc	44 Ru	45 Rh	46 Pd	47 Ag	48 Cd	49 In	50 Sn	51 Sb	52 Te	53 I	54 Xe
6	55 Cs	56 Ba	*71 Lu	72 Hf	73 Ta	74 W	75 Re	76 Os	77 Ir	78 Pt	79 Au	80 Hg	81 Tl	82 Pb	83 Bi	84 Po	85 At	86 Rn
7	87 Fr	88 Ra	•103 Lr	104 Rf	105 Db	106 Sg	107 Bh	108 Hs	109 Mt	110 Ds	111 Rg	112 Unb	113 Uut	114 Uuq	115 Uup	116 Uuh	117 Uus	118 Uuo
*Lanthanoids			*57 La	58 Ce	59 Pr	60 Nd	61 Pm	62 Sm	63 Eu	64 Gd	65 Tb	66 Dy	67 Ho	68 Er	69 Tm	70 Yb		
•Actinoids			•89 Ac	90 Th	91 Pa	92 U	93 Np	94 Pu	95 Am	96 Cm	97 Bk	98 Cf	99 Es	100 Fm	101 Md	102 No		

The essentials: Name: cadmium
Symbol: Cd
Atomic number: 48
Atomic weight: 112.411 (8) *g*
CAS Registry ID: 7440–43–9
Group number: 12
Group name: (none)
Period number: 5
Block: d-block

isotope. Elements can thus occur as several isotopes. Some have an unstable nucleus so that they also display radioactivity besides their chemical properties. The electron configuration of elements is described in orbitals assigned as s, p, d, and f shells or subshells, thus indicating the spatial configuration of the electrons. The s-orbital is spherically symmetrical around the nucleus of the atom of the element. The orbitals p, d, and f, are not spherically symmetrical. The chemical properties of an element depend on the specific electronic configuration of the atom, which varies in a systematic way according to the atomic number. This can be easily made clear from the very didactic display given on the website *http://www.webelements.com/index.html*.

3 COMPOUNDS OF METALLIC ELEMENTS

Metallic elements and metalloids form compounds in various oxidation states, yielding inorganic compounds such as salts and saltlike products, metal complexes (coordination compounds), and organometallic compounds. In metallic compounds, the atoms bind either in ionic or covalent bonds. Intermediate forms are also seen. Dissolved in water the metallic compounds dissociate into metal ions, mostly as cations. In some cases such as the permanganate ion (MnO_4^-), an oxoanion is formed. Metallic ions can form compounds with other metallic ions, forming alloys with two or more metals in varying proportions. Binary and multicomponent systems also exist in the crystalline phase (FeS is an example).

3.1 Covalent and Ionic Bonds

Two major types of chemical bonds exist (i.e., covalent and ionic). The covalent bond is defined as a region of relatively high electron density between nuclei that arises, at least partly, from the sharing of electrons and produces an attractive force and characteristic internuclear distance (McNaught and Wilkinson, 1997). Covalent bonds exist as homopolar and heterocovalent. When the two atoms of the diatomic molecule are the same (e.g., two hydrogen atoms), the electron density is distributed symmetrically between the two nuclei, and the covalent bond is homopolar. If the two atoms are not the same, the electron distribution will be asymmetrical, and the electron density will be displaced toward the atomic nucleus that is more electronegative (i.e., which has a higher capacity to attract electrons). This is a heteropolar covalent bond. The greater the difference in electronegativity of two atoms forming a bond, the more uneven the distribution of the electrons will be. In an extreme

case, a complete transfer of electrons from one atom to another occurs, thus forming an ionic bond. Metallic elements have low electronegativity. Chemical bonds are rarely entirely covalent or entirely ionic. Ionic bonds are predominantly formed in metal salts like chlorides (NaCl) or nitrates ($Ca(NO_3)_2$). Covalent bonds are predominantly, but not exclusively, formed between metals and carbon atoms as in organometallic compounds such as dimethylmercury (CH_3–Hg–CH_3).

3.2 Oxidation Number

Oxidation can be defined according to the following three criteria (McNaught and Wilkinson, 1997).

1. Oxidation is the complete removal of one or more electrons from a molecular entity (also called "de-electronation"), for example, the Zn^{2+} ion derives from the atom Zn of which 2 electrons have been removed.
2. This definition can be extended to chemical reactions in which a complete electron transfer does not occur and which, by custom and in current usage, are called oxidations. In this application, oxidation numbers are considered. Oxidation now is an increase in the oxidation number of any atom within a substrate, for example, $Fe^{2+} - e^- \leftrightarrow Fe^{3+}$. The oxidation number in an ion or a molecule is the charge the atom would have if the polyatomic ion or molecule was composed entirely of ions. Thus, for example, in MnO_4^-, manganese is considered to be in the oxidation state +7 (Mn^{VII}), and oxygen is assumed to exist as O^{2-} ion.
3. Oxidation is also the gain of oxygen and/or loss of hydrogen of an organic substrate. All oxidations meet criteria 1 and 2, and many meet criterion 3, but this is not always easy to demonstrate.

Alternately, an oxidation can be described as a transformation of an organic substrate that can be rationally dissected into steps or primitive changes. The latter consist in removal of one or several electrons from the substrate followed or preceded by gain or loss of water and/or hydrons or hydroxide ions, or by nucleophilic substitution by water or its reverse, and/or by an intramolecular molecular rearrangement.

This formal definition allows the original idea of oxidation (combination with oxygen), together with its extension to removal of hydrogen, as well as processes closely akin to this type of transformation, to be descriptively related to definition 1. For example, the oxidation of methane to chloromethane may be considered as follows:

$$CH_4 - 2e^- - H^+ + OH^- = CH_3OH \rightarrow \text{reversal of hydrolysis } CH_3Cl.$$

Oxidation number is used to define the state of oxidation of an element. Unbound atoms have a zero oxidation state. The oxidation number of a central atom in a coordination entity is the charge it would bear if all the ligands were removed along with the electron pairs that were shared with the central atom (McNaught and Wilkinson, 1997). It is represented by a roman numeral, for example, Cr^{III}, Cr^{VI}.

The oxidation state is a measure of the degree of oxidation of an atom in a substance. It is defined as the charge an atom might be imagined to have when electrons are counted according to an agreed-on set of rules: (l) the oxidation state of a free element (uncombined element) is zero; (2) for a simple (monoatomic) ion, the oxidation state is equal to the net charge on the ion; (3) hydrogen has an oxidation state of +1 and oxygen has an oxidation state of −2 when they are present in most compounds (exceptions to this are that hydrogen has an oxidation state of −1 in hydrides of active metals (e.g., LiH) and oxygen has an oxidation state of −1 in peroxides (e.g., H_2O_2); (4) the algebraic sum of oxidation states of all atoms in a neutral molecule must be zero. In ions, the algebraic sum of the oxidation states of the constituent atoms must be equal to the charge on the ion. For example, the oxidation states of sulfur in H_2S, S_8 (elementary sulfur), SO_2, SO_3, and H_2SO_4 are, respectively: −2, 0, +4, +6, and +6. The higher the oxidation state of a given atom, the greater is its degree of oxidation; the lower the oxidation state, the greater is its degree of reduction (McNaught and Wilkinson, 1997).

Another important reaction that metals undergo is the Fenton reaction, which is important in biological systems because most cells have some amounts of iron, copper, or other metals that can catalyze this reaction. Transition metals with redox potentials in a biologically accessible range, such as iron and copper, can accept and donate electrons in a catalytic fashion. The Fenton reactions results in generating oxidative species in the cell, as follows

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH + OH^-.$$

This is the iron-salt–dependent decomposition of dihydrogen peroxide, generating the highly reactive hydroxyl radical, possibly by means of an oxoiron(IV) intermediate. Addition of a reducing agent, such as ascorbate, leads to a cycle that increases damage to biological molecules (McNaught and Wilkinson, 1997).

3.3 Inorganic Compounds

Metallic elements form a great variety of inorganic compounds. They can be classified into binary and multielement compounds. The most important binary compounds, both from the technological and the

toxicological viewpoint, are oxides and sulfides. These are the chemical forms in which most metals appear in nature, the minerals and ores. The metal-containing aerosols produced in metallurgical processes often occur as metal oxides. However, in experimental toxicological studies, chlorides and acetates are the most commonly used metal compounds because of their high solubility in water and biological systems.

3.4 Metal Complexes

A metal complex or coordination compound is formed by the association of a metal atom or ion and another chemical species, called ligand, which may be either an anion or a polar molecule. The ligands such as BAL (2,3-dimercaptopropanol) and D-penicillamine ($(CH_3)_2C(SH)CH(NH_2)CO_2H$)) serve important biological functions, where –SH groups of the ligand easily bind to a metal. Because of this, they can be used as detoxifying agents in case of, for example, mercury exposure. Another example is EDTA (ethylenediaminetetraacetic acid) used in lead detoxification.

3.5 Organometallic Compounds

Organometallic compounds are classically compounds having bonds between one or more metal atoms and one or more carbon atoms of an organyl group. Organometallic compounds are classified by prefixing the metal with organo- (e.g., organopalladium compounds). In addition to the traditional metals and semimetals, elements such as boron, silicon, arsenic, and selenium are considered to form organometallic compounds. Examples are organomagnesium compounds MeMgI (iodo(methyl)magnesium); Et_2Mg (diethylmagnesium); an organolithium compound BuLi (butyllithium); an organozinc compound $ClZnCH_2C(= O)O$ Et) chloro(eth oxycarbonylmethyl)zinc; an organocuprate $Li^+(CuMe_2)^-$ (lithium dimethylcuprate); and an organoborane Et_3B (triethylborane). The status of compounds in which the canonical anion has a delocalized structure in which the negative charge is shared with an atom more electronegative than carbon, as in enolates, may vary with the nature of the anionic moiety, the metal ion, and, possibly, the medium. In the absence of direct structural evidence for a carbon-metal bond, such compounds are not considered to be organometallic (McNaught and Wilkinson, 1997).

4 SOLUBILITY

The solubility of metal compounds in water and lipids is of great toxicological importance, because it is one of the major factors influencing the availability and absorption of metals. The solubility of metal compounds in water depends on the presence of other chemical species, particularly H^+ or H_3O^+ ions (pH). Hence, it may vary widely, depending on whether the solvent is "pure" water or a biological fluid. In mammals, the biological fluids are slightly alkaline (pH 7.4), with the exception of the fluids in the gastrointestinal tract, which are acid (pH 2–6) in the stomach and almost neutral (pH 6.8) in the intestines. Biological fluids may also contain a variety of organic ligands that exert a further influence on the solubility. Experimental data on the solubility of metal species in biological fluids are limited. Some work has been published on aluminium (Dayde *et al.*, 2003; Desroches *et al.*, 2000; Harris, 1992; Salifoglou, 2002; Venturini-Soriano and Berthon, 2001), beryllium (Sutton and Burastero, 2003), and uranium complexes (Sutton and Burastero, 2004).

Although water is too simple a model for a biological medium, strategies used in solubility studies of elemental species in water may be copied to gain knowledge in complex systems. Some simple rules are governing the solubility of metal compounds/species in water, which may, indeed, be a useful indicator of solubility of these compounds in biological fluids. A simple rule used in chemistry divides various substances into "soluble or insoluble." "Soluble" substances have a solubility in water >1 g/100 ml^1 (10 g/L^1); "insoluble" substances have a solubility of <0.1 g/100 ml^1 (1 g/L^1). This distinction may not be meaningful if a substance is highly toxic. Within each group of the periodic table, the solubility of metal compounds/species generally decreases with increasing atomic numbers.

Usually nitrates, acetates, and all chlorides, bromides, and iodides of metals are soluble, except those of silver, mercury (I), and lead. All sulfates apart from those of barium, strontium, and lead are also soluble. All salts of sodium, potassium, and ammonium are soluble, except sodium antimonite, potassium hexachlorplatinate, and potassium cobaltinitrite.

Mainly insoluble are all hydroxides (except for those of alkali metals, ammonium, and barium), all normal carbonates and phosphates apart from those of alkali metals, ammonium, and the alkaline earth metals.

Solubility depends on factors such as pH, the presence of other ions, the oxidation state of the metal, and the rate of oxidation–reduction conversions (see section 5.2).

The solubility of sparingly soluble substances depends also on their particle size and aerodynamic diameter. Finely divided material is usually more soluble.

Generally, solubility of a substance is given as solubility in water, solvents, and acids. However, biological systems greatly influence the solubility. Thus, it is important to test the metal species of concern for solubility in the biological fluids, as mentioned previously.

5 PROPERTIES OF METAL IONS

5.1 Formation of Metal Ions

Metal ions are formed by the removal of one or more outer electrons from the neutral atom. The energy required for the ion formation depends on the environment in which this process takes place. The formation of ions in the gas phase requires a considerable amount of energy. Much less energy is required when the process takes place in water, because part of the ionization energy is provided by the energy of hydration (i.e., the energy that is gained when a positively charged metal ion binds dipolar water molecules). The number of water molecules that are bound directly to the metal ion (first hydration sphere) depends on the size and the charge of the metal ion and varies from 4 for Li^+ to approximately 10 for Ra^{2+}. Because further polarization of water molecules is contained in the first hydration sphere, additional water molecules will be attracted to form a second hydration sphere. This association can continue, but its extent decreases rapidly with distance from the ion. The size of the hydration sphere will depend on the polarizing power of the ion, which, in turn, depends on the charge/radius ratio. The hydrated ion is a dynamic system in which water molecules in the hydration sphere rapidly exchange with those in the bulk phase of the solution.

5.2 Redox Potential

Redox, or oxidation-reduction, processes involve the transfer of electrons from one reactant to another. The two processes, oxidation and reduction, are always coupled. This means that when one substance is oxidized (reducing agent), another must be reduced (oxidizing agent). Oxidizing or reducing power of an oxidation-reduction system is measured in terms of the standard electrode potential (i.e., the value of the standard emf of a cell in which molecular hydrogen under standard pressure is oxidized to solvated protons at the electrode). If the standard electrode potential, E^0 of a metal is large and negative, the metal is a powerful reducing agent, because it loses electrons easily. An example of a few standard electrode potentials of metals is given in Table 2. The actual electrode potentials, or redox potentials, depend on the concentration of metal ions, on the temperature, and on the presence of other ligands that can displace water from a hydrated ion. Oxidation-reduction reactions are of fundamental importance in biochemistry. Transition metals that can easily change their oxidation state play a very important role.

TABLE 2 Standard Electrode Potentials (Skoog *et al.*, 1994)

Reaction	E^0 at 25°C, V
$Ag^+ + e^- \leftrightarrow Ag$	+0.799
$Fe^{3+} + e^- \leftrightarrow Fe^{2+}$	+0.771
$Ni^{2+} + 2e^- \leftrightarrow Ni(s)$	−0.23
$Cd^{2+} + 2e^- \leftrightarrow Cd(s)$	−0.403
$Cr^{3+} + e^- \leftrightarrow Cr^{2+}$	− 0.41
$Zn^{2+} + 2e^- \leftrightarrow Zn(s)$	−0.763

5.3 Metal Ions as Lewis Acids

Lewis made a useful definition of acids and bases. An acid is an electron pair acceptor, and a base is an electron pair donor. This means that all positively charged metal ions can be classified as Lewis acids or electron acceptors. In the same way, the water molecule that is formally an electron donor can be classified as a Lewis base. The following equations illustrate this.

metal ion + water → hydrated metal ion

(Lewis acid + Lewis base → complex) hydrated ions + ligand → complex + water

The IUPAC compendium of chemical terminology (McNaught and Wilkinson, 1997) defines these terms as follows:

A Lewis acid is a *molecular entity* (and the corresponding *chemical species*) that is an electron-pair acceptor and therefore able to react with a *Lewis base* to form a *Lewis adduct*, by sharing the electron pair furnished by the Lewis base.

For example:

$$Me_3B + :NH_3 \leftrightarrow Me_3B\text{-}NH_3 + H_3$$

Lewis acid + Lewis base ↔ Lewis adduct
(Me, methyl; B, boron)

5.4 Hydrolysis

Hydrolysis is a reaction that can occur between the metal ion (M) and one or more water molecules in the coordination (solvation) sphere, in which a proton (hydrogen ion) is released and the solution becomes acidic.

$$(M(OH_2)_x)^{n+} \leftrightarrow (M(OH)(OH_2)_{x-1})^{(n-1)+} + H^+(aq)$$

Hydrolysis may proceed in several stages until the last coordinated water molecule is removed. The process of hydrolysis may be interrupted if at one stage an insoluble compound is produced. Hydrolysis occurs most readily with metal ions that strongly polarize the coordinated water molecules.

6 OTHER ASPECTS OF METAL CHEMISTRY OF BIOLOGICAL AND TOXICOLOGICAL INTEREST

6.1 Main Group and Transition Metals

According to the *IUPAC Nomenclature of Inorganic Chemistry* (Freiser and Nancollas, 1987), the main group metals are those belonging to the periodic system group 1 (alkali metals Na, K, Rb, Cs, Fr), group 2 (alkaline earths Be, Mg, Ca, Sr, Ba, Ra), group 13 (Al, Ga, In, Tl), group 14 (Ge, Sn, Pb), group 15 (Sb, Bi), and group 16 (Po). The transition metals are those belonging to group 3 (Sc, Y, La, Ac), group 4 (Ti, Zr, Hf), group 5 (V, Nb, Ta), group 6 (Cr, Mo, W), group 7 (Mn, Tc, Re), group 8 (Fe, Ru, Os), group 9 (Co, Rh, Ir), group 10 (Ni, Pd, Pt), group 11 (Cu, Ag, Au), and group 12 (Zn, Cd, Hg).

Elements with partly filled d- or f-orbitals are usually defined as transition elements. A broader definition would be elements in any oxidation state in which they form compounds with partly filled d- or f-orbitals. By this definition also Cu, Ag, and Au would be included. There are 56 transition elements of the d- and f-block. All have the same common properties:

a. They are all metals.
b. They exhibit variable oxidation states with a few exceptions.
c. Because of partly filled d- and f-orbitals, they form at least some paramagnetic compounds.
d. Their ions and compounds are colored in one or all oxidation states.

Properties b and c are of great biological importance because of their role in biological catalysis and by their electron transport function.

The transition elements are further subdivided into three main groups:

A. The main transition elements or d-block elements
B. The lanthanide elements
C. The actinide elements

The lanthanides and actinides are classified as elements of the f-series.

http://www.iupac.org/reports/periodic_table/IUPAC_Periodic_Table-14Jan05-CI.pdf

6.2 Metal-Containing Biological Molecules

Many metals play important roles for the biological activity of enzymes and vitamins when constituting either part of the structure or as a cofactor of these entities. Zinc, for example, plays an essential role in the zinc-dependent enzyme alcohol dehydrogenase, and cobalt is essential to vitamin B_{12}. Aspects on the biochemistry in this topic are given in Hughes' monograph (1972).

Metalloproteins (also called conjugated proteins) consist of a protein and a prosthetic group or cofactor that consists of a metal. Metalloenzymes are defined as holoenzymes and a prosthetic group or cofactor that consists of a metal.

Hemoglobin, hemerythrin, and myoglobin carry oxygen bound to iron. Examples of redox proteins are iron sulfur proteins like cytochromes *c* and *b5*. Redox enzymes are cyt-p450, catalase, and peroxidase.

A number of proteins are metal carriers, as in the case of the blood proteins shown in Table 3.

6.2.1 Metalloporphyrins

The metalloporphyrins include two important categories: the chlorophyll molecule and the molecules carrying the heme group. The ability of chlorophyll to absorb light is related to the conjugated polyene structure of the porphyrin ring. Magnesium ions that are coordinated to the nitrogen atoms of the four pyrrole rings have at least two functions. They provide the necessary structural rigidity, and they increase the rate of conversion of the singlet-excited state resulting from photon absorption into the triplet state that enables the transfer of the excitation energy into the redox chain.

The two main functions of iron-containing biological complexes are the transport of oxygen and the mediation in electron transfer chains. The heme group is in all cases associated with a protein molecule as in hemoglobin, myoglobin, cytochromes, and enzymes such as catalase and peroxidase. Cytochromes serve as electron carriers, and the heme-containing enzymes catalase and peroxidase catalyze the decomposition of hydrogen peroxide. Catalase is involved in the oxidation of mercury vapor.

TABLE 3 Examples of Metal-Carrying Proteins

Protein	Metal
Transferrin	Fe
Ceruloplasmin	Cu
Metallothionein	Zn, Cd, Hg, Cu
Albumin	Zn, Cd
Phosphoproteins	Ca

6.2.2 Non-Heme Iron Proteins

Non-heme iron proteins (e.g., rubredoxins, ferredoxins, hemerythrin, and high-potential iron proteins) contain strongly bound functional iron atoms attached to sulfur, but they do not contain porphyrins. All of them have a role in electron transfer.

Ferritin and hemosiderin are important iron-containing biological structures that both store iron in a protein structure. Transferrin binds ferric iron and transports it from ferritin to cells. In microorganisms, iron is transported by ferrichromes and ferrioxamines, structures containing cyclic or acyclic polypeptide chains.

6.2.3 Cobalt-Containing Biological Molecules

The best-known cobalt-containing biological molecules are vitamin B_{12} coenzymes (cobalamins). Cobalamins contain a cobalt atom, a macrocyclic ligand corrin, and a complex organic part constituting a phosphate group, a sugar, and an organic base also coordinated to the cobalt atom. Methylcobalamin is involved in the methane-producing bacteria and has been shown to transfer the methyl (CH_3) group to a number of metals and metalloids, including Hg(II), Te(III), Pt (II), Au(I) *in vitro*. It is considered the most likely methylator of mercury *in vivo*.

6.2.4 Metalloenzymes and Metal-Activated Enzymes

Some enzymes incorporate one or more metal atoms in their normal structure. They are called metalloenzymes, including many zinc metalloenzymes. The best known of these are carbonic anhydrase and carboxypeptidase. The zinc ion is bound in a distorted tetrahedral configuration, with two histidine nitrogen atoms, one glutamate carboxyl oxygen atom, and a water molecule as ligands.

Another group consists of the copper-containing metalloenzymes. Their structure is only known to a limited extent. Ascorbic acid oxidase and various tyrosinases are examples of this group. In lower animals (crabs, snails), the oxygen-carrying molecule is the copper-containing protein hemocyanin, which, however, does not contain any heme groups (Lloyd *et al.*, 2005; Pallares *et al.*, 2005).

Metalloenzymes containing molybdenum and iron (nitrogenases) play an important role in nitrogen fixation.

Metal ions can be bound to proteins in a reversible way. This is the case with metal-activated enzymes. Such systems are much less amenable to study than metalloenzymes, because they cannot be isolated with the metal in place. Most enzymes associated with phosphate group transfers or hydrolysis seem to be activated by Mg^{2+}.

6.2.5 Metallothioneins

Metallothionein (MT) is a low molecular mass protein of approximately 6500 Dalton with a high content of SH-groups from cysteine. Two distinct metal binding domains, the α- and β-clusters have been characterized in MT and are formed of the 61 amino acids in human MT. The genes coding for metallothioneins are present in most organisms, and their induction after exposure to metals plays an important role in the protection against metal toxicity. Both essential (zinc and copper) and nonessential (cadmium and mercury) metals can induce the synthesis of metallothioneins and also constitute part of the molecule. Thus, these proteins have a role in the metabolism of essential metals and protection against the toxicity of metals. The four major groups of metallothioneins consist of MT-1, -2, -3, and -4. Mammalian MT-1 and MT-2 are present and expressed in almost all tissues. Only MT-1 exists in many isoforms. MT-3 has seven additional amino acids for a total of 68, with differences in charge characteristics compared with MT-1 and MT-2. MT-3 was identified as a growth inhibitory factor (GIF) in brain. MT-4 consists of 62 amino acids and has one glutamate inserted. It is specific for squamous epithelium and expressed in keratinocytes. The 14 human MT genes are localized on chromosome 16q13-22. Of these, six are functional, two are not, and six have not been characterized. Metallothionein is important in the metabolism and kinetics of cadmium and copper, because these metals are transported by MT in the organism. Non-MT–bound cadmium is toxic and causes a toxic insult to the cell. MT also serves various important functions for zinc and mercury.

6.2.6 Lead-Containing Biological Molecules

Lead is interfering with the enzyme δ-aminolevulinic acid dehydratase (ALAD) by specific binding. Lead-binding proteins have been related to lead toxicity. For ALAD, genetic polymorphism is described. The polymorphism is important with regard to lead toxicity.

7 TOTAL ELEMENT ANALYSIS, ELEMENTAL SPECIATION, AND METALLOMICS

The adverse effects and toxicity of metals on a living organism depend on (1) the quantity and the chemical form *(species)* of the substance administered or absorbed, (2) the way it is administered *(inhalation,*

ingestion, topical application, injection) and is distributed in time *(single or repeated doses)*, (3) the type and severity of injury, (4) the time needed to produce the injury, (5) the nature of the affected organism(s), and (6) other relevant conditions (Duffus, 1993). A major share of the following paragraphs will cover the different aspects for identification and quantification of metal species as opposed to total element determinations in samples appropriate for toxicology. Addressing the chemical form of the element instead of the total trace element concentration renders the information gained through careful analysis much more valuable. The underlying reason for this is that the characteristics of just one species of an element may have such a radical impact on living systems (even at extremely low concentrations) that the total element concentration becomes of little value in determining the impact of the trace element. Good examples are mercury and tin. The inorganic forms of these elements are much less toxic (or even do not show toxic properties), but the alkylated forms are highly toxic.

This brings us to the definition of elemental speciation and fractionation.

The IUPAC has defined elemental speciation in chemistry as follows (Templeton *et al.*, 2000):

i. *Chemical species*. Chemical element: specific form of an element defined as to isotopic composition, electronic or oxidation state, and/or complex or molecular structure
ii. *Speciation analysis*. Analytical chemistry: analytical activities of identifying and or measuring the quantities of one or more individual chemical species in a sample
iii. *Speciation of an element; speciation*. Distribution of an element amongst defined chemical species in a system. When elemental speciation is not feasible, the term fractionation is in use, being defined as follows:
iv. *Fractionation*. Process of classification of an analyte or a group of analytes from a certain sample according to physical (*e.g.*, size, solubility) or chemical (*e.g.*, bonding, reactivity) properties.

As explained in the IUPAC paper (Templeton *et al.*, 2000), it is often not possible to determine the concentrations of the different chemical species that sum up to the total concentration of an element in a given matrix. Often, chemical species present in a given sample are not stable enough to be determined as such. During the procedure, the partitioning of the element among its species may be changed. The change can be caused by, for example, a change in pH necessitated by the analytical procedure or by intrinsic properties of measurement methods that affect the equilibrium between species.

New concepts are emerging in relation to the functional role metal species at trace and ultratrace levels play in biological processes such as signaling, gene expression, and catalysis. Therefore, the chemistry of a cell needs to be characterized not only by its genome in the nucleus and by its protein content, proteome, but also by the entirety of the metals and nonmetals among the different species in a biological system (metallome), examination of the metabolism of the elemental species and the speciation of the metallome (metabolomics), and examination of the metallomics underlying the mechanism that triggers biological/physiological/toxicological effects based on the metabolomics (Suzuki, 2005).

8 SAMPLING AND SAMPLE PREPARATION

8.1 General Considerations

The exposure of humans to toxic metals is estimated from measurements of the concentrations in environmental media (e.g., air, food, and water). Preliminary inventories of sources in both occupational and environmental settings are essential to the design of adequate sampling and analysis (Kneip and Friberg, 1986). Biological monitoring is done directly by measuring toxic substances in blood or urine or from tissues that are easily available (e.g., hair).

Sampling, subsampling, and sample handling must satisfy several conditions. The elemental composition should remain exactly the same as that of the original matrix to guarantee representativeness. The variance within the laboratory samples should be an unbiased estimate of the variance of the totality of the material. This implies that when the concentration of an elemental species has an estimated variance σ^2, the calculated variance on the result given by a laboratory should fall within the 95% confidence interval for the population σ^2.

The accuracy of all sampling operations together should be of the same order of magnitude as the accuracy of the subsequent analytical procedure (Sansoni and Iyengar, 1978). For example, it is, indeed, unthinkable to first contaminate your sample a 10-fold time with the elemental species you have to determine (e.g., contamination with lead ions) and then during all subsequent steps carefully determine the concentration of that species. The result is meaningless. The same when you first change the chemical species of the compound (lower pH so that metal-protein binding is disrupted) and then apply very carefully the full analytical procedure for analyzing that particular species.

Such are the first requirements for obtaining accurate and representative analytical data to be considered by physicians, epidemiologists, and toxicologists.

This chapter does not intend to give in-depth detail for every possible element in any feasible matrix. Such information is given in the specific chapters, metal by metal. Additional information can be found in publications in international journals and also in monographs and book chapters. Sample collection guidelines for trace elements in blood and urine have been published by IUPAC (Cornelis *et al.*, 1995). The article describes harmonized guidelines for collection, preparation, analysis, and quality control. The aim was to assist scientists worldwide to produce comparable data to be useful on a regional, national, and international scale. The guidelines cover the elements Al, As, Cd, Cr, Co, Cu, Pb, Li, Mn, Hg, Ni, Se, and Zn. Avoidance of contamination is a major issue when determining the trace elements in body fluids and tissues (Versieck, 1985). Informative chapters on sampling of clinical samples for trace element speciation purposes can be read in the *Handbook of Elemental Speciation* (De Cremer, 2003 and Muñoz Olivas and Cámara, 2003).

8.2 Air, Water, and Food

8.2.1 Air

Metals exist in ambient or workplace air in both vapor and particulate forms, depending on the specific metal and chemical species. In the case of particulate-borne metals, particle size distribution and chemical properties such as solubility are important in determining the site of deposition in the respiratory tract and the degree of absorption. These factors have been addressed in a number of studies (Baron, 2003).

Environmental surveys using stationary samplers did allow establishing the concentration of several metals in a number of cities and rural and remote locations (Van Dingenen *et al.*, 2004). More relevant estimates can be obtained by the use of personal samplers. The use of such devices for the determination of workplace exposures has become common practice (Schwela *et al.*, 2002; WHO, 1982). An interesting survey about sampling systems can be found in Dabek-Zlotorzynska and Keppel-Jones (2003). The choice of the filter substrate is very important. Filters should be mechanically and thermally stable and should not interact with the deposit, even when subject to a strong extraction solvent. The rule of thumb is that when no data are available from reliable studies by other research groups, the effect of sampling and storage conditions on the stability of the species in the matrix should be studied. Many species are thermodynamically unstable. The simple act of sampling and storing the species may alter them. The information is then irreversibly lost.

8.2.1.1 Ambient Air

Metal concentrations in ambient air are generally low, and intake through air is usually small in relation to intake of food. Exceptions may occur in the vicinity of plants emitting large amounts of metals (e.g., smelters) and in areas with heavy traffic. In the past, the intake of lead through inhalation exceeded that from food as a result of the use of leaded automobile fuels. Today, a new problem has occurred through the use of automobile exhaust catalysts containing Pt, Pd, and Rh (platinum group elements or PGE). Emission rates are estimated to be in the ng/km^1 range. The forms in which the PGEs are emitted are still unclear; however, a significant soluble fraction has been measured in automobile exhausts. Analysis of exhaust particles revealed the occurrence of metallic Pt(0) attached onto aluminium oxide together with a small amount of Pt(IV) (Rauch and Morrison, 2005).

8.2.1.2 Industrial Air

Occupational exposure takes place mainly by inhalation. Good sampling techniques and accurate analysis are thus essential for evaluating the exposure in workplaces. Concentrations in industrial air are usually much higher than in ambient air, which makes it easy to collect sufficiently large amounts for accurate measurements. Personal samplers are the preferred method. Special attention is needed concerning sampling techniques and storage of airborne metal species in the workplace (Dabek-Zlotorzynska and Keppel-Jones, 2003). The choice of the filter media plays a preponderant role. General criteria that must be considered in filter selection are: (1) representative sampling for particulates of $\geq 0.3\,\mu m$, (2) low hygroscopicity, because hygroscopicity exceeding 1 mg per piece leads to serious errors in weight concentration measurements and hence to the improper estimate of the environmental concentration, and (3) absence of impurities that might interfere with the analysis. As an example of the latter, glass fiber or Teflon filters were found to be unsuitable for the sampling of airborne dust with low platinum content (Alt *et al.*, 1993). Only polycarbonate and cellulose gave blank values as low as 5 pg Pt per total filter.

The absence of interaction between species and filter substrate is particularly relevant in the case of the analysis of Cr(III)/(VI) in air particulate matter. Spini *et al.* (1994) have reported the reduction of Cr(VI) to Cr(III) when cellulose filters were extracted with an alkaline solution containing a known amount of

Cr(VI). The same inconvenience was encountered by acid dissolution (H_2SO_4) of the filters, which can be explained by cellulose's well-documented reducing properties. Therefore, cellulose filters cannot be used for chromium speciation in airborne particulates. Polycarbonate membrane filters (Scancar and Milačič, 2002) and borosilicate microfiber glass disks (Christensen *et al.*, 1999) are suitable for this type of analysis.

Also crucial is the sample integrity during storage of particulate matter. Some changes can be anticipated (e.g., reduction of Cr(VI) because of interaction not only with the collection substrate but also with the air and with other compounds in the collected dust). Erroneous results may occur because of redox reactions. The enrichment of particles on the filter gives rise to enhanced contact of the Cr species with gaseous species (e.g., SO_2, NO_x, O_2, O_3) and/or with material collected on the filter (e.g, Fe^{II} [magnetite] and As^{III}-containing components) (Dabek-Zlotorzynska and Keppel-Jones, 2003). Storing the samples in closed polypropylene vessels under an inert atmosphere (nitrogen or argon) may minimize such changes (Christensen *et al.*, 1999, Dyg *et al.*, 1994). The shorter the time between collection and analysis, the better. The reverse, namely the oxidation of Cr(III) to Cr(VI), is most unlikely under the usual conditions of storage and sample treatment.

8.2.2 Water

In areas with tapwater, it might be interesting to determine the metals species in the cold as well in the hot water. Because water pipes may contain certain toxic metallic species, these can be released in larger amounts in warm water. The concentrations should be examined in the first portion of water after it was turned off for some time and compared with those in water that has been running for some time. Pb and Cd may accumulate in the tapwater when water is not used during longer periods. Very disconcerting are the organotin compounds, which are leached out of PVC tubing. Factors like hardness and pH should be determined, because they might be of importance for exposure evaluations.

It is evident that the sampling procedure must be contamination free and representative for the original sample (Emons, 2003). Whereas "contamination free" may be an overstatement, surely for naturally occurring species, whenever it may occur, the degree of contamination should be well documented and minimal compared with the expected concentration in the sample. When making exposure estimates, it should be kept in mind that water often comes from different suppliers during the day. The intake of drinking water may vary considerably, depending on individual and local habits and on climate. Sampling guidelines are available from WHO (1997).

8.2.3 Food

Food sampling strategies depend on the purpose of the study. This may be single food items that are analyzed for a given trace element, followed by an estimate of the amount of the element that could be ingested by people with different food consumption habits, using available food statistics. A second method is to collect certain classes of food (e.g., vegetables, dairy products, fish and meat products) in amounts similar to those that are actually consumed (as estimated for a nation, a region, or population group), analyze each class, and make estimates from that. Before analysis, each food should be treated as it would normally be (e.g., cooked, cured, fried). This approach is called the "market basket" method. Such surveys were done among others in the United States (Mahaffey *et al.*, 1975), Italy (Lombardi-Boccia *et al.*, 2003), Spain (Urieta *et al.*, 1996), and Japan (Maitani, 2004). A third method is called duplicate sampling. During a certain period, the people under study put meals identical to the ones they have eaten into a vessel. The trace elements can be determined in the deposited meals after homogenization and provide a total intake figure. For details on sampling and analysis of elements and their species in food, see Emons (2003) and Brereton *et al.* (2003).

Elemental speciation instead of total element determinations in nutrition has become very evident when evaluating human health risk assessment and has already substantially contributed to its improvement. Because this topic will be dealt with in great depth in the chapters dedicated to each element, only a few examples are cited here. Detailed knowledge is available about the different inorganic As^{III} and As^{V} species in food, as well as about the various organic As species (Gallagher *et al.*, 1999). The difference in toxicity between these compounds is large, going from the extremely toxic arsine to the totally inoffensive arsenobetaine, arsenocholine, and arsenosugars. A more detailed description can be found in the chapter on arsenic. Cadmium in food is originating for 70% from eating vegetables and meat such as liver and kidney. Relevant data should describe the Cd species in the edible parts. Cd is bound to high and low (<5000 Da) molecular mass compounds (Günther and Kastenholz, 2005). In meat, Cd is mainly bound to metallothionein. A third example concerns tin. Organotin compounds, such as tributyl tin, are known to be very toxic to marine organisms. They are originating from the use of antifouling paint

and are now monitored in crustaceans (oysters) and fish. Eventually, they could become harmful to man (Rosenberg, 2005).

8.3 Biological Monitoring

Biological monitoring consists of the continuous or repeated measurement of potentially toxic substances or their metabolites or their biochemical effects in tissues, secreta, excreta, expired air, or any combination of these to evaluate occupational or environmental exposure and health risk by comparison with appropriate reference values based on knowledge of the probable relationship between ambient exposure and resultant adverse health effects (Duffus, 1993). The purpose is to obtain an integrated estimate of the uptake of metal species through all pathways and media of exposure. The interpretation of the data requires knowledge of the absorption, metabolism, and excretion of the metal species in question. It becomes more and more evident that knowledge of total element concentrations in particular biological fluids and tissues is not sufficiently relevant. A typical example is arsenic, which is absorbed by humans as inorganic arsenic. It is methylated for the larger part first to monomethyl arsonic acid and next to dimethylarsinic acid. The inorganic and methylated species are the compounds to be specifically monitored in the urine of people exposed to inorganic arsenic from drinking water or through inhalation. This will allow discrimination against arsenic uptake from eating fish and seafood, where the element is mainly present as nontoxic arsenobetaine and arsenosugars (Buchet, 2005; Francesconi, 2002). Arsenobetaine progresses unaltered throughout the gastrointestinal tract and is excreted in the urine. Arsenosugars, however, are metabolized, and approximately 12 metabolites have been documented (Raml *et al.*, 2005). Before starting on speciation of the arsenic species, one should be aware that this element is easily subject to contamination from reagents, dust, and laboratory ware at the $\mu g/L^1$ level. If contamination occurs, it will most probably be in the form of inorganic arsenic and not organic arsenic. Another aspect to consider is the stability of the species. An extensive study by Feldmann *et al.* (1999) on the stability of common arsenic species such as arsenite (As^{III}), arsenate (As^{V}), monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), and arsenobetaine in urine shows that low temperature conditions (4 and −20 °C) are suitable for the storage of samples for up to 2 months. For longer periods (4–8 months) the stability of the arsenic species was dependent on the urine matrix. Whereas the arsenic species in some urine samples were stable up to 8 months at both 4° and − 20 °C, other urine samples showed substantial changes in the concentrations of arsenite, arsenate, MMA, and DMA. The use of additives did not improve the stability of the arsenic species in urine. Moreover, the addition of 0.1 mol/L^1 HCl to urine samples produced relative changes in the inorganic arsenite and arsenate concentrations. Another research group (Jokai *et al.*, 1998) investigated the effect of storage on the stability of solutions of some organic and inorganic As species at room temperature and at 4 °C. The results indicated that organic arsenic species are stable during short-term storage, whereas solutions of inorganic species were only stable in refrigerated conditions.

Another example where speciation has become the rule is the monitoring of mercury. Exposure to the toxic alkylated species must be discerned from that of exposure to elemental mercury or its inorganic salts (Horvat and Gibičar, 2005). Methylmercury is bioaccumulating to mg/kg^1 levels in the top predators of the food chain, making up 90–100% of the total Hg concentration. Exposure to mercury vapor is highly toxic, because it is easily absorbed in the lungs into the bloodstream, from where a major share crosses the blood–brain barrier and even the placenta barrier (see Chapter 33).

One more element in which only the measurement of species is most relevant is tin. The widespread use of organotin compounds (OTCs) has led to their entrance into various ecosystems and in the food chain. Because of the high toxicity at even very low levels, tributyltin and triphenyltin have received great attention. These compounds (as well as the complete family of OTCs) are very persistent and represent a significant problem for the coming years. The analytical method to monitor the extremely low concentrations of these compounds in humans is possible, but, at present, the analyses require substantial preconcentration and sample cleanup. Therefore, they are time consuming and costly, not to say impossible, if too large a sample of blood or tissue is requested (Rosenberg, 2005).

9 SEPARATION TECHNIQUES

Species separation is mainly achieved by one of the following well-known techniques: liquid chromatography (LC), gas chromatography (GC), capillary electrophoresis (CE), and gel electrophoresis (GE). The choice will be determined by the chemical properties of the species, the available skills and infrastructure in the laboratory and, last, but not least, by the available resources.

9.1 Liquid Chromatography

The sample is introduced into a chromatographic column packed with a stationary phase while a liq-

uid mobile phase is continuously pumped through the column. The stationary phase is usually a chemically modified silica or polymer. The analytes interfere to a different extent with the stationary phase and the mobile phase. This determines the length of time each analyte resides in the column. Usually the LC system is coupled to a specific detector. Such a setup is perhaps the most common in elemental speciation analysis. High-performance liquid chromatography columns (HPLC) form a widely used subset of LC, with small diameter particles (3–5 µm) as the stationary phase, the mobile phase being pumped under increased pressure. A good overview of the role in elemental speciation can be found in Ackley and Caruso (2003). The most common types of LC are size exclusion chromatography (SEC), ion-exchange chromatography (IC), and affinity chromatography (AC). Today, it is also possible to couple an LC setup to a soft ionization system to obtain structural information. Examples of a soft ionization technique are electrospray mass spectrometry (ES-MS) (Chassaigne, 2003) and tunable plasma (Leach *et al.*, 2003). Figure 1 shows an example of the separation of Ni speciation analysis in the hyperaccumulating plant *Sebertia acuminata* (Schaumlöffel *et al.*, 2003). The authors first applied size exclusion chromatography on the plant extract and measured Ni with ICP-MS, followed by chromatography of the m/z fraction containing Ni on Superdex peptide HR (30 cm × 10 mm) column also coupled to ICP-MS. Next, electrospray ionization mass spectrometry (ESI-MS) was done of the fractions corresponding to the major peak. This allowed the identification of the compound as a complex of Ni with nicotinamide.

Multiple procedures are described in the literature for the separation of specific elemental species or groups of species. As an example, approximately 100 chromatographic conditions have been listed in the literature for the separation of organotin compounds (Harrington *et al.*, 1996), selenium species (Montes-Bayón *et al.*, 2000), arsenic species (Ali and Jain, 2004; B'Hymer and Caruso, 2004), mercury species (Harrington, 2000), elemental species bound to proteins (Templeton, 2005), elemental species bound to humic acids (Heumann, 2005), etc. Variations in the choice of the column and the elution conditions are legendary. Enrichment and derivatization of the species have been comprehensively outlined by Bouyssiere *et al.* (2003). Liquid chromatography will surely remain the major separation technique in the decades ahead. The electronic databanks prove to be very helpful in putting together a procedure that is ideally suited for the combination of matrix, analyte, and the available infrastructure of the laboratory.

9.2 Gas Chromatography

Only volatile and thermally stable species qualify for separation by gas chromatography. Very few compounds fulfill these requirements. Fortunately, the analyst can resort to chemical reactions that transform nonvolatile compounds into volatile, thermally stable ones. This process is referred to as "derivatization" (García Alonso and Encinar, 2003). Liu and Lee (1999) have written a comprehensive review on chemical modification or derivatization of analytes in speciation analysis.

Naturally occurring volatile species are dimethylmercury (Me_2Hg), dimethylselenium (Me_2Se), tetramethyltin (Me_4Sn), trimethylantimony (Me_3Sb), trimethylbismuth (Me_3Bi), methylated arsines, tetraalkylated lead compounds in sewage sludge, and many more gases from municipal waste disposal sites. This list is not exhaustive. Feldman (1997) has done interesting research on these compounds, describing innovative ways to convert nonvolatile into volatile species by derivatization techniques. Various separation schemes have been developed. Most common is the cryogenic trapping and sequential thermal desorption from the packed columns. This method is not very selective, but unstable compounds can be preserved for a long time before analysis. Next comes gas chromatography on packed columns, offering an improved separation of the analytes through interaction with the column, combined with separation on the basis of their volatility (Szpunar *et al.*, 1996). Gas chromatography with capillary columns offers a much-improved resolution. Their very small loading capacity forms the limiting factor for their exploitation.

The most common detector for this type of speciation is inductively coupled plasma mass spectrometry (ICP-MS), additionally inductively coupled plasma atomic emission spectrometry (ICP-AES). It is also possible to do isotope dilution measurements and isotopic ratio patterns when a high-resolution ICP-MS is available as the elemental detector.

9.3. Capillary Electrophoresis

The principle of separation by capillary electrophoresis (CE) is based on differences in the electrically driven mobility of charged analytes, similar to conventional electrophoresis. A high voltage electrical field (typically 20–30 kV) is applied along an open tube column with small internal diameter (Michalke, 2003).

This technique can be used as a primary or as a secondary separation technique (e.g., after HPLC) when it is referred to as a two-dimensional technique. Taking into account the very small loading capacity of CE, the

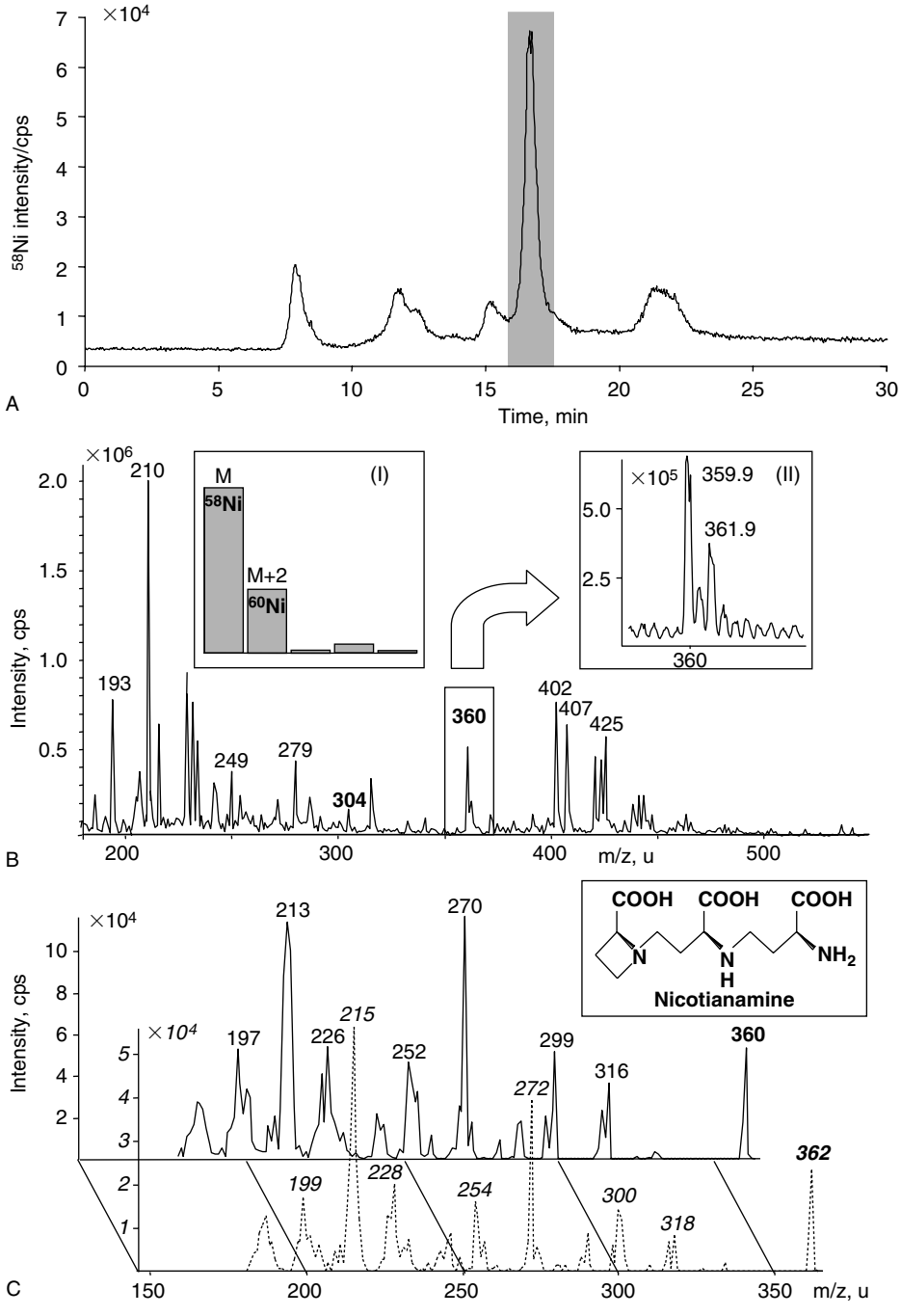

FIGURE 1 Ni speciation analysis in the hyperaccumulating plant *Sebertia acuminata*. (a) SEC HPLC - ICP MS chromatogram of the latex extract, elution with 5 m*M* ammonium acetate buffer (pH 6.8) from the Superdex peptide HR (30 cm × 10 mm) column; (b) ESI-MS spectrum of the fractions corresponding to the major peak (shaded area) in (a) inset (I): theoretical Ni isotopic pattern, inset (II): observed Ni isotopic pattern; (c) ESI MS/MS spectrum of the *m/z* 360 and 362 ions leads to the identification of a Ni-complex with nicotinamide, inset: structure of the ligand (Schaumlöffel *et al.*, 2003).

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two-dimensional approach will yield far more interesting results, bringing the high resolution of CE to its full potential. The system is often connected to ultraviolet (UV) detection for molecular information but also to ICP-MS or ES-MS (electrospray mass spectrometry) for either elemental or molecular information.

Different separation modes exist in CE: capillary zone electrophoresis (CZE) with separation on the

basis of the charge/mass ratio, capillary isoelectric focusing (CIEF) based on the isoelectric point, capillary isotachophoresis based on analyte conductivity, and micellar electrokinetic capillary chromatography (MECC) based on hydrophobicity.

CE analysis offers high resolution and high speed, and it is easily adaptable for automation and quantitative analysis. It has been successfully used for the speciation of many compounds (Alvarez-Llamas *et al.*, 2005), among others Cr(III)/Cr(VI) (Jung *et al.*, 1997), selenium and arsenic compounds (Sun *et al.*, 2004), Se in human milk (Michalke, 2000), and metallothionein of Cu, Cd, and Zn (Profrock *et al.*, 2003).

9.4 Gel Electrophoresis

The field covered by gel electrophoresis (GE) for elemental speciation consists of charged macromolecules to which a metal or metalloid is bound, covalently or not. These macromolecules can be proteins, humic acids, or other samples such as DNA or dyes. There are practical limitations because of the small amount of material that can be brought onto the gel and, consequently, the limit of detection of the species. For protein separation its resolution is unsurpassed (Chéry, 2003).

The first prerequisite during the separation procedure is again the preservation of the elemental species. This is not evident, considering the nature of the many reagents needed to operate gel electrophoresis. Not all publications hitherto included such a stability study, and neither did they address the contamination that may occur during the process. Very prominent is (e.g., the Pt) contamination of the samples because of the release of Pt ions from the Pt electrodes during electrophoresis. Substitution of Pt by Ag solved this problem when searching for Pt species (Lustig *et al.*, 1999). Other critical parameters are the choice of buffer and pH. More about the precautions to be taken during such separations can be found in section 9.5.

In case the metal is covalently bound, such as Cu in ceruloplasmin, denaturing conditions can be used during electrophoresis. The primary structure of the molecule will be preserved. This is not the case for noncovalently bound elements, for which nondenaturing conditions or native electrophoresis should be applied, to prevent the loss of the basic structure of the complex and stripping of the metal. Another factor that may even jeopardize the stability of covalently bound elements is oxidation of residues of proteins, as has been documented in the case of selenoproteins (Chéry *et al.*, 2001; 2005).

The method can be hyphenated with powerful detection methods such as MALDI-MS (matrix-assisted laser desorption ionization mass spectrometry) for molecular detection or with LA-DRC-ICP-MS (laser ablation dynamic reaction cell inductively coupled plasma mass spectrometry) for elemental detection (Chassaigne *et al.*, 2004). A more tedious way, but reliable for quantitative measurements, consists of cutting out zones of separated proteins in the gel and measuring the element offline with ICP-MS, by use of ETV (electrothermal vaporization) as the sample introduction system (Chéry *et al.*, 2002).

9.5 Precautionary Measures in Elemental Speciation

Whenever speciation analysis is called for, it is of paramount importance to conserve the species in the same form and ratio as in the original matrix (De Cremer, 2003). Before separating and analyzing the species, it is, therefore, necessary to define the conditions under which they remain stable. In practice, it turns out that these conditions are very close to those occurring in the sample. A well-documented example is the noncovalently bound vanadium (V)–transferrin complex, the principal vanadium species in serum with a log K value of 6.5 $mole^{-1}$. This is a relatively low-stability constant compared with those of Fe(III) and Al(III) with transferrin (log $K(Fe^{3+}) = 22.7\,mole^{-1}$ and $K(Al^{3+}) = 12.9\,mole^{-1}$). The V-transferrin complex was studied at varying pH, salt molarity, and acetonitrile concentrations (De Cremer *et al.*, 1999). All experiments were carried out by use of ultrafiltration. It turned out that only around the physiological pH is the percentage of vanadium bound to transferrin high, whereas in extreme acid and basic media, the binding is completely disrupted. This behavior can be explained by protonation and deprotonation of some amino acids at the protein-binding site. Out of this behavior it was concluded that the addition of an acid for preservation purposes (e.g., to urine) would lead to misleading results. Contrary to these findings, a study of the vanadate–albumin complex, showed that the addition of acid increased the binding capacity of vanadium to albumin at lower pH values. The addition of high amounts of salt caused a similar rupture of the transferrin–vanadate bond. Salts are added to the buffer during chromatographic techniques such as anion-exchange or hydrophobic interaction chromatography. The salts are added to generate a gradient, which governs the elution behavior of the analytes from the column. From these observations it was concluded that the use of hydrophobic interaction chromatography (high salt concentration at the starting point) is not recommended in the case of vanadium–transferrin complexes. In anion exchange chromatography, high salt concentrations are also used, but at the end of the chromatographic run. As such, limited use of this technique can be considered. There were,

however, also significant differences observed between the various kinds of salt. The addition of high amounts of acetonitrile in the buffer also disrupts the vanadium–transferrin bond, precluding the use of reversed-phase chromatography, because it usually requires high acetonitrile concentrations in the eluent.

Before embarking on a separation procedure, it is essential to investigate the stability of the trace elemental species in the different media. If not, the chromatograms will fail to give the original distribution of the metal species and, worse, they will only yield meaningless artifacts.

As mentioned in section 9.2, derivatization (chemical modification) of a compound is common practice before gas chromatography. When studying elemental species, care should be taken that the original moiety in which the trace element is sitting is not disrupted as a consequence of the derivatization. Liu and Lee (1999) warn about the problem that is sometimes encountered by using chemical modification for speciation analysis because of the loss of speciation information in the original sample. For example, when the fraction of free versus bound ions is of interest or when the species and its complexes originally existing in the sample are less stable than the complexes formed as a result of the added complexing agent (Olesik *et al.*, 1995), the use of that complexing agent will cause problems in identifying the original species. Therefore, attention must be paid to choosing modification methods and appropriate reagents and in controlling operational conditions governing the modification process.

When proteins are subjected to lysis, it is necessary to check whether the sidechains carrying the element remain unaltered. As mentioned in the previous section, it is fundamental to test the integrity of the elemental species when exposed to any reagent, and more so in the case of the harsh conditions encountered in gel electrophoresis. The procedures that can be used for this purpose are gel filtration and ultrafiltration (Chéry *et al.*, 2001).

In all these endeavors a main impediment is the lack of suitable standards for identifying and quantifying metal-binding biomolecules, which commonly appear during toxicological investigations.

10 DETECTION METHODS

10.1 General Aspects

During the past decade, substantial progress has been made in improving the sensitivity of the detection methods of the commercially available equipment.

There are two lines of use: total element and elemental speciation analysis. In routine work, most of the applications go for total element measurement. In case of liquid samples, a simple dilution of the sample may be sufficient to allow a direct measurement of the element(s) with calibration versus standard solutions. In case of difficult matrices (e.g., salts, organic compounds such as proteins), it may be necessary to apply the standard addition method. For solid samples, such as tissues, it is feasible to apply electrothermal vaporization (ETV) of the sample in a graphite oven and introduction of the fumes into the element detector ETV-AAS (atomic absorption spectrometry) and ETV-ICP-MS (inductively coupled plasma–mass spectrometry). Calibration is done versus a similar matrix calibrated for the element(s) of interest and undergoing the same procedure. This method has been developed (e.g., the measurement of Hg in solid samples by ETV-ICP-MS). In this particular case, the prerequisite for a similar matrix calibrated for the element can be circumvented by use of isotope dilution mass spectrometry. The direct determination of Hg in solid samples, such as hair and tissue, was done with a ^{200}Hg-enriched gaseous phase for calibration based on isotope dilution (Resano *et al.*, 2005). All matrix effects (suppression) observed during Hg vaporization were dealt with, allowing accurate values to be obtained in practically the same operating conditions for all sample types. The method presents interesting features such as a low sample consumption (a few milligrams), a high sample throughput (10 min/sample), a low limit of detection (6 ng/g^1), and a reduced risk of analyte losses or contamination.

In elemental speciation analysis, the trend is to go for online, also called hyphenated, systems. The most convenient methods for online coupling are inductively coupled plasma mass spectrometry (ICP-MS), hydride generation atomic absorption spectrometry (HG- AAS), and atomic fluorescence spectrometry (AFS). ICP-MS is the most versatile but also the most expensive "detector." It can be coupled to LC systems, gas chromatography, and capillary electrophoresis. This potentially multielement method is then only used for the detection of a single element. For economical reasons, it is, therefore, worthwhile to consider what other single element detection methods such as atomic absorption and fluorescence spectrometry have to offer.

10.2 Current Methods for the Detection of Metals

10.2.1 Atomic Absorption Spectrometry

Theory and practice of atomic absorption spectrometry (AAS) can be learned from the handbook by Welz and Sperling (1998), among others.

The basic principle underlying AAS is that atoms formed in the ground state in a gas will absorb radiation at characteristic ground-state energy levels. A light beam with the spectral composition of the ground state lines of the element will be specifically absorbed by the ground-state atoms of that element. For example, ground-state cadmium atoms thus absorb radiation at a characteristic wavelength of 228.8 nm, one of the ground-state cadmium lines, and lead atoms absorb at 283.3 nm. The light source is a high-intensity, ground-state line emitting hollow cathode lamp of the specific element. The decrease in intensity of the light beam compared with a blank is proportional to the amount of the element in the ground state in the plasma.

There are two main methods for atomization of a sample in AAS: the flame (F-AAS) and the graphite furnace method (GF-AAS). The flame method uses different gas mixtures for creating a high temperature atomization flame (e.g., air-acetylene). Different types of flames are required for different metals. The liquid sample is aspirated directly into the flame. Because of the relatively low atomization of the flame (approximately 500 °C), the method may not be sensitive enough to measure most elements in biological materials. In the graphite furnace, temperatures of approximately 2000 °C prevail, increasing by several orders of magnitude the number of atoms and hence the detection sensitivity. Typically, the detection limit for Cd by F-AAS is $1\,\mu g/L^{-1}$ and only 8 ng/L^{1} with GF-AAS. A small aliquot of the sample is injected into the oven. A matrix modifier may be added to assist the charring and atomization processes.

Nonspecific absorption may occur because of the presence of matrix atoms and molecules in the flame or the furnace. Salts such as sodium chloride and phosphates are especially apt to cause interference. This may be avoided by background correction, for which a deuterium lamp is generally used. A more sophisticated method uses the Zeeman effect that splits the lines with a magnetic field. In case the background correction fails, or when a concentration step is needed, it is necessary to mineralize the sample and to extract the element. A common chelating agent is APDC (ammonium pyrrolidine dithiocarbamate), which is extracted into MIBK (methylisobutyl ketone). This procedure has been used for several elements (e.g., cadmium, lead, and nickel).

For mercury with its high volatility at room temperature, the best detection method is cold vapor atomic absorption spectrometry.

Elements such as As, Se, Sb, and Hg that can easily be transformed into hydrides can be very advantageously measured by hydride generation (HG)-AAS. This method allows elimination of background interferences to a very large extent.

The difficulty, if not the impossibility, of making flame atomic absorption (F-AAS) and graphite furnace atomic absorption spectrometry (GF-AAS) an online method for measuring the elements in liquid chromatography fractions make them less popular for elemental speciation purposes. They have, however, earned their merits in the field. GF-AAS has been used for the offline measurement of elements in the elution fractions of LC, although insufficient detection limits proved to be a serious drawback in the case of many clinical applications in which the concentrations of the elemental species in the biological fluids and tissues are very low (Zhang and Zhang, 2003).

When species can be converted to hydrides, as is routinely done for Hg, Se, As, and Sb, hydride generation atomic absorption spectrometry is a very interesting and cheap online detection technique. It has been applied for the speciation of arsenic in persons with abnormally high arsenic concentrations in serum, such as dialysis patients (Zhang *et al.*, 1996; 1998). An online method was developed for the speciation of arsenic species in human serum, including monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), arsenobetaine (AsB), and arsenocholine (AsC). The method is based on cation-exchange liquid chromatography (LC) separation, UV-photooxidation for sample digestion, and continuous hydride generation (HG) atomic absorption spectrometry (AAS) for the measurement of arsenic in the LC eluent. By developing the technique of argon segmented flow in the postcolumn eluent, a substantial improvement in chromatographic resolution for the separation of these four arsenic species was obtained. The LC separation, photooxidation, hydride generation, and AAS measurement can be completed online within 10 minutes. The detection limits for MMA, DMA, AsB, and AsC in serum were 1.0, 1.3, 1.5, and 1.4 $\mu g/L^{1}$ of arsenic, respectively. The concentration of the four species was determined in six serum samples of patients with chronic renal insufficiency. Only AsB and DMA were significantly detected by this method. The main part of arsenic in human serum is AsB. No MMA, AsC, or inorganic arsenic was detected.

Atomic absorption spectrometry with a quartz tube atomizer is a very sensitive, specific, rugged, and comparatively inexpensive detector for GC. Gas chromatography coupled with atomic absorption spectrometry has been described as a sensitive instrumentation for mercury speciation (Emteborg *et al.*, 1996). Online solid-phase extraction, coupled to graphite furnace AAS, has also been explored.

Cold vapor atomic absorption spectrometry (CV-AAS) is the most widely used technique for measuring Hg species. Direct coupling of solid-phase

microextraction and quartz tube-atomic absorption spectrometry has been used for the selective and sensitive determination of methylmercury in seafood (Fragueiro *et al.*, 2004).

It can be anticipated that these simple, rapid, and inexpensive procedures, based on AAS detection, will be readily accepted once routine laboratories get involved in speciation analysis.

10.2.2 Atomic Fluorescence Spectrometry

When species can be converted into hydrides, such as is routinely the case for Hg, Se, As, and Sb, atomic fluorescence spectrometry (AFS) becomes a very economical elemental detection technique. This method is based on measuring the intensity of the specific resonance fluorescence of the atom (Kirkbright and Sargent, 1974). It is, however, necessary to keep in mind that the conversion of the different species of an element into hydrides is not happening to the same extent and at the same rate. This has been documented (e.g., in the case of As). The conversion of methylated arsenic species into methylated hydrides gives a different response than the conversion of inorganic arsenite or arsenate to AsH_3 (Zhang *et al.*, 1996).

10.2.3 Atomic Emission Spectrometry

Inductively coupled plasma atomic emission spectrometry (ICP-AES) has become the most common emission spectrometric technique. It is sometimes referred to as ICP-optical emission spectrometry (OES). The argon-based plasma is compatible with aqueous aerosols and offers high energy for drying, dissociation, atomization, and ionization of the analytes. The temperature reached by an argon ICP is 5500–6500 K, high enough to destroy to a great extent the molecular bonds and to ionize many elements. The high degree of excitation results in a high atomization yield and thus high sensitivity. The standard configuration of an ICP includes a pneumatic nebulizer for the formation of the aerosols and a spray chamber acts as a filter selecting droplets with a maximum cutoff diameter. The light emitted by the atoms on their return from the excited to a lower energetic state is resolved into a line spectrum by either a polychromator or a monochromator, depending on the equipment. The wavelength is specific for the atom and the intensity for its concentration. The incidence of possible interferences caused by matrix constituents is very large and requires a careful study.

More information on ICP-AES can be found in specialized handbooks (Boumans, 1987; Dean, 2005)

ICP-AES is in principle multielemental, although in the case of elemental speciation, it will most often be used as a single element detector. It is easy to couple online with LC, because it can accept a continuous flow of eluent. The disadvantages are the overall inefficiency of the nebulizer and the plasma's sensitivity to organic solvents. The poor tolerance of the plasma source to common mobile phases, such as ion-pair reagents, limits the applicability of the technique. The fact that many ion exchange chromatography elutions are not isocratic (i.e., the elution is effected under variable, usually increasing, ionic strength) requires special protocols to circumvent the problem of varying analyte response during the elution (Zhang and Zhang, 2003).

10.2.4 Mass Spectrometry

10.2.4.1 Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)

ICP-MS is a remarkably powerful technique for (ultra) trace element determinations. It is characterized by extremely low limits of detection and a wide linear dynamic range, multielement capability, and high sample throughput (Vanhaecke and Köllensperger, 2003). This method is based on measuring m/z ratios. The very low detection limits are due to the very high degree of atomization in the argon plasma at approximately 7000 K (Dean, 2004). This extreme temperature makes it far superior to the graphite furnace as an atomization source for atomic absorption spectrometry. When a quadrupole mass spectrometer is used, there are problems caused by spectral interference, because the resolution is limited to $\Delta m/m = 1$. For instance, when measuring ^{52}Cr (most abundant Cr isotope θ = 83.8%), mass 52 will experience interference from the isobars of $^{40}Ar^{12}C^+$, $^{35}Cl^{16}OH^+$, $^{36}S^{16}O^+$, occurring in samples having a high C, Cl, S,…content. ^{50}Cr and ^{54}Cr suffer from interferences from high background counts because of $^{36}Ar^{14}N^+$ and $^{38}Ar^{16}O^+$, respectively.

Today two major tools exist to reduce the spectral interferences so as to become negligible. The dynamic reaction cell allows chemical reactions in a collision cell so that the interfering isobars are neutralized or transform the analyte into another more heavy polyatomic. Another very reliable, but very expensive tool to ban isobaric interferences is the high-resolution ICP-MS with $\Delta m/m = 1/4000$ till $1/10000$ (Houk, 2003).

Besides spectral interferences, there may be numerous nonspectral interferences, which are matrix-induced signal suppression or enhancement. An additional hurdle is signal instability and/or drift. In case of total element determination, these, together with the nonspectral interferences, can often be corrected for by use of a carefully selected internal reference. To all blank, sample, and standard solutions an

equal amount of an internal reference element (internal standard) is added. It is assumed that this element undergoes the same suppression or enhancement as the analyte element(s). Usually an internal reference element with a mass number close to that of the analyte is chosen. It is, however, recommendable to consult the specialized literature, because much research has already been done for each specific element to describe optimal conditions.

ICP-MS is ideal to work online with HPLC (Ponce de Léon *et al.*, 2002). Similar difficulties caused by the influence of the eluent on the plasma can be anticipated and need careful consideration as mentioned in the section on ICP-AES.

An alternative way for sample introduction is solid-sampling electrothermal vaporization followed by ICP-MS detection. An interesting application is the direct determination of methylmercury and inorganic mercury in fish tissue with nonspecific isotope dilution (Gelaude *et al.*, 2002).

10.2.4.2 Plasma Source Time-of-Flight Mass Spectrometry

Plasma source time-of-flight mass spectrometry is a powerful tool for elemental speciation analysis through the use of a modulated or pulsed ionization source that provides both atomic and molecular fragmentation information (Leach *et al.*, 2003). Its use has been documented, among others, for the analysis of organotin compounds and the oxidation states of various elements.

10.2.4.3 Glow Discharge Plasmas as Tunable Sources for Elemental Speciation

Glow discharge plasmas offer a number of interesting possibilities as speciation detectors for gaseous and liquid sample analysis (Marcus, 2003). The plasma works at sufficiently low temperatures (kinetic temperatures in the range of 100–500°K) so as not to cause dissociation of the molecular species. The detector can be optical emission spectrometry or mass spectrometry. The technique has been successfully applied for the speciation of, for example, organotin compounds.

10.2.4.4 Electrospray Mass Spectrometry

This technique offers soft ionization of metal-containing species followed by tandem mass spectrometry for the precise determination of the molecular mass of the original species and of the individual fragments. This method is ideal to obtain structural molecular information about the species. There is a need for excessive sample cleanup to obtain high sensitivities (Chassaigne, 2003).

The method allows the coupling of HPLC online, on condition of using a suitable eluent. This method has been successfully applied for the speciation of organoarsenicals, selenium species, metallothioneins, etc.

10.2.5 Electrochemical Methods

Electrochemical methods are based on the measurement of electrical signals associated with molecular properties or interfacial processes of chemical species (Town *et al.*, 2003). Because of the direct transformation of the desired chemical information (concentration, activity) into an electrical signal (potential, current, resistance, capacity) by the methods themselves, they are easy and cheap. The two major difficulties in the application of electroanalytical techniques to complex real-world samples have been the lack of selectivity and the susceptibility of the electrode surface to fouling by surface-active materials in the sample. A variety of electroanalytical techniques that differ in the mode of excitation signal–response characteristics are currently being used: potentiometry, fixed potential methods, amperometry, various forms of voltammetry, and electrochemical detection in liquid chromatography and flow-injection analysis. These methods have been applied for the quantification of various oxidation states of several elements ((Fe(III)/Fe(II), Cr(VI)/Cr(III), Tl(III)/(I), Sn(IV)/(II), Mn(IV)/(II), Sb(V)/(III), As(V)/(III), and Se(VI)/(IV)). Electrochemical methods have also been used to investigate organometallic species or metal complexes in equilibrium with each other (e.g., butyltin species in harbor water measured by adsorptive stripping voltammetry with tropolone). The ideal is to perform *in situ* measurements with minimal sample perturbation. Despite many difficulties well known to specialists in the field, the sensitivity of electroanalytical methods makes them very powerful tools for many applications.

10.2.6 Spectrophotometry

Spectrophotometry usually involves the formation of a complex of the trace element with a selective reagent that absorbs light in the visible and UV region. Selective reagents have been used for measuring many trace elements and also to distinguish between oxidation states of certain elements. Most of them, however, suffer from interferences from either colored substances, from substances that also form complexes with the chosen reagent, or from oxidizing-reducing agents.

Spectrophotometry is still used for the measurement of Cr(VI) in welding dust. The selective determination is based on the formation of a complex between Cr(VI) and ammonium pyrrolidinedithiocarbamate (ADPC) (Andrle and Broeckaert, 1993). A major disadvantage of

the ADPC method is that interferences by other colored species such as Fe(III) or Cu(I), or species forming complexes with ADPC such as V, Mo, and Hg are possible.

10.2.7 Biosensors for Monitoring Metal Ions

Different types of biosensors exist: whole cell biosensors, enzyme- and apoenzyme-based biosensors, antibody- or protein-based sensors, and protein-based capacitive sensors (Bontidean *et al.*, 2003). They are a versatile tool for monitoring metal ions in the environment with high sensitivity and selectivity. Biosensors based on metal-binding proteins are an excellent tool to measure the bioavailable concentrations of metals.

10.2.8 Direct Measurement of Metals in Solid Samples (Particle Characterization)

The characterization of aerosol particles, their size distribution, and chemical composition is of great importance in occupational health monitoring to evaluate health hazards for workers exposed to dust from foundries, calcination ovens, powder handling, milling, etc (Ortner, 2003). The instrumentation necessary for these investigations is very specialized and quite expensive. The procedures routinely used are high-resolution scanning electron microscopy (HR-SEM) and electron probe microanalysis (EPMA). A series of explicit methods exist for particle speciation (Ortner (2003). In addition, there is the possibility of direct speciation of solids with X-ray absorption fine structure spectroscopy (XAFS), an element- and species-selective method (Welter, 2003). Only with the invention of synchrotron radiation as a source of very intense X-rays to XAFS spectroscopy in 1974 did it become possible to measure XAFS spectra within a reasonable time and in diluted samples. The region above the edge in the spectrum is normally divided into two subregions, the first 50–100 eV above the edge is called X-ray absorption near edge structure (XANES), the region above the XANES region is called extended X-ray absorption fine structure (EXAFS) (Welter, 2003). Between explicit methods for particle speciation, there exist valence band X-ray spectrometry by EPMA-WDX (electron probe microanalysis-wavelength-dispersive X-ray detector), TEM (transmission electron microscopy), and XPS (X-ray–induced photoelectron spectrometry (Ortner, 2003).

10.2.9 Neutron Activation Analysis

Neutron activation analysis (NAA) is a very sensitive and reliable multielement method (Ehmann and Vance, 1993). Today, it is only sparingly used as a consequence of the closure of a major share of the nuclear research reactors all over the world. NAA is based on the principle that when materials are irradiated in a nuclear reactor or in another neutron source, some of the atoms are converted into radioactive isotopes. The type and energy of their radiation and their decay rate are specific. A quantitative estimation can be made by comparing the element's radioactivity with suitable standards irradiated simultaneously. Gamma-spectrometry with high-resolution germanium detectors coupled to multichannel analyzers has become the method of choice for measurement of the induced radioactivity. The standardized k_o method offers unique possibilities, because it has modeled NAA into a very flexible procedure (Decorte *et al.*, 1994).

NAA has played a leading role in trace element analysis because the danger of contamination and loss of elements can be virtually eliminated.

This method is most interesting for the analysis of those elements that are converted into radioactive isotopes (i.e., they have an abundant isotope with a high cross-section for thermal neutrons, such as arsenic). Other elements, such as lead, have an extremely low sensitivity for NAA. In the latter case, it is necessary to resort to another technique such as F-AAS, GF-AAS, ICP-AES, ICP-MS.

NAA is divided into two broad categories: (1) instrumental neutron activation analysis (INAA) consisting of irradiation, measurement, and evaluation of the spectra; and (2) radiochemical activation analysis (RNAA), including irradiation of the samples, followed by radiochemical separation of the elements, measurement of the induced radioactivity, and evaluation of the spectra. In the case of INAA, advantage is taken of the decay properties of the isotopes so that the short-lived ones are measured first. As they progressively decay, the longer-lived isotopes can be measured. When analyzing biological material, a major matrix-induced radioactivity prohibits the measurement of the radioactivity attributable to the trace elements. Because of their overwhelming amount of gamma-rays and/or Bremmstrahlung, the ^{24}Na, ^{32}P, ^{38}Cl, and ^{82}Br isotopes (the list is not exhaustive) have to be removed from the isotopes of interest, such as ^{76}As, ^{64}Cu, ^{69m}Zn, ^{122}Sb, ^{51}Cr because these interferences are so outspoken when analyzing serum, packed cells, tissue, bone, and milk, RNAA is the only way out. INAA is feasible for long-living isotopes, such as ^{65}Zn, ^{75}Se, and ^{124}Sb; RNAA requires highly skilled technicians, is time consuming, and requires a heavy infrastructure. INAA may cover many months to allow the decay of the shorter living isotopes. INAA for very short-living isotopes (half-lives seconds or minutes) may be very fast but is generally not conceivable for the very low concentrations encountered in biological material because of the radioactivity induced into

major elements. For instance, ^{47}Ca ($t_{1/2}$ 4.5 d) and ^{32}P ($t_{1/2}$ 14.28 d) produce intense Bremsstrahlung radiation, which prevents the determination of several longer-lived but low-energy isotopes. Under such conditions, RNAA has to be done.

Interesting literature is available on the International Atomic Energy (IAEA) website (2004).

10.2.10 Spark Source Mass Spectrometry

Spark source mass spectrometry (SSMS) used to be the most sensitive widely used multielement technique until the 1960s. The basis for SSMS is the formation of ions when the sample is subjected to a high-energy discharge (Morrison, 1979). The apparatus uses a vacuum spark in which a high-voltage radio frequency discharge (20–100 kV) is produced between two closely spaced electrodes containing the material to be analyzed. This sparking results in vaporization and ionization of sample constituents. The repetition rate and duration of the radio frequency spark source is variable to meet the various analytical requirements. Nearly all masses are integrated simultaneously over a period of time to provide high sensitivity. For SSMS, the sample has to be electrically conductive. This is achieved for nonconducting biological material by blending them with high-purity graphite followed by briquetting to form electrodes that sustain the vacuum spark. Two major problems arise. First, the graphite used for making the electrodes also contains trace element impurities, and they may exceed the concentrations in the biological sample. The second problem lies with the interferences caused by the organic ions of differing complexity obtained from many possible combinations of C, H, O, N, S, P, etc. Therefore, to take full advantage of this multielement method, the samples are ashed before analysis. This will, however, cause the loss of volatile elements such as mercury and selenium, among others.

11 CALIBRATION

The calibration of the method forms a very critical point during the analysis. Calibration will be mostly done in the relative way (Heumann, 2003). The signal caused by the detection of the element in a sample will be compared with a set of calibration samples with known content. Because of the practical advantages of linear calibration graphs, they are always favored. Linear calibration graphs can be obtained by measuring only a few calibration standards and, in addition, are easily described by a simple mathematical function:

$$S = kc + b,$$

where S is the signal response of the detection method, k the calibration factor, c the concentration of the calibrant, and b the intersection with the y-axis. The k value reflects the sensitivity. The higher the k value for a given concentration range, the better the sensitivity.

External calibration modes, where the sample and the corresponding calibrant are separately measured, can only be used when there is no effect (either quenching or enhancement) by the sample matrix on the analyte signal. Internal calibration (i.e., standard addition method) becomes then compulsory to include possible influences of matrix composition on the signal intensity of the detection system (Skoog *et al.*, 1994). Two or more aliquots of the sample are transferred to volumetric flasks. One is diluted to volume, and the signal is measured. A known amount of analyte is added to the second, and its signal is measured after dilution to the same volume. Data for other additions may also be obtained. First, it should be checked that a linear relationship between the signal and concentration exists (and this should be established by several standard additions). If several additions are made, the signal can be plotted versus the added concentrations. The resulting straight line can be extrapolated to signal zero. The intercept with the concentration axis gives the concentration of the analyte in the sample. The addition method has the advantage that it often compensates for variations caused by physical and chemical interferences in the sample solution.

In the case of total element determinations using F-AAS, GF-AAS, HG-AAS, HG-AFS, ICP-AES, and ICP-MS, spectrophotometry, electrochemical methods, and biosensors for monitoring metal ions, it may be possible to calibrate versus the external mode. It is compulsory, however, to check the validity of this assumption by comparing the results of at least one series of samples versus those obtained by the more labor-intensive standard addition method.

In case of neutron activation analysis, external calibration is the rule. The one-point calibration curve is applicable, similarly as in isotope dilution mass spectrometry. ES-MS-MS is a stand-alone case as far as calibration is concerned, because this method is primarily used for obtaining structural information of the species. Calibration is, of course, evident when calibrants of the species are available.

Speciation analysis is usually the result of a separation of species followed by elemental detection. The standard addition method applied to the original sample, before the separation or enrichment of the species, is only justified when an equilibrium has been reached between the analyte in the sample and the standard. This also supposes that the calibrant is commercially available or can be synthesized by the laboratory.

Whereas most of the relevant inorganic elemental species are commercially available (e.g., selenite and selenate), there is often a lack of organoelemental species such as selenosugars. There is also a total lack of isotopically labeled elemental species needed for the species-specific isotope dilution technique, which contains the elemental species to be determined in a different isotopic composition from that of the sample. The synthesis of the corresponding spike must be carried out in the laboratory. The spiked calibrant is added to the sample before all analytical sample treatment steps. This method will allow coping with any loss of substance, because the isotopic ratio of the isotope diluted elemental species does not change. It also guarantees a similar response to that of the analyte from the sample. When no spiked calibrant is available, external calibration in ICP-IDMS is still very interesting with a species-unspecific standard added continuously just before the detection. Such a spike does not allow for any losses during the separation procedure, but it allows correction of any variation in response of the analyte in, for example, the eluent of the LC. Because the amount of spike is constant, any variation in response of the spike will be used to recalculate the response of the analyte from the sample. This assumes that detector systems working at high temperatures, such as those with inductively coupled plasma excitation, should not show significant differences in the detector response for various elemental species (Heumann *et al.*, 1998).

12 REFERENCE MATERIALS

The use of reference materials is the major monitoring tool for the performance of all analytical work. The ISO (International Organisation for Standardisation) Guide 30 gives the following definitions for reference materials (ISO, 1991).

Reference material (RM): a material or substance one or more of whose property values are sufficiently homogeneous and well established to be used for the calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials.

Certified reference materials (CRM): a reference material, accompanied by a certificate, one or more property values are certified by a procedure which establishes traceability to an accurate realization of the unit in which the property value is accompanied, and for which each certified value is accompanied by an uncertainty at a stated level of confidence.

For determining total element and even some elemental species in matrices of toxicological importance, a relatively wide choice of CRMs is available. There are several serum, blood, urine, tissue, water, working place dust, and environmental matrices issued by internationally recognized bodies. The complete inventory of these products can be found on the web pages of these institutes: EVISA (European Virtual Institute for Speciation Analysis), IAEA (International Atomic Energy Agency), IRMM (Institute for Reference Materials and Measurement of the European Commission), NIST (National Institute for Standards and Technology of the USA), NRCC (National Research Council Canada), NIES (National Institute for Environmental Studies in Japan), and VIRM (Virtual Institute for Reference Materials).

When choosing a reference material, care should be taken to not only use a matrix that is the same or very similar to the matrix of the sample to be monitored but also that the concentration of the analyte in the RM is within the concentration range expected during the analysis of the actual sample. For example, a liver reference material with a concentration of an element in the mg/kg^{-1} range is not suitable as a reference material for analysis of blood or urine where the concentration of the analyte is in the $\mu g/L^{-1}$ range.

13 QUALITY ASSURANCE

Because the analyst provides scientific evidence for taking important decisions, he or she has to assure that all actions have been taken to ensure that any factor that has an influence on the final result has been considered and reported in a permanent record (Prichard, 1995). This should cover the several steps related to procedures before analysis (e.g., sampling, avoidance of contamination or losses during sampling, storage of the sampled material, that is, preanalytical quality assurance). Chemical changes of a substance after sampling may cause problems if the specific chemical form of a substance is to be monitored. Recording and treatment of data are to be included in both the preanalytical and analytical phase.

It must also be the policy to deliver analytical results that are fit for purpose. This implies a thorough discussion with the end user of the results before starting the analysis. It is vital to understand why the work is being done, what will happen to the results, and what decisions will be taken, depending on the reported numerical values. It goes without saying that the analyst should make the measurements to the best of his or her abilities. The highest level of precision is, however, not always required. The result should be precise and accurate enough for the intended purpose. Every result should carry an estimate of the uncertainty of the value.

13.1 Definitions

The terminology and symbols of practical statistical methodology required to process and interpret results assessed from a sample can be found in the ISO Standards Handbooks: *Terminology and Symbols, Acceptance Sampling* (1995a) and *Interpretation of Statistical Data and Process Control* (1995b). It is beyond the scope of this chapter to give a detailed account of the important actions to be taken. The comments about quality are therefore limited to some practical concepts:

- *Quality control (QC)*: The planned activities designed to verify the quality of the measurement.
- *Quality assurance (QA)*: The planned activities designed to ensure that the quality control activities are being properly implemented. These should fully reflect the need of the user.
- *Quality system*: A set of procedures and responsibilities that a company or organization puts into force to make sure that the analytical chemist has the facilities and resources to carry out the measurements that will satisfy the end user. The quality system should only be as comprehensive as is required to meet the needs of the users.
- *Accuracy (trueness and precision)*: The closeness of agreement between a test result and the accepted reference value. The term accuracy, when applied to a set of results, involves a combination of random components and a common systematic error or bias component.
- *Trueness*: The closeness of agreement between the average value obtained from a large series of test results and an accepted reference value.
- *Precision*: Closeness of agreement between independent test results obtained by applying the experimental procedure under stipulated conditions. The smaller the random part of the experimental errors that affect the results, the more precise the procedure. A measure of precision (or imprecision) is the standard deviation. Precision is often misused for accuracy. This problem will be avoided if one recognizes that precision only relates to dispersion, not to deviation from the conventional true value (McNaught and Wilkinson, 1997).
- *Method validation*: This applies to a defined protocol, for the determination of a specified analyte and range of concentrations in a particular type of test material, used for a specific purpose (Thompson *et al.*, 2002).

13.2 Sources of Error

Incorrect analytical results may be due to one or more of the following (Prichard, 1995): unsuitable methodology, contamination, interferences, calibration errors, sampling errors, losses and degradation, incompetence, or lack of care.

Avoidance of contamination is crucial in trace element analysis. Potential sources of contamination are the laboratory environment (including the analyst), the reagents, the utensils, and this as from the sampling until the detection of the analyte. Similarly, losses of the substance to be monitored can occur as a consequence of adsorption to sampling equipment, volatilization, decomposition of the species, or other problems.

There is no definite method that always gives accurate and precise results. Even so-called definitive methods such as IDMS and NAA may give inaccurate results if not properly performed. A definitive method is a method of exceptional scientific status that is sufficiently accurate to stand alone in the determination of a given property. Such a method must have a firm theoretical foundation so that systematic error is negligible relative to the intended use (McNaught and Wilkinson, 1997). Erroneous results may be obtained even with approved methods if they are wrongly executed, used outside the tested calibration range, or with matrices that were not included in the original validation process. For example, the presence of fat can cause problems if the digestion technique was not designed for a fatty matrix. Many analysts have a tendency to introduce subtle changes into the approved procedure: changes to sample mass, reagent ratios, times and temperatures, recommended purity of the reagents or columns, etc. The extent to which a method may be modified without change in performance is what is meant by its robustness.

It must also be stated that there is no measurement method that is exempt of interferences. These will be mainly due to one or more of the many other compounds of the sample or to the reagents, including the gases. Interferences possibly occurring during ICP-MS measurements are well documented. Fortunately, many problems, albeit not all, could be solved by the application of high-resolution ICP-MS (e.g., nonspectral interferences are not solved) or a dynamic reaction cell when using quadrupole ICP-MS (section 10.2.4.1). Inaccurate results may also be related to a lack of competence or concern among the analysts. Human factors are often very important and can introduce errors that may influence the entire chain of events.

13.3 Results of Interlaboratory Testing

The need for quality assurance/quality control procedures (QA/QC) has become very evident during interlaboratory testing, in which participants have been asked to analyze samples containing (for the participating laboratories) unknown concentrations.

Control of the actual concentration of the analyte in the samples for external quality control (EQC) should be established by reference laboratories, preferably

using different methods. It is recommendable that the reference laboratories also use methods other than those used for routine analysis to avoid systematic errors, which may be common in reference and routine laboratories. Duplicate analyses at reference laboratories are another way to check the analytical performance.

Interlaboratory testing schemes for routine measurements are now very well established in different countries and even on an international scale. New initiatives are under way for elemental speciation measurements (EVISA: www: speciation.net).

13.4 Elements of Quality Assurance

For each element/elemental species and in each particular matrix, a quality assurance plan should be worked out. This plan must specify in detail the different internal (IQC) and external quality control (EQC) procedures that will be used to guarantee an accurate performance. IQC is conducted by the laboratories themselves, whereas an outside laboratory or agency is involved in EQC procedures. Both IQC and EQC should include the analysis of a number of reference materials certified for the elements or species of interest. To test the quality of the laboratory, the control samples should be analyzed with the methods used for routine analysis, and the CRM samples should be treated in the same way as the other samples. If more care and control were given to these samples than to the others, the results will not reflect the quality of the routine analyses, often being more precise and accurate.

The IQC samples can be either certified reference material, such as those marketed by one of the organizations mentioned in section 12, or could be taken from specially prepared EQC samples.

QA procedures exist for sampling and sample handling. The sampling plan should never be allowed to be out of the control of the analyst. The method of sampling may be laid down in international and national standards or in a set of guidelines. Examples are the guidelines for sampling blood, urine, and tissues published by IUPAC (Cornelis *et al.*, 1995), the Codex Alimentarius Commission guidelines on the sampling of food (2004), and the guidelines about sampling of drinking water issued by WHO (1997). Whenever applicable, they should follow legal and statuary requirements, such as having been issued for the sampling of food.

It is further necessary that the measured sample has the same composition as the sample received. Holding time is defined as the maximum period of time that can pass from sampling to measurement before the sample has changed significantly. When degradation is a possibility, holding time is a very important element to be taken into account in the storage decision. Properties of the analyte such as volatility, sensitivity to light, thermal stability, and chemical reactivity all have to be considered in designing the sampling strategy.

13.5 Statistical Considerations

It has become common practice to report a result together with its uncertainty (i.e., an estimate that characterizes the range of values within which the true value is asserted to lie). This is described by the standard deviation or the standard error of the result. When analyzing a certified value in a reference material, the reported value should lie between the boundaries indicated by the standard error on the certified value.

13.6 Reporting of Quality Assurance Data

Today all published data have to be accompanied by quality assurance data. This may be helpful to explain large variations in levels obtained in different studies by methodological errors rather than by actual differences in concentrations. In cases in which extremely high (or low) values are reported without quality control, it is obvious that the data cannot be regarded as valid. Laboratories may well meet acceptance criteria on one occasion and for one metal, whereas 1 month later, or for another metal, results may be far from the true values. It is, therefore, important that quality assurance procedures form an integral part of any project in which trace metal analyses take place. It should be up to the individual investigators to prove that their data are reliable.

The data should be documented in a form that is easily understood by the layman. The customer requires information from the analyst to prove a point. The data need to be fully documented, although details such as a complicated chromatogram of which only one peak is of interest may be of little benefit.

It is also compulsory to report the method's detection limit for the analyte. Expressed as the *concentration*, c_L, or the quantity, q_L, it is derived from the smallest measure, x_L, that can be detected with reasonable certainty for a given analytical procedure. The value of x_L is given by the equation

$$x_L = \bar{x}_{bi} + k s_{bi}$$

where~$_{bi}$ is the mean of the *blank measures*, s_{bi}, is the standard deviation of the blank measures, and k is a numerical factor chosen according to the confidence level desired (McNaught and Wilkinson, 1997).

When the concentration of the analyte is well below the permitted level, pragmatism does not require a high

degree of accuracy. On the contrary, when the concentration of the analyte is close to the allowed maximum, accuracy is of paramount importance.

14 CONCLUSIONS

Today's available analytical techniques allow measuring accurately total element concentrations in most matrices, even at very low concentrations, and also a number of elemental species. Measurement of the right parameter with adequate accuracy is fundamental to assess the toxicity of metals. Standardized sampling procedures and good programmes for the quality control of analytical results of total element concentrations are well implemented in most laboratories. Speciation analysis is already well developed for many species of anthropogenic origin but needs to be further developed in the case of noncovalently bound compounds.

It is quintessential that the results are representative for the real exposure level. This is the only way to draw correct conclusions with regard to dose-response relationships and health hazards.

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CHAPTER

3

Routes of Exposure, Dose, and Metabolism of Metals*

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ABSTRACT

The chapter first describes the main sources of exposure through air, food, and water but also points to unusual sources such as medical implants. Special attention is given to the processes of lung deposition and clearance of inhaled gases, vapors, and particulates, including ultrafine particles. In contrast to the extensive studies in the lung, absorption of metal in the gastrointestinal tract is less well understood. A diagrammatic example is given of the summation of all the absorption processes as they contribute to the total body burden. Since the publication of the first edition, new information has become available on the mechanisms of transport and distribution of metals in the body. In particular, it has been shown that several metals can cross cell membranes by specific carriers and ion channels intended for endogenous substrates. One well-documented example is the chromate oxyanion that is structurally similar to the sulfate anion and thereby gains entrance into the cell by the sulfate carrier. The fecal excretion of several metals occurs as the end result of extensive enterohepatic recirculation. In the case of certain organometallic species, the gut microflora may play a critical role converting the metal to the inorganic form, which is excreted in the feces. The renal accumulation and excretion of metals has also received considerable attention. The renal accumulation of cadmium in the form of its complex with the small molecular weight protein, metallothionein, still remains one of the best-documented mechanisms. Toxicokinetic models continue to be useful in providing a quantitative description of the overall body turnover of metals. They can be useful in establishing dose-response relationships where, for example, the range of half-times of elimination of a metal can contribute to the overall variance in the dose-response relationship. In addition to the observationally based models, pharmacokinetic models can be developed based *a priori* on physiological and mechanistic considerations. The chapter concludes with a consideration of indicator media that best reflect the dose to the critical organ.

1 INTRODUCTION

When evaluating the potential adverse effects of metal exposures, it is important to understand the mechanisms by which metals and their compounds are delivered from the source to the site of action in the human body. When such mechanisms constitute a result of events taking place in the ecosystem, they are dealt with mainly in Chapter 13. This chapter focuses on mechanisms and factors of importance for the uptake and distribution of metals when they come in contact with the human organism.

Airborne metals occur most frequently as aerosols, and metal particles inhaled into the respiratory tract frequently cause adverse health effects both to the lungs and other systems to which the metals are transported.

*This chapter is based on the chapter by Camner *et al.* with the same title in the 2nd edition of this Handbook.

Other absorption routes and factors governing distribution and excretion of metals are also dealt with.

Several new and exciting developments have taken place since the time of writing the previous version of this chapter. The disposition of ultrafine particles (including nanoparticles) in the lung is now an area of considerable interest and current research. Metal–protein complexes have long been a topic of interest. For example, nonspecific binding to serum albumin has been assumed to restrict metal movement from the blood compartment. However, an increasing number of publications indicate that many nonessential metals bind to proteins that normally bind essential metals. Such binding to these essential metal-binding proteins may affect the biological activity of nonessential metals in both intracellular and extracellular fluids. The mechanisms of transport of metals across cell membranes continue to be a subject of exciting research findings. Early in the history of metal toxicology, it was assumed that the free ionic species crossed into the cell through passive diffusion. Today, the picture is changing to one in which metals are the substrates of selective protein carriers. Some of these carriers are tailored to carry certain essential metals, but other carriers normally handle other endogenous substrates such as amino acids or inorganic anions. The terms "ionic mimicry" and "molecular mimicry" have been coined to describe these types of transport of the nonessential metals.

The metabolism and distribution of metals in the body continue to be a topic of interest but with fewer new developments. The term "metabolism of metals" is now usually restricted to those biochemical reactions that change the oxidation state of the metals or that form organometallic complexes, whereas this term was previously used to include the kinetics of metal disposition in the body. The previous usage is still acceptable and appears in some of the chapters of this handbook. An increasing number of metals have been found to undergo enterohepatic recirculation. This has proved to be a complex biochemical and physiological process involving the secretion into bile from the liver by specific transport processes, metabolic changes to the metal as it proceeds down the biliary and intestinal tract, followed by partial reabsorption in the gallbladder and intestine. This process not only plays a key role in the fecal excretion of metals but also affects the subsequent tissue disposition of the reabsorbed species.

An increasing emphasis on the use of biomarkers of exposure follows a long history of use of urine and blood, but the advent of molecular biology has opened up new vistas. For example, chromium–DNA complexes may serve as biomarkers of exposure. New analytical techniques and instrumentation open up an exciting field in the speciation of metals, whereby specific compounds of metals can be recognized in biological fluid and tissues. Speciation of metallic ions and complexes is important in the field of biomarkers of exposure, because only specific species of a metal can be transported into cells and across the blood–brain and placental barriers.

Some details on how the dose rate may influence mathematical models for dose-effect and dose-response relationships are discussed in Chapter 8. Sometimes, the influence of dose rate may be explained by the rates for absorption, transport, biotransformation, and excretion, which control the dose reaching their site of effects. These factors are dealt with in this chapter. As noted in another review (Goyer and Clarkson, 2001), there is a "need for quantitative information regarding dose and tissue levels as well as greater understanding of the metabolism of metals particularly at the tissue and cellular levels where specific effects might occur."

2 EXPOSURE

2.1 General Aspects

Air, food, and water are the major exposure media for humans. Air is especially important for occupational exposures, although the skin may also be exposed, and food and drink may be indirectly contaminated. All three exposure media can be important for the general population. In heavily industrialized regions and areas of high traffic density, air may well be the dominant route of exposure to certain metals. Water can be the dominant medium in geographical areas where metals such as arsenic or uranium are naturally present in soils and rock formations. Food can be the major exposure medium under a variety of circumstances, including accidental contamination and dietary habits. High natural background exposure may occur, such as to methylmercury in populations dependent on fish or marine mammals for their major source of protein. Infants and children may experience different degrees of exposure from air, water, or food compared with adults. Calculations of intake per kilogram of body weight for children compared with adults is twofold for air, threefold for water, and sixfold for food. The twofold difference for air is because the lung volume is lower in newborns but the alveolar surface area is larger per kilogram of body weight. In addition, the higher frequency of breathing in newborns gives them higher uptake of inhaled substances than adults. It is not clear how nose breathing, which is more frequent in early life, affects these factors.

Other routes of human exposure are through implanted medical devices such as hip and knee replacements made of Co/Cr/Mo alloys (cf Chapters

24 and 25) and dental amalgam fillings (cf Chapter 33). Other unusual routes of exposure such as hemodialysis and other medical therapeutic procedures will not be reviewed here (see Chapter 9).

2.2 Exposure by Inhalation

Metals as elements or metallic compounds constitute a certain part of the earth's crust. In air, metals may occur as aerosols and, in some instances, as vapor. Particles in ambient air from combustion sources are often composed of a mixture of carbon compounds and metals (USEPA, 2004). Particles coming from the earth's crust may contain Al, Mg, and Fe, whereas particles formed from burning fossil fuels contain transition metals and others, such as Cr, Co, Ni, Cu, Zn, As, Se, and Pb (USEPA, 2004). Metals have a vapor pressure, which is of very limited interest in regard to exposure, with the possible exception of mercury. However, the role of vapor pressure in environmental transport of metals may be considerable, particularly for mercury. It is known, for example, that mercury vapor is evaporated more efficiently from the continents than from the oceans (see Chapter 13).

Release of mercury vapor from dental amalgam restorations constitutes a special case. It has been shown that chewing and brushing of teeth gives rise to a release of small amounts of mercury, partly in the form of mercury vapor and partly as ionic mercury. This release can contribute significantly to the exposure of the general population to mercury vapor (Abraham *et al.*, 1984; Aronsson *et al.*, 1989; Brune and Evje, 1985; Hero *et al.*, 1983; WHO, 1991).

In certain industrial environments, human exposure to metals in vapor form may predominate (e.g., exposure to mercury vapor in chlor alkali plants or in mercury mines and to nickel carbonyl in some nickel refineries). Various natural and man-made sources contribute metal-containing aerosols to the atmosphere. A number of metals are thus found in the fly ash from coal-fired power plants. Depending on the source of the coal, the fly ash may contain different concentrations of Pb, Cd, Zn, As, etc. (Coffin and Stokinger, 1977). With oil-fired power stations, the dominating metals in the emitted aerosols are vanadium and nickel contained in oil (Ahlberg *et al.*, 1983), but they also may emit mercury (Boylan *et al.*, 2003).

Leaded gasoline (tetraethyl lead), which is still used as fuel in motor vehicles in some countries, gives rise to air pollution in the form of lead-containing aerosols, particularly in cities and along highways. In some countries where the addition of organolead compounds has been banned, organo manganese compounds are used for improvement of the octane of gasoline, and increased exposure to Mn aerosol is likely to occur. For smokers, inhalation exposure to the metal compounds that occur in tobacco smoke is of great importance. In this case, there is combined exposure to various metal compounds in aerosol form and a number of nonmetal gases and vapors.

In most industries, metal aerosols occur much more commonly than metal vapors. Examples of industrial exposure are lead or cadmium exposure in storage battery factories; exposure in metal smelters and refineries; exposure to all types of metals (Fe, Mn, Mo, Ni, Cr, etc.) that occur during the manufacture and use of steel; and exposure to solid particles of virtually all metals during grinding, finishing, and polishing of metal products.

Aerosols containing metals may be generated by various processes in the working and general environment. For an understanding of some of these processes, knowledge of the chemical and physical properties of metals is necessary. Formation of condensation and disintegration aerosols of metals is considered to depend, in part, on the electronic and crystal structure of the metals and their compounds (Brakhnova, 1975). For the formation of disintegration (pulverization) aerosols, the crystallochemical characteristic cleavage of a metal or its compound is of particular importance. Whereas, for example, graphite exhibits, under the action of mechanical forces, perfect cleavage along planes parallel to crystal faces, this is not the case for most of the metals, which display less perfect or indistinct degrees of cleavage. With decreasing particle size, the total surface of the particles increases. Thus, per 10 g of material, particles of 0.1-μm diameter have a surface 100 times greater than particles of 1-μm diameter. The increased surface area means that the number of disturbed metal–metal bonds increases and, as a consequence of this, there is an increase in the reactivity of the particles, their solubility in body fluids, and the possibility of a transition to an ionic state and interaction with the biological molecules (proteins, nucleic acids, etc.). In addition, these small particles have a greater ability to penetrate deeply into the respiratory tract (see Section 3.1.3), where they are less likely to be removed by mucociliary clearance and more likely to be absorbed into the blood.

Condensation aerosols, also known as metal fume, may be formed in various metallurgic processes. When metal vapors of high temperature react with atmospheric oxygen, metal oxides are formed. When a mixture of metals with different melting and boiling points is heated, compounds with lower melting or boiling points are released first (for example, manganese oxides), either as vapors or aerosols. A similar situation arises when tobacco is burned in a cigarette.

The metals in the tobacco will be released into the mainstream smoke as aerosols or may be retained in the remaining portion of the cigarette, depending largely on the melting point of the metal compounds in tobacco.

In various industrial processes, toxic and irritant gases are associated with metal oxide aerosols (fumes), including oxides of sulfur, oxides of nitrogen, hydrogen sulfide, and hydrogen selenide.

2.3 Exposure Through Food and Drinking Water

Human exposure to metal compounds in the general environment is usually greater through food and drink than through air. Even in occupational circumstances, exposure to metals by ingestion may be of importance, although absorption after inhalation is usually of primary importance in industry. Metals and their compounds occur naturally in food and drinking water, because they are intrinsic components of the earth's crust and of various biota. Depending on geological variation and agricultural and ecological processes, there are great geographical differences in metal intakes among populations living in various parts of the world. A striking modern example is the contamination of drinking water wells with arsenic in large areas of Bangladesh involving the exposure of millions of people (Wasserman *et al.*, 2004).

Industrial processes may, in some instances, add substantially to natural exposures, particularly in areas close to the sources of emission. A now classic example of local pollution contaminating the food supply is the outbreak of methylmercury poisoning that broke out in fishermen and their families eating fish caught from a large ocean bay in Minamata, Japan (for details, see Hunter, 1969). Mercury in various chemical forms had been discharged into the bay from a chemical plant manufacturing acetaldehyde. A mercury compound was used as a catalyst. Methylmercury either produced from other forms of mercury by a natural biomethylation process and perhaps released as such from the factory was bioaccumulated in the aquatic food chain to such an extent that consumption of fish from the bay resulted in cases of severe poisoning and even fatalities.

Attention has recently been directed to the increased metal content of crops from fields fertilized with metal-rich sewage sludge. The agricultural use of sewage sludge has, therefore, been regulated in some countries. In this context, it should also be noted that phosphate fertilizers, widely used in agriculture, might also contain some undesirable metals (e.g., cadmium). Furthermore, the acidification of soils by various processes including certain fertilizers as well as acid rain can increase the uptake of certain metals (e.g., cadmium) in crops (Nordberg *et al.*, 1985a). A metal discharged into the environment may be transformed into other chemical species by processes in the environment. The most well-known example is mercury as discussed previously and in Chapter 33.

Children may be exposed to metals in different ways than adults. The exposure of infants and children to lead is a well-documented example. Infants and toddlers crawling or playing close to floors can be exposed to lead dust and even larger particles released from indoor lead paint or entrained into the house from exterior paint and contaminated soils (for details, see Chapter 31). Liquid metallic mercury is occasionally spilled in the home as a result of breakage of mercury thermometers or through ethnic-religious practices with this metal. Mercury that evaporates from the liquid droplets forms the most concentrated layers of vapor near the floor where infants and toddlers can receive high exposures. Infants may be exposed indirectly from contaminants in the mother's tissues by ingestion of breast milk.

3 DEPOSITION AND ABSORPTION

This section will focus mainly on the two major routes of entry into the body, namely, inhalation and oral ingestion. Only limited new information has become available on absorption through the skin since the review by Wahlberg (1971). Although this route of exposure is usually less important than inhalation and ingestion, significant uptake through the skin has been reported, for example, of organic and inorganic thallium compounds (cf Chapter 41) and of cobalt in the hard metal industry (cf Chapter 25). Also, skin exposure may be important in sensitization of T lymphocytes to beryllium, with subsequent disease from inhalation exposure and immunological response. (Tinkle *et al.*, 2003). Fatal outcomes have been reported from skin absorption of organometallic compounds in rare instances (Nierenberg *et al.*, 1998).

After a metal has been taken into the lung by inhalation or into the gastrointestinal tract through food and drinking water, the metal will deposit on the walls of the airways or will be taken up in the mucosa of the gastrointestinal tract. The amount deposited will depend on physical characteristics of the aerosol or the chemical form of the metal in food and drinking water. After deposition has taken place, a certain fraction of the deposited amount will be transferred through the walls of the lung or gastrointestinal tract into the systemic circulation.

The physicochemical properties of metals in exposure media, such as air, food, and water, play an important role in determining the extent of absorption into the body. This has been investigated in great detail with respect to inhalation of metal aerosols. Less information is available in the physicochemical status of metals in other media, especially food.

3.1 Deposition and Absorption After Inhalation

In addition to chemical form, evaluation of the effects of airborne metal particles is complicated by the fact that these particles vary considerably in size, shape, and density. These physical characteristics, as well as the pattern of inhalation, determine the extent to which deposition and retention of the particles will occur.

The behavior of airborne particles greater than 0.5 μm is frequently described in terms of its equivalent aerodynamic diameter, which depends on the shape, size, and density of the particle. The aerodynamic diameter is equal to the diameter of a spherical particle of unit density (1 g/ml) with the same settling velocity in air as the particle in question. Under 0.5-μm aerodynamic diameter, particle deposition is governed more by diffusion, with particles striking molecules of gas and impacting airway walls as the result of Brownian motion. For such particles, size is usually defined by Stokes diameter, which for irregular particles is the diameter of a sphere with the same aerodynamic resistance. Unlike aerodynamic diameter, Stokes diameter is independent of mass (US EPA, 2004).

A given aerosol can be expected to contain particles of many different sizes and shapes. One method of categorizing the particles in an aerosol is by natural clustering into certain size distributions. Figure 1 shows an idealized frequency distribution of particles of different sizes that might be observed in traffic, with the particles falling into four peaks or modes of larger, intermediate, and smaller particles. Vapor may form small particles by the mechanisms of condensation or nucleation. Very small particles rapidly accumulate into larger particles by coagulation. The largest particles have a different source, usually generated

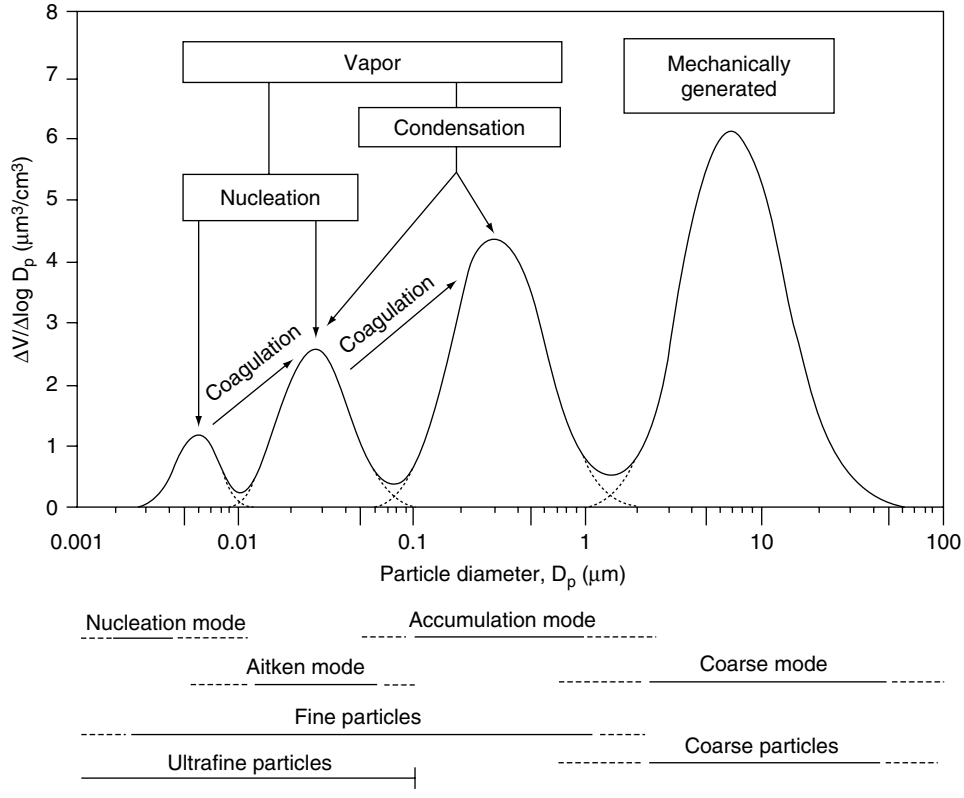

FIGURE 1 A volume or mass distribution of particles such as might be observed in traffic, according to particle diameter, showing four major modes of particle diameter. The largest particles are mechanically generated from geological dust and other sources, whereas the smaller particles originate from combustion and other sources and are formed from vapor by condensation or nucleation and then further coagulate into larger particles. The four modes, from smallest to largest, are the nucleation, Aitken, accumulation, and coarse modes. This is an idealized distribution. (USEPA 2004).

by mechanical forces on solid materials to break them down into particles.

The size distribution of larger or intermediate size particles in aerosols is often log-normal.

The mass median diameter is the diameter of a particle with the median mass, that is, half of the mass lies on either side of the median diameter. The mass median diameter is a measure of the mass distribution of the aerosol. The mass median aerodynamic diameter (MMAD) is important in determining pulmonary deposition of particles with penetration to the small airways and alveoli of particles less than 10-μm aerodynamic diameter. Particles greater than 10 μm aerodynamic diameter are efficiently filtered by the nasal passages, whereas particles smaller than this may penetrate to the alveoli, which are more sensitive to injury and may serve as a route for translocation to other parts of the body.

An alternate convention when describing the size of particles in ambient air pollution is to divide particles into categories of total suspended particulate (TSP) comprising all particles suspended in air; particulate matter less than 10-μm mean aerodynamic diameter (PM_{10}, which are likely to penetrate beyond the upper airway filtering mechanisms) and particulate matter less than 2.5-μm ($PM_{2.5}$), which are more likely to be deposited in the terminal bronchioles and alveoli (as shown in Table 1) and to have systemic effects.

In occupational health, particles are sometimes categorized into size categories according to the portions of the respiratory system they can reach when inhaled. Using this classification, "respirable particles" with a mass median aerodynamic diameter less than approximately 5-μm are considered more hazardous, because they can penetrate into and deposit in the alveolar or gas-exchange region. Thoracic particles are those that can penetrate the upper airways to the thoracic airways and beyond, whereas inhalable particles are all particles that can be deposited anywhere within the respiratory tract.

All particles, even very small particles, are efficiently filtered by the upper airways when breathed through the nose, but when the nasal passages are bypassed and particles are breathed through the mouth, there is a particularly high deposition for ultrafine particles, less than 100-nm diameter. In healthy subjects breathing through a mouthpiece at rest, the percentage of inhaled ultrafine carbon particles deposited (number deposition fraction) varied from 55% for particles 65 nm MMAD to 80% for the smallest particles 8 nm MMAD (Daigle *et al.*, 2003). This contrasts with a deposition fraction for fine particles under the same circumstances of well under 50% (Beckett *et al.*, 2005). The deposition fraction of particles further increases with exercise and is greater in subjects with mild asthma than in normal subjects (Chalupa *et al.*, 2004).

The biological fate and also the toxicological effects of deposited particles depend on the site of deposition in the airways and on particle characteristics, including size, solubility in airway lining fluid, and other physical characteristics (Oberdörster and Driscoll, 1997).

For a more detailed discussion of aerosol properties, generation, and measurement, see Mercer (1973), Stockham and Fochtman (1977), and USEPA (2004).

TABLE 1 Calculation of Total Absorption into the Body as a Function of Two Different Rates of Alveolar Absorption and Different Particle Sizes for a Specific Deposition and Clearance Model

Particle (MMAD) (μm)	Alveolar deposition (%)	Tracheobronchial - Total absorption Size (%) nasopharyngeal into body when alveolar absorption is:		
			100%	50%
0.1	50	9	50.4	26.7
0.5	30	16	30.8	16.6
2.0	20	43	22.2	12.6
5.0	10	68	13.4	8.6
10.0	5	83	9.2	6.8

From Task Group on Metal Accumulation, 1973.
MMAD, Mass median aerodynamic diameter.
Gastrointestinal absorption is assumed to be 5%.

3.1.1 Absorption of Gases and Vapors

The ability of vapors and gases to penetrate the airways depends on their solubility in water. The higher the solubility in water, the higher up in the tracheobronchial tract the gas will be absorbed. For example, sulfur dioxide is highly soluble in water, and more than 99% of concentrations found in typical air pollution episodes is absorbed in the nose (Speizer and Frank, 1966). Other less important factors determining the efficiency of uptake of vapors and gases include the rate of airflow (increased absorption with increased airflow) and the concentration of the gas (Fiserova-Bergerova, 1983). However, vapors and gases of metallic compounds are usually insoluble in water and will reach the alveoli, where they can penetrate the air–blood barrier.

3.1.2 Deposition of Particles

Most of the absorption of less soluble particles occurs in the lower part of the respiratory tract. However, it has been shown that some transfer of metals in the form of nanoparticles (ultrafine particles) and/or solutions may occur by a direct transfer from the olfactory

mucosa in the nose into the olfactory nerve in the brain, as shown in rats (e. g for cobalt) (Persson *et al.*, 2003).

Particle size is the primary determinant both for how many particles are deposited in the respiratory system and for the region of the respiratory systems in which they are deposited (Schulz *et al.*, 2000). The nose is an efficient filter not only for water-soluble gases but also for particles. It is a much more efficient filter than the mouth for particles of approximately 1 μm or larger (Heyder and Rudolf, 1977; Task Group on Lung Dynamics, 1966). During heavy physical work, breathing through the mouth is necessary (Proctor, 1977). Even during calm breathing, inhalation through the mouth occurs to some extent, and this affects the amount of particles deposited in the lungs (Camner, 1981; Camner and Bakke, 1980).

The main mechanisms for deposition of particles in the lung are impaction, sedimentation, and diffusion (Raabe, 1982). The effect of impaction increases with particle size and air velocity, and it is most important in the nose, throat, and the larger bronchi (i.e., where the velocity of the air is highest). The effect of sedimentation increases with particle size and decreases with air velocity. It is most important in the smaller airways and the alveoli. The effect of diffusion is of importance only for particles smaller than a few tenths of a micrometer, and the effect increases with decreasing particle size. Electrostatic effects may also be important for the deposition of particles, at least under some conditions. Melandri *et al.* (1977) have shown that unipolar electrostatic charges on particles increase the total deposition because of electrostatic attraction between the particle charge and the image charge on the airway wall.

Because the nose very effectively humidifies the air, the relative humidity is already 98–99% in the subglottic space (Ingelstedt, 1956). Particles that are hygroscopic can increase in size by increasing their water content and thus be deposited in the lungs quite differently from what could be expected from measurements at normal relative humidity. For example, particles of sodium chloride have been shown to increase seven times in diameter when they are inhaled (Dautrebande and Walkenhorst, 1964).

Several theoretical models for the deposition of particles in the lung have been proposed. The first was published in 1935 by Findeisen. The International Commission on Radiological Protection (ICRP) has used Findeisen's model with some smaller modifications (Task Group on Lung Dynamics, 1966). The model agrees fairly well with several experimental results (Raabe, 1982; Task Group on Lung Dynamics, 1966; Task Group on Metal Accumulation, 1973). A more recent model is also useful (National Institute for Public Health and the Environment, 2002). Particles greater than 100-μm aerodynamic diameter have a low probability of entering the human respiratory system, and particles less than 50-μm frequently enter. Under most circumstances, most inhaled particle mass is exhaled in the same breath, and the remainder is deposited in the respiratory system.

Particle size is the most important determinant of the location of deposition of inhaled particles (Schulz *et al.*, 2000). The upper airways—head airways and tracheobronchial region—serve as the site of deposition of much of the inhaled particle mass and serve to protect the more vulnerable alveolar region from exposure to inhaled particles. Particles greater than 10-μm aerodynamic diameter are mainly deposited in the head airways, also known as the extrathoracic region. Particles less than 10-μm diameter may be deposited in the tracheobronchial airways or the alveolar region. Particles deposited in the larger bronchi are more likely to be cleared to the pharynx by the mucociliary ladder and swallowed into the gastrointestinal tract, whereas particles deposited in small terminal and alveoli are less efficiently cleared.

Some examples of numerical values of deposition in tracheobronchial and peripheral lung structures (alveolar) for particles with varying mass median aerodynamic diameter are given in Table 1. Theoretical models predict the deposition in different parts of the respiratory tract as a function of particle size. In real situations there are always large variations in the particle size. However, theoretical calculations with the ICRP model show that estimates obtained using the aerodynamic mass median diameter give a rather good approximation of the deposition of an aerosol even when large size variations occur.

More sophisticated theories of particle deposition have been developed (e.g., the model by Yu and Taulbee, 1977). This model agrees very well with theoretical estimates of the total deposition by Heyder *et al.* (1975). The particle-size range of 0.3–3 μm was used during several breathing patterns. Compared with the ICRP model, the model by Yu and Taulbee gives lower values for deposition.

One difficulty with the theoretical models is that even if they give a good average estimate of deposition, they do not take into account the large individual differences that are known to exist. The part of the total deposition in the lung that is deposited in alveoli has, for example, been shown to vary at least by a factor of two, even among relatively young healthy male nonsmokers (Albert *et al.*, 1967; Camner and Philipson, 1978; Lippmann *et al.*, 1971). The penetration of particles (6 μm) to the alveoli has been found to correlate with lung function parameters as forced expiratory volume in 1 second (FEV_1) and airway resistance (Svartengren *et al.*, 1984; 1986).

3.1.3 Clearance of Particles from the Respiratory System

Mechanisms for clearance or translocation of particles depend on the regions of deposition. Sneezing, nose wiping or blowing, mucociliary transport, dissolution and absorption into the blood or lymph, or endocytosis by macrophages or epithelial cells may remove particles deposited in the extrathoracic or tracheobronchial regions. Particles deposited in the alveolar region may be removed by dissolution and absorption into the blood or lymph, endocytosis by macrophages or epithelial cells, or translocation into the systemic circulation (Schlesinger, 1995).

3.1.3.1 Tracheobronchial Clearance

3.1.3.1.1 Mucociliary Clearance The upper airways, the nasal passages, the paranasal sinuses, the auditory tube, and the upper part of the pharynx are provided with ciliated epithelium as are the lower airways from the lower parts of the larynx down to and including the terminal bronchioli. The upper airways cilia drive mucus backward and downward to the pharynx, and the lower airways cilia drive mucus upward to the pharynx whereafter it is swallowed. Lucas and Douglas (1934) proposed a two-layer model for mucociliary transport. The lower layer, in which the cilia beat, was proposed to consist of a low viscous secretion (the sol layer) and the upper layer of a highly viscous secretion (mucus or gel layer). Most particles deposited on the ciliated epithelium of the large airways are rapidly cleared (within hours to days) by this mechanism.

The importance attached to mucociliary transport in the airways has been motivated by theoretical considerations. If mucociliary transport is impaired, the concentration of particles per unit surface area should increase, and the particles should remain for a longer time on the area. People with congenitally immotile cilia have been identified and studied by Eliasson *et al.* (1977), Levison *et al.* (1983), Mossberg *et al.* (1983) and Pedersen and Stafanger (1983). Studies on these persons provide a unique opportunity to evaluate the importance of mucociliary transport. Such patients often have chronic infections in the upper and lower airways since childhood and commonly have bronchiectasis and obstructed airways.

In healthy humans, the tracheal mucus transport rate has been estimated to be in the range of about 4–20 mm/min (Santa Cruz *et al.*, 1974; Yeates *et al.*, 1975). The velocity is lower in the more peripheral airways. The particles deposited in the most peripheral ciliated airways are eliminated from the lung usually within 24 hours (Albert *et al.*, 1967; Camner and Philipson, 1978).

Mucociliary clearance of inhaled particles depends on genetic factors (Bohning *et al.*, 1975; Camner *et al.*, 1972). Clearance can also be affected by many different agents (e.g., tobacco smoke and other air pollutants, drugs, and infectious agents). The effect of tobacco smoke on mucociliary transport has been the subject of numerous investigations. In many *in vitro* and *in vivo* studies in animals, an immediate impairment of the transport rate was seen after exposure to cigarette smoke (see e.g., Asmundsson and Kilburn, 1973). In man, however, the average short-term effect of smoking cigarettes is an increased mucociliary transport (Albert *et al.*, 1976; Camner *et al.*, 1971). The average effect of long-term cigarette smoking is impaired mucociliary transport (Camner and Philipson, 1972; Lourenco *et al.*, 1971). However, some individuals who have smoked for 20–30 years do not have impaired clearance develop. The impaired clearance after long-term smoking is at least partially reversible (Camner *et al.*, 1973d).

Inhalation of agents other than tobacco smoke, carbon dust, sulfur dioxide, or sulfuric acid can immediately give rise to an increased mucociliary transport (Camner *et al.*, 1973a; Newhouse *et al.*, 1978; Wolff *et al.*, 1975). It is well known that inhalation of all three of these pollutants can give increased airway resistance mediated by the vagus. Because the administration of cholinergic compounds increases mucociliary transport, it seems probable that the vagus has a role in mediation of the effect of pollutants (Camner and Philipson, 1974; Camner *et al.*, 1974). High concentrations of sulfuric acid mist (980 $\mu g/m^3$) produce a transient slowing of clearance (Leikauf *et al.*, 1981).

Because more than 99% of SO_2 at common ambient concentrations is absorbed in the nose, its effect on mucociliary transport should also be most pronounced in this organ. Andersen *et al.* (1974) found a reduced transport after exposure to approximately 15 mg SO_2/m^3 for 3 hours. A tendency toward reduced mucociliary transport in the nose was seen even after exposure to 3 mg/m^3 over a period of 6 hours. In experiments on animals for longer exposure times, SO_2 reduced mucociliary transport in rats after exposure to about 3 mg/m^3 for 770 hours (Ferin and Leach, 1973), and in dogs after exposure to approximately 3 mg/m^3 for 12 months (Hirsch *et al.*, 1975). NO_2 has also been reported to impair mucociliary transport in animals after long-term exposure. In rats, exposure to approximately 12 mg $N0_2/m^3$ for 6 weeks caused decreased transport (Giordano and Morrow, 1972).

Not only administration of cholinergic compounds but also of adrenergic compounds has been shown to increase the mucociliary transport rate in man to a high degree (Camner *et al.*, 1976; Foster *et al.*, 1976;

Konietzko *et al.*, 1975; Yeates *et al.*, 1975). A marked effect of adrenergic compounds is interesting, because it may suggest that levels of catecholamines in the blood affect the mucociliary transport velocity. Wolff *et al.* (1977) showed that physical exercise increased bronchial clearance in healthy men. This could have been caused by an increase in catecholamine during exercise.

Both acute and chronic diseases of the lung have been shown to be associated with impairment of mucociliary transport. During acute infections with influenza A virus or mycoplasma pneumoniae, patients had markedly reduced mucociliary transport (Camner *et al.*, 1973b; Jarstrand *et al.*, 1974). Approximately 1 month after the onset of the infection, a definite improvement of clearance was seen in both groups of patients. Observations of cases with mycoplasma infection suggest that some impairment persists even 5–15 months after the onset of the disease and that persons with slow clearance more easily contract the disease than those with fast clearance (Camner *et al.*, 1978). Patients with chronic bronchitis usually have a clearly impaired clearance (Camner *et al.*, 1973c; Santa Cruz *et al.*, 1974; Togio *et al.*, 1963) and impaired mucociliary transport. In these patients, coughing is an effective elimination mechanism that seems to compensate for the reduced mucociliary transport (Camner *et al.*, 1973c), but only partly, because patients with immotile cilia syndrome do not have healthy lungs. An increase in tracheobronchial secretion is necessary for coughing to be effective (Camner *et al.*, 1979).

3.1.3.2 Peripheral Lung Clearance

Particles deposited distal to the ciliated epithelium, as well as some particles deposited on this epithelium, are cleared much more slowly from the airways. This may occur through the interaction of several mechanisms, which include dissolving of particles in epithelial lining fluid, interstitial fluid, or phagocytic cells; transport by phagocytic cells (alveolar macrophages, polymorphonuclear leukocytes) to ciliated epithelium, lymphatics, or alveolar spaces; and translocation out of the lung of dissolved material through epithelial lining fluid, interstitial fluid, lymphatic fluid, or blood. Phagocytosis of particles is most effective for particles between 0.5 and 2 μm (Bair *et al.*, 1994; Kreyling and Scheuch, 2000).

The most distal lymph vessels do not reach the level of the air–blood barrier or of the interalveolar septa (Lauweryns and Baert, 1974). However, some elimination of particles takes place through the lymphatics.

Inhaled ultrafine carbon particles (less than 100-nm diameter) are deposited in the alveoli in high proportion, but it is still unclear how rapidly and to what degree they can pass into the blood of the systemic circulation (Mills *et al.*, 2006; Nemmar *et al.*, 2001).

3.2 Absorption After Ingestion

Metals in the gastrointestinal lumen may originate from food and drink or may be transferred to the gastrointestinal tract by mucociliary clearance (Figure 2). The metal may also enter the gastrointestinal tract by secretion through intestinal mucosa and in bile and pancreatic fluids. The subsequent reabsorption of metals secreted in bile amounts to an enterohepatic cycle that will be discussed later. The metal may be absorbed and sequestered inside intestinal mucosal cells only to be returned to the intestinal tract when the cells exfoliate. Because intestinal cells turn over at a high rate, this process can prove to be an important limitation of the degree of absorption into the bloodstream. Not illustrated in Figure 2 is the role of intestinal microflora that are capable of methylation and demethylation and

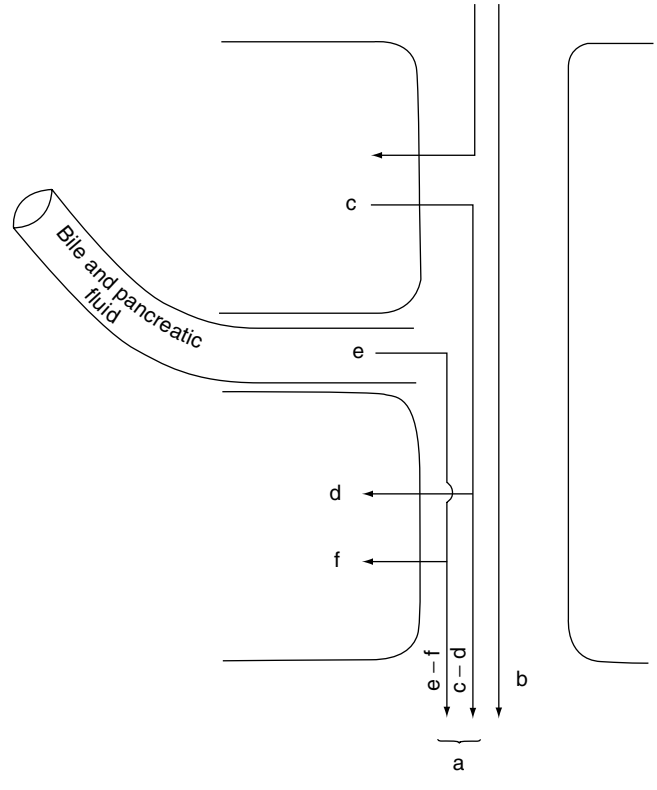

FIGURE 2 Routes for ingested metals. (a) Metal ingested and absorbed; (b) metal ingested but passing unabsorbed through gastrointestinal tract; (c) excretion of metal through intestinal mucosa; (d) reabsorption of metal excreted through intestinal mucosa; (c–d) Excretion minus reabsorption through intestinal mucosa; (e) Excretion of metal through bile and pancreatic fluid; (f) reabsorption of metal from bile and pancreatic fluid (enterohepatic circulation). g = (e – f) + (c – d) = net gastrointestinal excretion. g + b = fecal content of metal.

changing the oxidation state of metal. The metabolic interaction between metals and intestinal microflora may affect the degree of intestinal absorption and fecal excretion of the metal.

Depending on the source, metals may be presented to the fluids of the gastrointestinal tract in elemental form or as inorganic or organometallic compounds. The chemical form in which the metal occurs is of great importance, because absorption can be entirely different for different compounds of the same metal. This is particularly so when inorganic and organometallic compounds of the same metal are compared. Methylmercury, for example, is almost completely absorbed (approximately 90–100% absorption), whereas inorganic Hg (II) salts are absorbed to an extent of 10% or less.

Another consideration with regard to exposure to metals through the gastrointestinal route is the physical form of the metal. In food, metal or metal compounds may sometimes occur as particles of various sizes mixed with other components of food. Whereas the particle size is not as important as it is in the case of the lung, it may sometimes be of great importance for solubility and subsequent absorption. In addition, nonmetal compounds present in food may influence the uptake of metals from food. Such interacting substances should also be examined when evaluating metal exposures through food.

Knowledge about the chemical compounds of metals in foodstuffs is, therefore, of great importance. Established analytical methods can achieve some degree of speciation of metals, for example, distinguishing between the methylated and inorganic forms. However, identification of specific metal compounds and complexes remains a task for the future. A promising start has been made in the case of methylmercury, where a sophisticated instrumental method was used to identify methylmercury bound to cysteine residues in protein in fish meat (Harris *et al.*, 2003). Cadmium in the form of cadmium metallothionein has been studied in human and animal tissues (Lu *et al.*, 2005; Nordberg and Nordberg, 2000).

A chemical is absorbed from the gastrointestinal lumen by passive diffusion or by specialized (metabolic-dependent) transport systems and, in addition, ultrafiltration may contribute to transport into the lumen. With respect to absorption by passive diffusion, the lipid solubility of the molecule and its ionization are important (see reviews by Rozman and Klaassen, 1996 and WHO, 1978). The pH in the unstirred surface mucus layer adherent to the intestinal epithelium reflects the presence and activity of acid/base transporters in the apical membrane of the enterocytes. It determines the degree of ionization and thus the rate of absorption of weak acids and bases and is slightly alkaline or neutral in the duodenum, slightly acidic in the jejunum, and neutral in the ileum and the large intestine (Allen and Flemström 2005). Furthermore, diseases affecting intestinal electrolyte transport and mucosal paracellular permeability to ions, like celiac disease, may increase or decrease surface pH.

Inorganic salts of metals are usually not lipid soluble and are thus poorly absorbed by passive diffusion. For absorption of metals to occur, it is, in most instances, necessary that the metals are dissolved in the luminal fluids of the gastrointestinal tract, and subsequently they may be bound to molecules that facilitate their absorption. Absorption mechanisms for some essential metal ions sometimes serve to transfer nonessential metals into the body. Examples of such mechanisms have been shown for thallium salts (Leopold *et al.*, 1969) and for cadmium ions, the latter metal ion being transported by the DMT-1 transporter, also transporting iron and other divalent essential metals (Tallkvist *et al.*, 2001; see also Chapters 30 and 47).

The large differences in gastrointestinal absorption of inorganic salts of metals from less than 10% for ionized cadmium, indium, tin, and uranium to almost complete absorption (90–100%) for water-soluble inorganic salts of arsenic, germanium, and thallium may be related to the presence or absence of suitable transport systems. The importance of solubility in the fluids of the gastrointestinal tract has already been alluded to, and, for obvious reasons, this has been most easily demonstrated for metals whose soluble salts are readily absorbable.

The proportion of an oral dose of a metal salt that is absorbed is often influenced by dose level. For many essential metals (e.g., Cu, Fe), small doses are absorbed to a greater extent than larger doses. This is because homeostatic mechanisms—absorption and excretion—serve to keep concentrations of these metals at physiological levels. For nonessential metals (e.g., Cd), there is some evidence indicating the opposite (i.e., small doses are absorbed to a lesser degree than moderate [nontoxic] ones) (Engstrom and Nordberg, 1979a). For both essential and nonessential metals (e.g., Fe, Cd), excessive doses affect the integrity of the gastrointestinal mucosa with subsequent increase in uptake.

For several metals (Pb, Cd, Fe, Co, Hg), it has been shown that gastrointestinal absorption is greater in newborn or young animals than in adults (Engstrom and Nordberg, 1979b; Ezekiel, 1967; Kello and Kostial, 1977; Kostial, 1983; Kostial *et al.*, 1971). This may be related to the high pinocytotic activity of the immature intestinal mucosa or a greater (paracellular) leakiness of the young epithelium. One of the important factors in childhood susceptibility to lead exposure in the home is the high rate of intestinal absorption.

The composition of the diet is of concern, because components like phytate, ascorbic acid, other metals may have great influence on absorption (see Chapter 7). The transfer of metals from the intestinal lumen into the mucosal cells is not always associated with further transport into the organism (systemic uptake). The binding of metals by the low molecular weight protein, metallothionein, in the mucosa and subsequent loss by mucosal shed has been suggested as an important mechanism in homeostatic regulation of the systemic uptake of an essential metal-like zinc (Nordberg and Kojima, 1979; Richards and Cousins, 1976). High oral doses of zinc induce metallothionein synthesis in the mucosal cells and block the uptake of copper by the intestinal mucosa. This is used clinically to avoid tissue accumulation of copper in patients with Wilson's disease (see Chapter 26). A homeostatic mechanism for iron implies that iron is stored in intestinal cells bound to ferritin (see Chapter 30).

3.3 Total Absorption

Figure 3 describes the pathways of metals after inhalation or gastrointestinal intake and includes retention in the lung or intestinal compartment. Corresponding mathematical expressions for total absorption or uptake (I), and concentration (amount) of metal in lung (E), or intestine (E2) are also given.

As mentioned in the previous sections, aerosols deposited on the mucociliary escalator will be transferred to the gastrointestinal tract, and the uptake there will be governed by the factors discussed in Section 3.2. It is important to remember that for the effects of metals that occur as a result of systemic uptake, both the part of metal that is absorbed directly through the lung and the part that is absorbed after gastrointestinal translocation of inhaled particles will have to be taken into account, as well as those amounts of metal that are absorbed from the gastrointestinal tract from exposure through food and drink.

4 TRANSPORT, BIOTRANSFORMATION, AND DISTRIBUTION

The transport and distribution of metals will, to a large extent, depend on the form in which the metal occurs in the blood, which is the main transporting

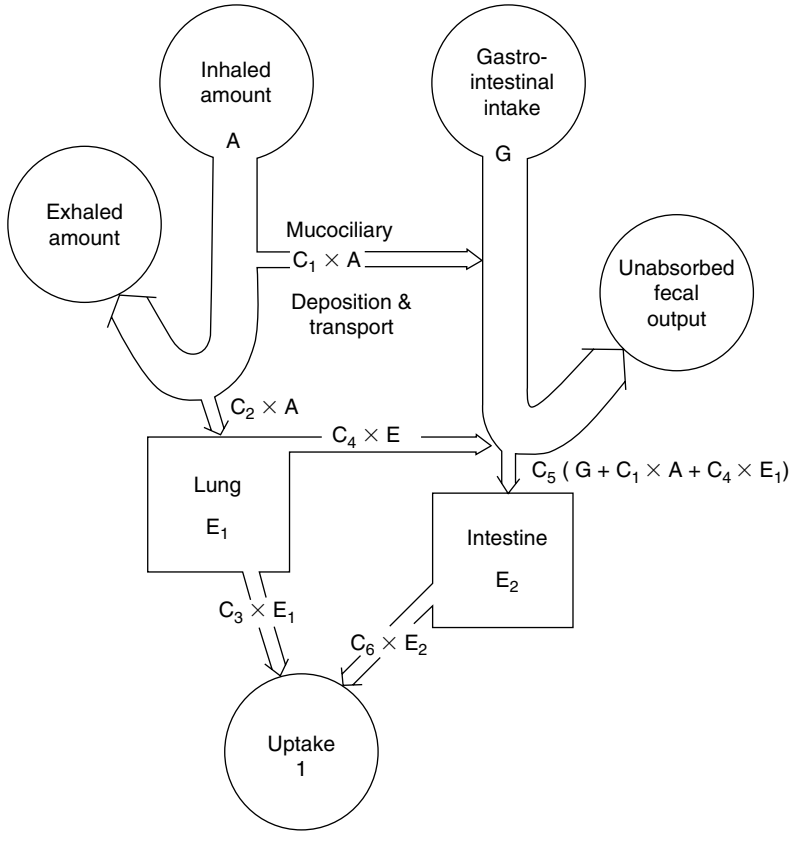

FIGURE 3 The pathways of metals after inhalation or gastrointestinal intake including retention in the lung or intestinal compartment. Corresponding mathematic expression for total absorption or uptake (1) and concentration (amount) of metal in lung (E_1) or intestine (E_2) are also given.

medium in the body. The lymph may also, in certain instances, constitute an important route for transport of metals, for example, from the lung into the blood circulation. Among metals, there are very large differences between the fraction of an absorbed dose that is transported to various organs and the fraction that is excreted. The following basic factors are of importance for distribution as presented diagrammatically in Figure 4: the protein bound and "diffusible fraction" in plasma, interstitial, and intracellular fluid; the rate of organ vascular perfusion; the rate of biotransformation; the permeability of cell membranes to the metal as it occurs in plasma; and the availability and turnover rate of intracellular ligands for the metal.

Protein binding of metal ions in plasma and body organs varies greatly among metals. Germanium is thought not to bind to plasma protein, but cadmium and mercury are protein-bound to at least 99%. Beryllium may be transported in the form of a colloidal phosphate, adsorbed to plasma α-globulin. Metals may also bind to proteins that normally handle essential metals, and, in some instances, protein-binding metal may serve a transport function for the metal (see review by Nordberg, 1982). For example, transferrin normally binds and transports Fe^{+3}, but transferrin also binds In^{3+}, Mn^{3+}, Ga^{3+}, Bi^{3+}, and Al^{3+}. A slow release of cadmium bound to metallothionein (mol. Wt., 6500) is thought to be of importance for the selective uptake of filtrated cadmium in the proximal tubule of the kidney by tubular reabsorption (Foulkes, 1978; Nordberg and Nordberg, 2000; Nordberg *et al.*, 1975; 1985b).

The diffusible fraction in plasma is of special importance to renal accumulation and excretion. As discussed previously, the Cd–metallothionein complex is filtered at the glomerulus as the initial step in renal uptake. Another example is the renal accumulation of uranium present in plasma as a bicarbonate complex. Its renal accumulation and excretion will be discussed in the following section.

The term "diffusible fraction" does not necessarily imply that metals move from one body compartment to another by passive diffusion. Instead, the term stands for the small molecular weight or ultrafiltrable fraction that oftentimes includes small molecular weight compounds of the metal that may be transported on specific protein carriers. For example Figure 5 illustrates the structure of the arsenate and chromate oxyanions. Such structures mimic those of the endogenous anions phosphate and sulfate, respectively, and are transported across cell membranes by the protein carriers for the endogenous anions. This type of "ionic mimicry" also accounts for transport of monovalent cations such as Tl^+ on K^+ and Li^+ on Na^+ carriers.

Methylmercury-cysteine is carried into the endothelial cells of the blood–brain barrier on the large neutral amino acid carrier. The process is inhibited by large neutral amino acids such as methionine and is so selective that only the complex of methylmercury with the L optical isomers is transported (Figure 7).

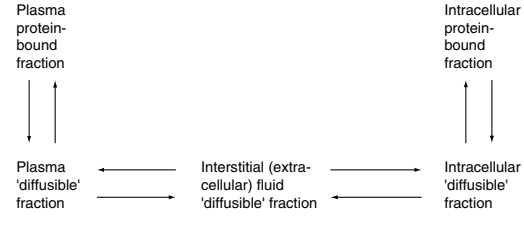

FIGURE 4 Model for the exchange of metal between blood and other tissues. (From Task Group on Metal Accumulation, 1973).

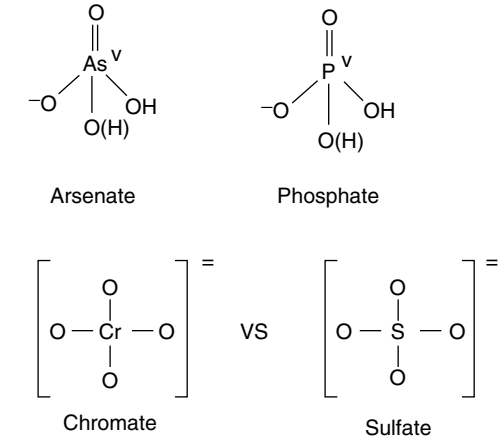

FIGURE 5 A diagrammatic comparison of the structures of the oxyanions of arsenate and chromate with those of the endogenous oxyanions of phosphate and sulfate, respectively.

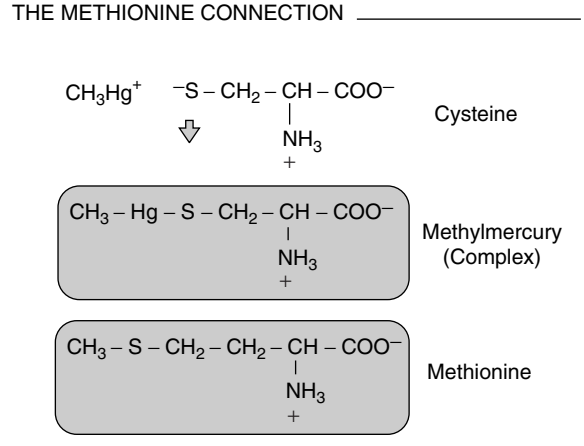

FIGURE 6 The structure of the methyl mercury–cysteine complex structurally mimics that of the large neutral amino acid, methionine.

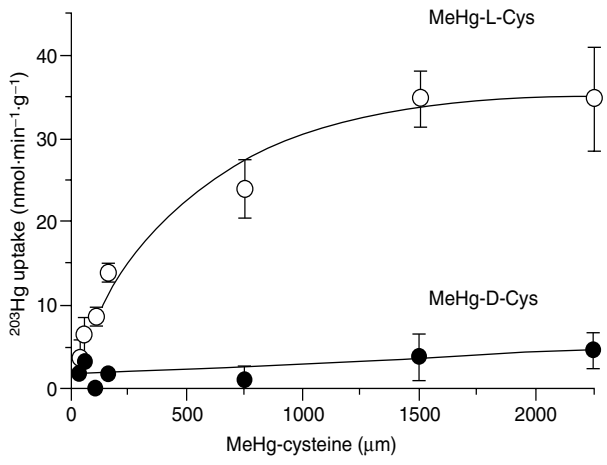

FIGURE 7 The uptake from plasma to brain in rats of the L and D optical isomers of the mercury–cysteine complex. (From Kerper *et al.*, 1992).

Studies on methylmercury secretion into bile indicate that methylmercury is extruded from cells as a complex with glutathione on the reduced glutathione carrier (Ballatori and Clarkson, 1982; 1983; Refsvik and Norseth, 1975). Thus, transport across the endothelial cell of the blood–brain barrier probably involves a stepwise process: transport into the cell on the large neutral amino acid carrier, the exchange of methylmercury from a cysteine thiol to one on glutathione because the latter is present inside the cell at much higher concentrations than cysteine, and finally transport out of the cell on the glutathione carrier.

Some aspects illustrating the importance of biotransformation (formation or breakdown of metal–carbon bonds [e.g., breakdown of organomercurials to inorganic mercury] or change of oxidation state of a metal in the biological system) are given in Chapter 33. The importance of the oxidation of mercury vapor to mercuric ion for the toxic effects on the CNS is described in Chapter 33 in some detail. Exposure to mercury vapor also exemplifies the importance of a rapid vascular perfusion for distribution. The rapid perfusion of the brain makes it possible that physically dissolved mercury vapor is transported from the lung to the brain before Hg^0 is oxidized to Hg^{++} in the blood. The vapor can penetrate the blood–brain barrier and will thereafter be oxidized to Hg^{++}, which will be "trapped" in the cells of the CNS and exert its toxic effect. Hexavalent chromium is an example of transport into the cells followed by metabolic reduction to trivalent chromium that is assumed to induce mutations and ultimately carcinogenesis by a direct reaction with DNA (Figure 8).

The methylation and/or demethylation of metallic compounds is of great importance for toxicity. Whereas methylation processes are of importance as a detoxifying process for arsenic in most animals and man (Buchet *et al.*, 1981; Vahter and Norin, 1980), demethylation serves as an important step in the detoxification and elimination of mercury from animals and man after exposure to methylmercury, as will be discussed in more detail in the next section. The breakage of one carbon–metal bond in tetraethyl tin, on the other hand, yields a highly toxic metabolite (triethyl tin) believed to be responsible for the toxic effects (Cremer, 1959).

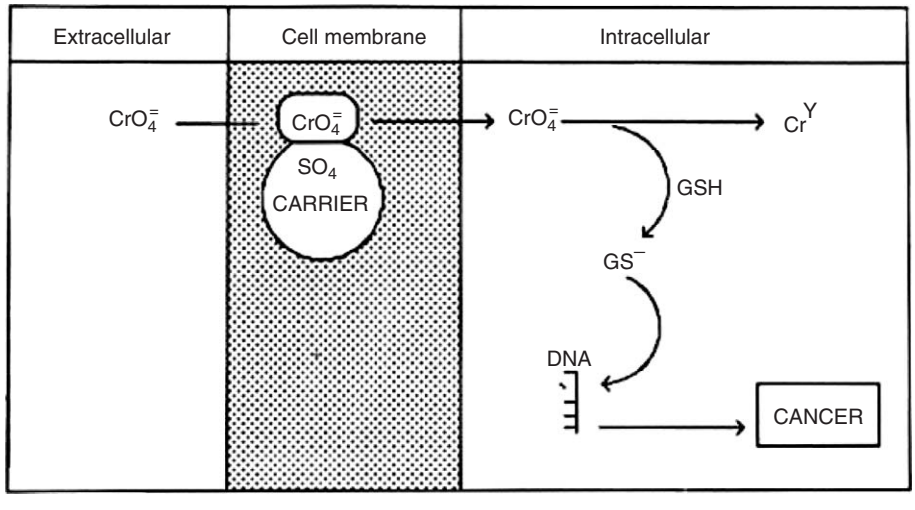

FIGURE 8 Diagrammatic representation of the entry of the chromate oxyanion into the cell of the sulfate carrier and its subsequent reduction to trivalent chromium. The latter forms complexes with DNA to produce mutations that may ultimately lead to cancer.

The metabolism of tetra to triethyl lead is a similar example (Cremer, 1958), because triethyl lead is considered to be the toxic metabolite of tetraethyl lead (Grandjean and Grandjean, 1984). The metabolism of these organometallic compounds is mediated through microsomal enzymes.

5 PATHWAYS AND MECHANISMS OF EXCRETION

Various routes exist by which a metal may be excreted from the body. The most important excretory pathways are the gastrointestinal and renal ones. Other processes of excretion such as salivary secretion, perspiration, exhalation, lactation, exfoliation of skin, loss of hair and nails, and bleeding may be of interest in special cases, both as a significant means of elimination and as an index of exposure and body burden. However, these will not be considered here.

5.1 Gastrointestinal Excretion

Routes by which metals may be excreted through the gastrointestinal tract are shown in Figure 2. Excretion through the intestinal mucosa may occur as an active or passive process by which metals are transported by the cells of the mucosa into the lumen of the gastrointestinal tract. Both inorganic mercury and cadmium seem to be excreted by this route as indicated by autoradiographic studies (Berlin and Ullberg, 1963a,b). Cells of the intestinal mucosa turn over rapidly, and passive loss of metal bound to cells that are shed and excreted in feces is another route of excretion that may be of considerable importance in certain cases.

The other main route of gastrointestinal excretion is through bile (and pancreatic secretions). Biliary excretion has been demonstrated for both inorganic and organometallic compounds of many elements (e.g., Al, As, Cd, Co, Hg, Pb, and Te). Many factors influence the extent to which metals are excreted in bile. An obvious example is the difference depending on valence state that has been shown for arsenic. Whereas arsenate—As(V)—was excreted to an extent of approximately 1% in 2 hours, approximately 10 times more of an injected dose of arsenite—As(III)—was excreted through the bile of rats (Cikrt and Bencko, 1974). Because the concentration of As in bile was several hundred times greater than the one in plasma, the biliary excretion is likely to involve an active transport system (Klaassen, 1974).

A variety of chemicals and drugs influence biliary excretion of metals. Compounds capable of inducing liver enzymes stimulate secretion of methylmercury into the bile (Klaassen, 1975; Magos and Clarkson, 1973; Magos *et al.*, 1974,). Low molecular-weight thiol compounds such as cysteine, reduced glutathione (GSH), and penicillamine also stimulate biliary excretion of methylmercury (Norseth, 1973). Ballatori and Clarkson (1983) have produced further evidence that methylmercury secretion into bile is intimately linked with the secretion of reduced GSH in bile; inhibitors of GSH secretion, such as bromosulfophthalein and its conjugates, also inhibit methylmercury secretion; sex differences in GSH secretion are mirrored by similar differences in methylmercury secretion, and developmental changes in GSH secretion during and just after the suckling period (Ballatori and Clarkson, 1982) seem to explain changes in methylmercury secretion. A similar relationship between inorganic mercury secretion and GSH in bile also seems to exist (Ballatori and Clarkson, 1984a, b). The preceding observations suggest that the liver plays an active role in the formation and secretion of methylmercury complexes and that factors affecting liver metabolism will influence biliary excretion. The role of the liver enzymes with regard to secretion of other metals remains to be investigated.

Metals secreted in bile may be reabsorbed further down the gastrointestinal tract and, consequently, become available for re-excretion in the bile. This process of biliary secretion followed by subsequent reabsorption in the biliary tree and intestinal tract is usually referred to as enterohepatic circulation. As discussed previously, it is known that many metals are secreted in bile. Some degree of subsequent intestinal absorption is likely to occur. Thus, to varying degrees, enterohepatic recirculation must occur with many metals. Enterohepatic circulation is known to occur for As^{3+}, CH_3Hg^+, Hg^{++}, Mn^{++}, and Pb^{++}.

The process can be quite complex. Methylmercury is a well-documented example. It is secreted into bile as a complex with reduced glutathione. Animal data indicate that this first step is dramatically age dependent—it being completely absent during the suckling period. The glutathione complex is hydrolyzed to its constituent amino acids by the enzyme glutathione transpeptidase as it passes down the biliary tree. Methylmercury is released as a complex with cysteine. As discussed in a prior section, this complex of methylmercury is readily transported across cell membranes on the large neutral amino acid carrier. Some absorption back into the bloodstream takes place in the gallbladder, and the remainder enters the intestinal tract. At this juncture, further reabsorption takes place across the mucosal cells. However, methylmercury also comes into contact with intestinal microflora present in the ileum and the large intestine. Rowland *et al.* (1983) have demonstrated that methylmercury is degraded to inorganic mercury by microflora in the gut and that

such demethylation is a key step in the fecal excretion process. Changes in diet or doses of antibiotic that affect the composition of gut flora also affect the fecal excretion rate of methylmercury in rodents. Inorganic mercury is poorly absorbed and passes directly into the feces. The process of biliary secretion followed by demethylation accounts for approximately 90% of the total elimination from the body.

The process of enterohepatic recirculation preserves the body pool of bile acids. For drugs, the recirculation has been most extensively studied in the area of clinical pharmacology. It can result in the appearance of at least two concentration peaks in the blood compartment; first, because of the initial dose of the drug and the next, because of the enterohepatic circulation. The process is subject to genetic control, as well as to physiological changes with age and disease. The cycle can be broken, and fecal excretion increased by oral doses of a nonabsorbable binding agent. Resins binding methylmercury in the gut have been used in this way and successfully tested in cases of human poisoning. Much remains to be learned of the role of the enterohepatic cycle with respect to metals.

Most studies on biliary excretion of metals have been performed on rats. It should be remembered, however, that there might be considerable quantitative differences in the proportion of biliary excretion in relation to other excretion routes among various animal species and between such species and man.

5.2 Renal Excretion

It is also important to understand detailed mechanisms of renal excretion of metals: the renal pathway is a major route of excretion for many metals, and the urinary concentration of metals is frequently used as an index of body burden. An understanding of this excretory route may also make it possible to influence such excretion and thereby speed up the elimination of metals, thus providing a means for treatment of poisoning.

Classical renal physiology has related renal excretion mainly to the "ultrafiltrable fraction" in plasma. The complex physicochemical state of metals in blood, involving in some instances colloidal solutions and protein binding of various types, has to be considered to understand the renal excretory mechanisms. The glomerular ultrafiltrate contains various ions and compounds from plasma, ranging in size up to plasma albumin. Only a small proportion of plasma albumin appears in the glomerular filtrate, and proteins with larger molecules are retained in blood. Macromolecules with relatively low molecular weight, such as inulin (mol. wt., 5000) and metallothionein (mol. wt., 6500), pass the glomerular membrane. Metals bound to such low-molecular-weight proteins may thus be cleared from plasma into the tubular fluid. The filterable fraction of metal in plasma may be influenced by some factors (e.g., changes in the concentration of some ions and other substances that occur normally in plasma).

The renal clearance and the ultrafiltrable fraction in plasma have been determined for several metals (Cr, Cu, Ni, Ra, Tl, U, and Zn). Renal clearance was found to be lower than the calculated glomerular filtration, which may be explained by tubular reabsorption of part of the metal present in tubular fluid (see review by Nordberg, 1982). Cadmium bound to metallothionein is very efficiently reabsorbed in the renal tubule, and only a small fraction is excreted in urine. Urinary excretion of beryllium is considered to take place through tubular secretion. The role of tubular secretion or other forms of transtubular transport may be important for the renal accumulation and urinary excretion of mercury and lead.

Changes in the urinary pH give rise to changes in the urinary excretion of metals such as uranium and lead. Uranium is the first and still the most dramatic example of the influence of acid–base balance on renal accumulation and excretion of this metal (Voegtlin and Hodge, 1960). Uranium was shown to be present in plasma as the uranyl cation, UO_2^{++}, partly protein bound and in part complexed with bicarbonate anions (Figure 9).

The bicarbonate complex is readily filtered through the glomerulus to enter the proximal tubular fluid. Here, dependent on the pH, it dissociates to release the uranyl cation that can then bind to the proximal tubular cells. In this way, when present at sufficient concentration, damage occurs to the resorptive mechanisms as evidence by the appearance of aminoaciduria (Clarkson and Kench, 1956). The undissociated bicarbonate complex is not absorbed and passes directly into urine. Pretreatment with sodium bicarbonate maintains a high pH in the tubular fluid such that little dissociation takes place and virtually all the uranium passes into the urine. Acidification of the animal by pretreatment with ammonium chloride resulted in a low tubular pH, almost complete dissociation of the complex, a large renal uptake, and a small excretion of uranium (Figure 10). The urinary excretion of lead is also affected by pH. The concentration of some amino acids such as cysteine and histidine may increase the filterable fraction of some metals (mercury, copper, or nickel).

Magos and Clarkson (1977) have reviewed interrelationships between renal injury and urinary excretion of metals. The increased excretion of heavy metals because of exfoliation of tubular cells has been

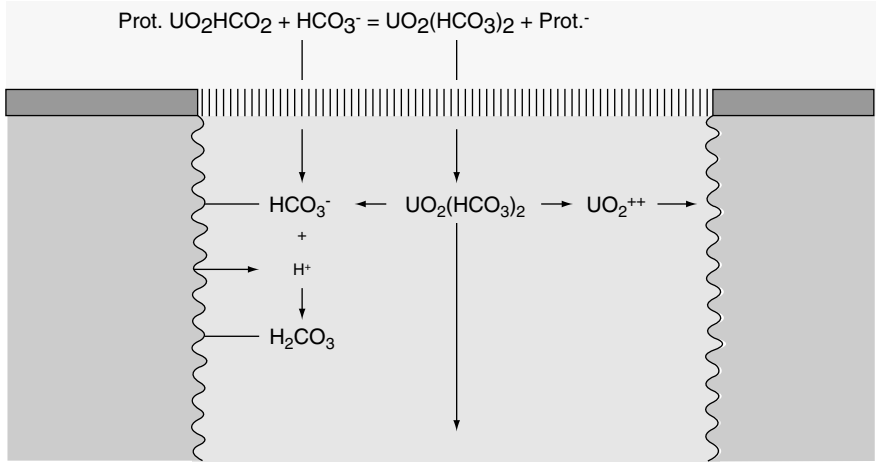

FIGURE 9 A diagrammatic representation of the renal uptake and urinary excretion of uranium present in plasma as the uranyl cation complexed with bicarbonate anions.

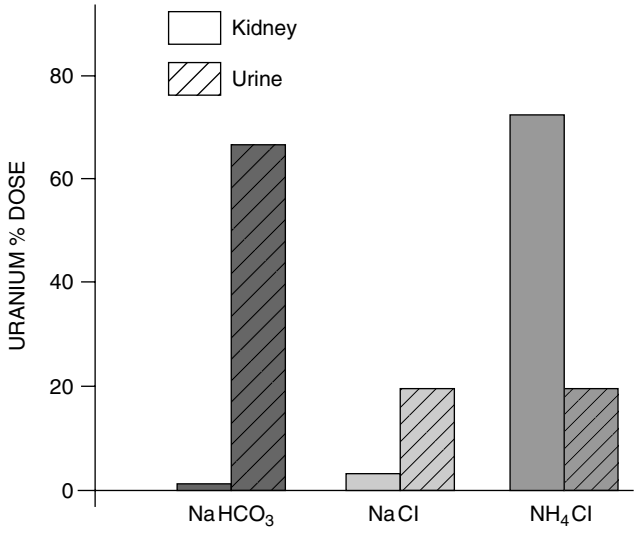

FIGURE 10 The effect of alkalosis and acidosis on the kidney level and urinary excretion of uranium.

extensively studied. Thus, any agent that elicits exfoliation of cells will increase urinary excretion of a metal. In contrast, acute renal failure will dramatically reduce urinary excretion of metals.

5.3 Excretion Rate—Biological Half-Time

The rate of excretion or clearance of a metal compound from an organ or from the body as a whole depends on a number of specific processes. When the course of an elimination process is governed only by the concentration gradient, the process can be represented with reasonable accuracy by a single exponential function.

According to the Task Group on Metal Accumulation (1973), the concentration in the organ under consideration may be expressed as

$$c_1=(c_0 \times \exp(-bt), \qquad (1)$$

where c_1 = concentration in the organ at time t; c_0 = concentration in the organ at time 0; b = elimination constant; and t = time. The biological half-time (i.e., the time it takes for the concentration to be reduced to half of its initial value) is then

$$T_{1/2}=\ln 2/b, \qquad (2)$$

where $T_{1/2}$ = biological half time; and $1n\ 2$ = natural logarithm of 2 = 0.693. The concept of biological half-time has been much used in evaluations of metal toxicity, and this is illustrated further in the following section of this chapter, as well as in the chapters on the various metals, where available data on the biological half-times are given.

The biological half-time varies greatly among metals. When discussing half-time, it is customary to lump together various parts of the body that have the same elimination rate into one "compartment," and one may speak of, for example, the "soft-tissue compartment" or of a compartment with a half-time of, for example, 5 days. Data on the half-time of a compartment may be obtained by actual measurements of elimination from that compartment after the termination of exposure, and in such a case, the anatomical structures comprising the compartment will be known. Such measurements may even be possible in humans through external scanning techniques after ingestion or inhalation of substances labeled with radioisotopes. When chemical analysis is used, it is usually necessary to limit the investigation to studies on blood, urine, and feces.

As discussed later, knowledge of the population variance in excretion rates (biological half-times) is essential for a quantitative estimate of risk. This information is lacking for most metals. Methylmercury is the best-studied example, where it has been shown that many factors influence the magnitude of biological half-time. Species differences range more than two orders of magnitude—from approximately 7 days in the mouse to more than 700 days in certain marine mammals (for a review see Clarkson, 1972). Half-times in humans (average value of approximately 70 days) are bimodally distributed and cover approximately a fivefold range—approximately 33–189 days (WHO, 1991). Lactating women—average half-time 42 days—have significantly shorter half-times than non-lactating adult women—average 76 days (Greenwood *et al.*, 1978). Biological half-times in suckling infants are not known, but experiments on mice (Doherty *et al.*, 1977) indicate that no elimination of methylmercury takes place during the suckling period. Other experiments on mice indicate that dietary changes can alter biological half-times (Rowland *et al.*, 1983).

6 TOXICOKINETIC MODELS AND THEIR USE FOR ESTABLISHMENT OF DOSE-RESPONSE AND DOSE-EFFECT RELATIONSHIPS

A toxicokinetic (or pharmacokinetic) model describes the processes of absorption, distribution, biotransformation, and excretion quantitatively as a function of time. Such a model is sometimes named "metabolic model." Such models may help us in a general manner to understand the occurrence of adverse effects of chemical compounds. They may also be used together with toxicodynamic knowledge, particularly when critical concentrations in the critical organ can be estimated, to define exposures that are associated with a certain risk of adverse effects. In instances where this is possible, the toxicokinetic model constitutes a very important part of a total model for risk estimation. Such models may be of great value, because they provide a means of calculating expected dose-response relationships and may be helpful as a basis for setting exposure limits in industry and in the general environment (see Chapter 14).

Toxicokinetic models may also serve a useful purpose by enabling conversions of information about concentration in biomarker media (e.g., blood, urine) to intakes and vice versa. However, the most important use of such models is to calculate the concentration in the critical organ under various conditions of exposure. The following sections deal with models of different complexity and with different types of exposure.

6.1 One-Compartment Model

6.1.1 Description

The one-compartment model can be described by the same mathematical expression as used in the foregoing section (5.3), where biological half-time is defined. Equation 1 describes the course of the elimination process from a compartment when there is no further influx (e.g., after a single dose). In this equation, *c* stands for the concentration of metal in the compartment. The same mathematical expressions are also valid when the amount of metal in the compartment is studied. When additional doses are given at time intervals that are several half-times long, the doses will not appreciably influence each other, and the concentration curve in the organ will take a course that is a repetition of the one after a single dose (Figure 11). When the interval between doses is shorter, there will be an accumulation of metal in the organ (Figure 12a). This will also be the case if there is continuous exposure (Figure 12b). The situation with continuous exposure is the one that represents the greatest accumulation hazard and, therefore, deserves special attention. The accumulated amount (A) of metal in a compartment can be expressed mathematically as

$$A = a/b[1 - \exp(-bt)], \qquad (3)$$

where A = accumulated amount; a = fraction of daily intake taken up by the organ; b = elimination constant; and t = time of exposure. At steady state

$$(t = 8)$$

$$A = a/b \qquad (4)$$

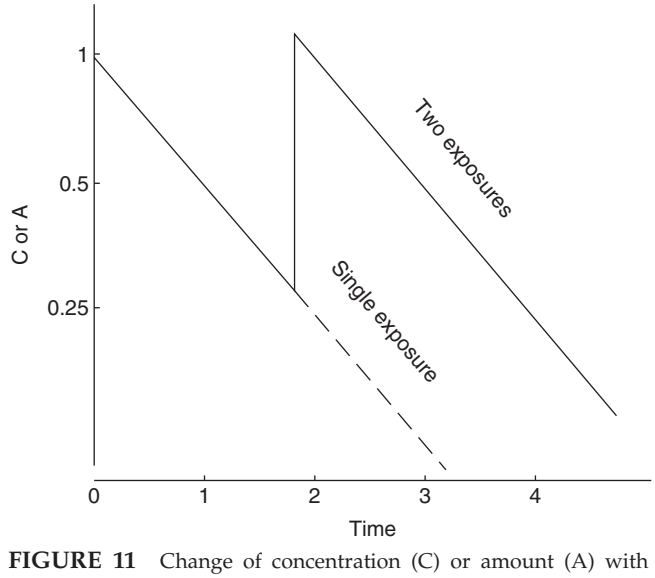

FIGURE 11 Change of concentration (C) or amount (A) with time in a compartment after single exposure or after two consecutive exposures with a relatively long time interval in relation to the biological half-time.

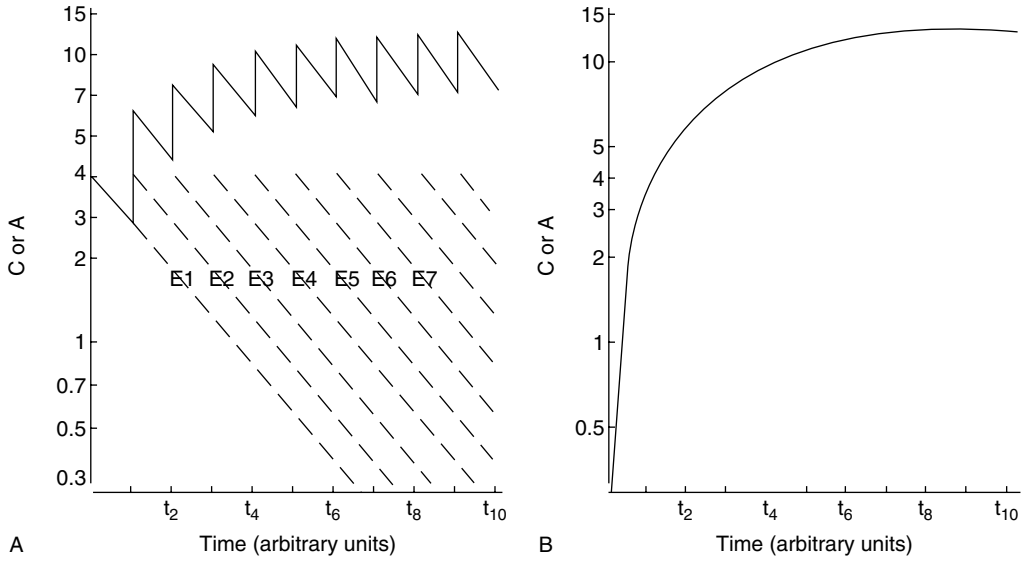

FIGURE 12 (a) Simulation of the cumulative plasma concentration of a chemical (C) or amount in the body (A) after repeated administration or exposure (from WHO, 1978). (b) Plasma or organ concentration in relation to time during continuous exposure.

When t is sufficiently long (i.e., corresponding to five times the biological half-time or more), the steady state can be regarded as reached from a practical point of view.

These mathematical expressions are based on the assumption that intake and absorption are constant. If either intake or absorption changes systematically with age, type of exposure, period in time, etc., the equation must be modified. Some examples of this will be given.

6.1.2 Use of One-Compartment Model for Toxicokinetic (TK)–Toxicodynamic (TD) Modeling of Dose-Response or Dose-Effect Relationships

The one-compartment model—as well as more complex models—has been used extensively for description of distribution and elimination of drugs and various organic chemicals (Teorell, 1937; WHO, 1978). In radiation protection, the one-compartment model has been successfully applied in estimating radiation doses to body tissues that are used to recommend maximum permissible exposure (ICRP, 1968; 1971; 1979; 1982). Radiation protection calculations were based on "standard man" and thus did not provide for individual variations. The "standard man" and "reference man" were specified by the International Commission on Radiological Protection (1959; 1975).

As mentioned previously, interindividual variation in intake, absorption, distribution, and excretion may greatly influence the accumulation in critical organs and should be taken into account to obtain correct predictions (Nordberg and Strangert, 1985). Systematic changes in organ weights and daily intake should be considered when estimating accumulation. Such factors have been considered in calculations regarding cadmium accumulation in the kidney (Kjellstrom and Nordberg, 1978, 1985) and have also been taken into account by the Task Group on Reference Man (IRCP, 1975) and by Choudhury *et al.* (2001). Age-dependent changes in organ composition and organ function should also be taken into account when they are of importance for the modeling process. Renal changes have, for example, been identified as important in modeling the kinetics of cadmium (Kjellstrom and Nordberg, 1978; Travis and Haddock, 1980). For dose-effect evaluations regarding neurological effects of methylmercury, the one-compartment model has been successfully used for calculations of accumulation in the CNS (Berglund *et al.*, 1971). The use of this model and the information concerning interindividual differences in biological half-time (Shahristani and Shihab, 1974; as shown in Figure 13), and information concerning interindividual differences in threshold body burden for elicitation of clinical symptoms and signs of poisoning (see Figure 14; from Bakir *et al.*, 1973), Nordberg and Strangert (1976) calculated the risk of poisoning in relation to long-term daily intakes of methylmercury (see Figure 15). Nordberg and Strangert (1978; 1985) have further discussed principles for risk estimations by use of interindividual variation of metabolic and organ sensitivity parameters.

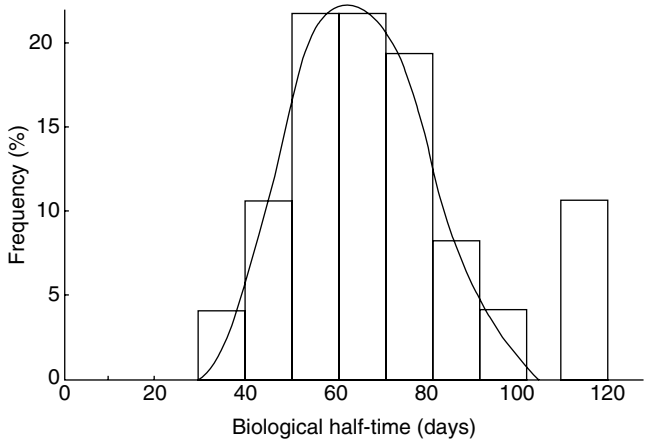

FIGURE 13 Population distribution curve of biological half-time of methylmercury obtained by measurement of hair concentrations. (From Shahristani and Shihab, 1974).

6.2 Multicompartment Models and Physiologically Based Models

Atkins (1974) and the WHO (1978) have discussed the use of multicompartment models for interpretation of experimental data on essential trace elements and drugs. It has been recognized that for an adequate and useful quantitative description of the toxicokinetics of many metals—including inorganic lead, cadmium and mercury—multicompartment models are required (Task Group on Metal Accumulation 1973; Task Group on Metal Toxicity, 1976). It has also been recognized that multicompartment models for these toxicologically important metals would be useful as a basis for interpretation of data from environmental and biological monitoring programs and for calculation of dose-response relationships (i.e., for toxicokinetic/toxicodynamic TK/TD modeling).

For animal data, Cember (1969) and Nordberg and Skerfving (1972) developed a model for retention and accumulation of inorganic mercury in the kidney. Matsubara-Khan (1974), Matsubara-Khan and Machida (1975), Shank *et al.* (1977), and Marcus (1982) developed models for accumulation of cadmium in various organs of experimental animals. Several mathematical models have been advanced for the description of the retention of alkaline earth metals (Sr, Ba, Ra) in the body and particularly in bone (Harrison, 1981; Marshall and Onckelinx, 1968). Because lead is also a bone-seeking element, similar models have also been considered for this metal. Piotrowski (1971) discussed a model for the kinetics of lead. A power function was required to fit the accumulation of lead in tissues. A multicompartment model with spatial diffusion of lead in bone was developed by Marcus (1983) and adapted to lead disposition in dogs. For humans both physiologically based (O'Flaherty

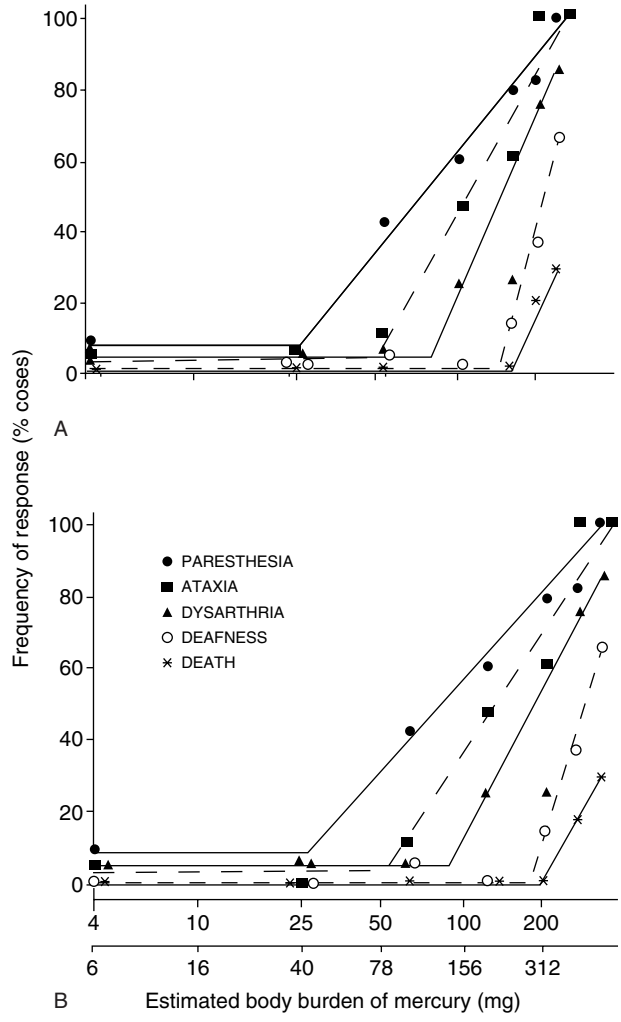

FIGURE 14 The relationship between frequency of signs and symptoms and the estimated body burden of mercury (A) at the time of onset of symptoms and (B) at the time of cession of ingestion of methylmercury in bread. Both scales on the abscissa are for body burden of methylmercury and were calculated in different ways as described by Bakir *et al.* (1973). For the top scale, use was made of the observed relationship between mercury concentration in blood and the ingested dose. The bottom scale was estimated from the relationship between the mercury concentration in blood and the ingested dose as reported by Miettinen (1972).

1993) and compartment models have been developed and used in assessments of human uptake of lead from environmental sources (US EPA 1994; 2002; see also Chapter 31). Several parts of human lead toxicokinetics have alternative mathematical descriptions. This is true for the relationship between exposure levels and blood lead levels and the relationship between bone lead and blood lead (cf further discussion in Chapter 31).

The concept of "physiologically based" models has been advanced since the early 1990s in metal toxicology. These models use information about physiological

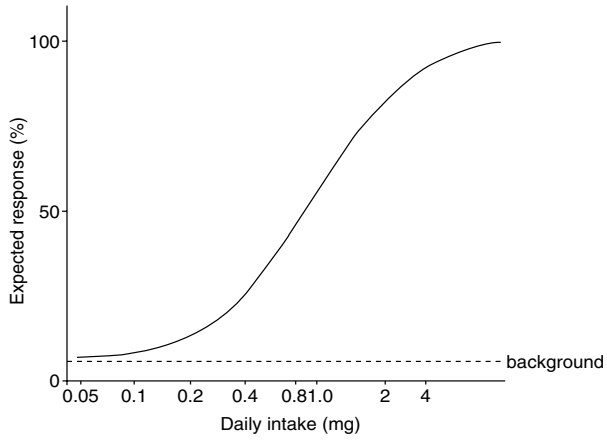

FIGURE 15 Relationship between intake of methylmercury and risk of poisoning in adult persons.

processes (e.g., bone formation and incorporation of bone-seeking elements into bone and oxidation-reduction processes changing the oxidation state of metallic compounds in human body fluids). All physiological processes that have an important impact on the toxicokinetic model for the element under consideration are included in such models. When such models are developed for pharmacologically active substances, they are named physiologically based pharmacokinetic models, PBPK models. Such modeling has been developed for the toxicokinetics of lead in humans (O'Flaherty 1993; 1995) and for chromium toxicokinetics in humans after ingestion (O'Flaherty *et al.*, 2001). These models, like multicompartment models based on knowledge of metal binding in plasma and tissues (see later), both try to use as much as possible of actual data and understanding of the metabolic and toxicokinetics behavior of metallic compounds in human tissues and body fluids.

A multicompartment model for cadmium has been developed and applied to the interpretation of data on human cadmium exposure. This model for cadmium has been used for a long time with some modifications (cf Section 6.2.1) and will, therefore, be used as an example of such a model. This model (Choudhury *et al.*, 2001; Kjellstrom and Nordberg, 1978; 1985; Nordberg and Kjellstrom, 1979) is briefly described in the following section.

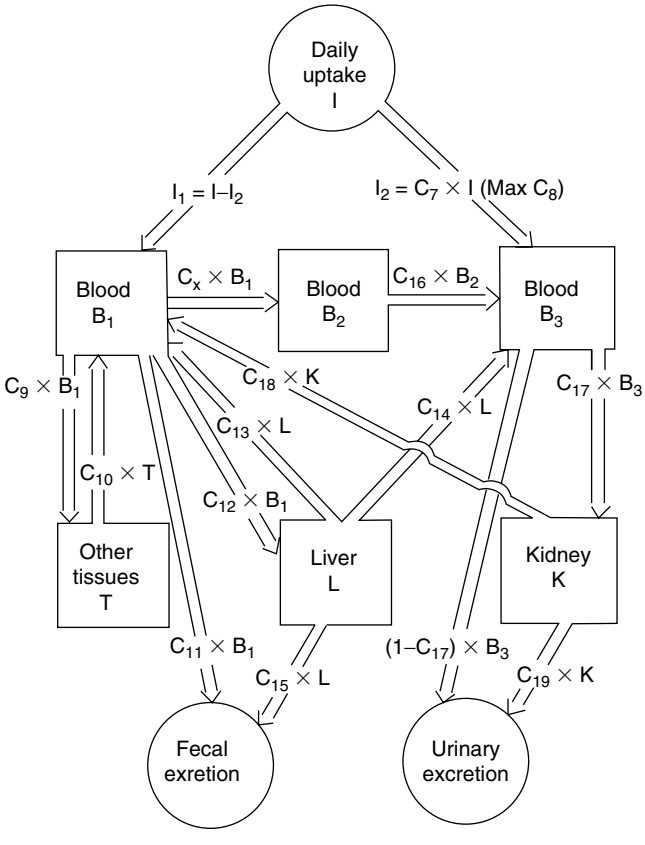

FIGURE 16 Flow scheme describing the movement of cadmium among various compartments. (From Nordberg and Kjellstrom, 1979).

6.2.1 Description of a Multicompartment Model for Cadmium

The flow scheme describing the model is shown in Figures 3 and 16. The first part of the model is identical with the general pathways for absorption described in Section 3.3 and in Figure 3.

The amount transferred daily to the systemic circulation (daily uptake I) will be distributed among three blood compartments: B_1, representing cadmium bound to albumin and other proteins (except metallothionein); B_2, representing cadmium in erythrocytes; and B_3, cadmium bound to metallothionein in plasma. The amount of cadmium that can be bound to metallothionein directly in plasma (I_2) is limited, because the amount of circulating metallothionein is extremely small. Other binding sites in plasma are abundant, however, and it is assumed that cadmium not bound to metallothionein in plasma will instead be bound to these other binding sites ($I_1 = I–I_2$). The amount of cadmium reaching B_1 will be further transferred to the various tissues in the body, and only a small proportion of the cadmium from these compartments will return to B_1, because tissue binding is much stronger than binding to ligands in B_1. Part of the cadmium in B_1 will be excreted in feces through the intestinal mucosa ($C_{11} \times B_1$). Part of the liver cadmium ($C_{14} \times L$) will be released in the form of metallothionein to the blood (B_3), and a very small proportion will be excreted in the bile ($C_{15} \times L$). The kidney derives its cadmium content

from the metallothionein fraction in plasma. A very minor part of B_3 (approximately 5%) is transferred to the urine. Because renal tubular reabsorptive capacity diminishes with age, C_{17} is partly age dependent.

The flow of cadmium among the compartments in the model described by Kjellstrom and Nordberg was derived by a series of equations. An improved mathematical approach that uses the same model for Cd flow in the human body (Figure 17), has been described by Choudhury *et al.* (2001). The equations describing intercompartmental transfers of Cd were implemented as differential equations in Advanced Continuous Simulation Language (ASCL v 11), and improved growth algorithms and other adjustments were used. A Monte Carlo simulation was used to propagate variability in daily intake through the model. On the basis of data concerning intake of Cd in the US population, levels of Cd in urine in the US population were computed by this model. When comparing their results with those measured in NHANES II, they found good agreement in men as shown in Figure 17, whereas the urine Cd values in women generated by the model were lower than those observed in NHANES II. Data demonstrating a higher gastrointestinal absorption in women than men have been published since the Kjellstrom-Nordberg model was developed in the 1970s. This information was used and gastrointestinal uptake in women was adjusted to 10% (instead of 5% in both men and women originally used by Kjellstrom and Nordberg). With this modification, Choudhury *et al.* (2001), found reasonably good agreement between observed and model generated values in females also.

6.2.2 Use of Multicompartment and Physiologically Based Models for TK/TD Modeling

Toxicokinetic (TK)—toxicodynamic (TD) modeling describe quantitative estimations of expected dose-response relationships by the use of TK/TD data. Early contributions to this field that used data on cadmium were those published by Nordberg and Strangert (1985), described and discussed in more detail in Chapter 23. More recently, multicompartment (IEUBK model) and physiologically based models (e.g., O'Flaherty, 1993) have been used to assist in risk assessment of metal exposures in humans, but published estimates of expected dose-response relationships are not available.

7 USE OF INDICATOR MEDIA FOR ESTIMATION OF EXPOSURE OR CRITICAL ORGAN CONCENTRATION

To estimate the risk of poisoning for an individual or a group of individuals, it would be desirable to measure the dose at the site of effect (i.e., the concentration in

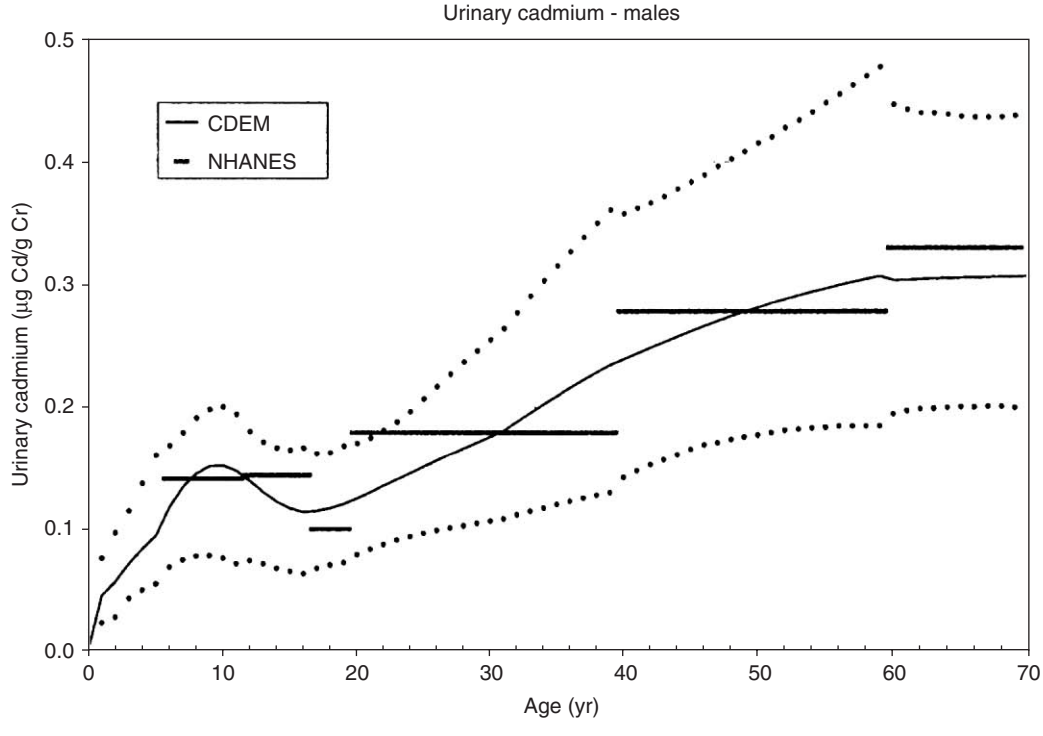

FIGURE 17 Urine levels of Cd in relation to age in the US population and a comparison with values calculated by Choudhury *et al.* (2001).

the critical organ). In living individuals, this is seldom possible. However, because metal concentrations are also found in other biological media such as blood, urine, or hair, these are often used as indicators of the concentration of the metal in the critical organ and, thus, provide an indication of risk of adverse effects (review by Clarkson *et al.*, 1983). When useful biological indicator media can be found (i.e., when metal concentrations in such media correlate accurately with the appearance of effects), biological monitoring provides a more accurate means for evaluating the risk than does measurement of exposure. Often, such measurements are also more easily and conveniently performed than measurements of exposure. Measurement of the concentration of lead in the blood of a worker, for example, is less time-consuming and gives more accurate information about the risk to this person than does measurement of lead in air, even when a personal air-sampling device is used over an entire working shift. The reason for this is that the blood determination reflects an integrated exposure over a longer period and the soft tissue and critical organ concentration of lead, and thus is more directly related to the appearance of adverse effects. It also integrates uptake by various routes such as inhalation, dermal uptake, and gastrointestinal absorption.

Biological monitoring programs can be designed on various scales and have different objectives. The example with lead, just mentioned, refers to the use of blood sampling for lead analysis in the control of occupational lead exposure in industry. A worldwide pilot project to monitor the present exposures and body burdens of a number of metals and some organochlorine pesticides by analysis of human biological samples has been performed under the auspices of the World Health Organization and the United Nations Environment Programme (Friberg, 1983; WHO, 1977).

When biological monitoring is used, it is important to consider the results obtained in the light of knowledge of the toxicokinetics for the particular metallic compound under study. In some instances, monitoring of an unsuitable biological medium may give a false impression of safety. Although the determination of mercury concentration in urine is a useful tool to estimate risks for groups of workers exposed to elemental mercury in vapor form or inorganic mercury salts, such determination gives no information of value for individuals exposed to alkylmercurials.

Head hair is a useful indicator medium in the case of certain metals. The usefulness of head hair is best illustrated in the case of methylmercury (Clarkson

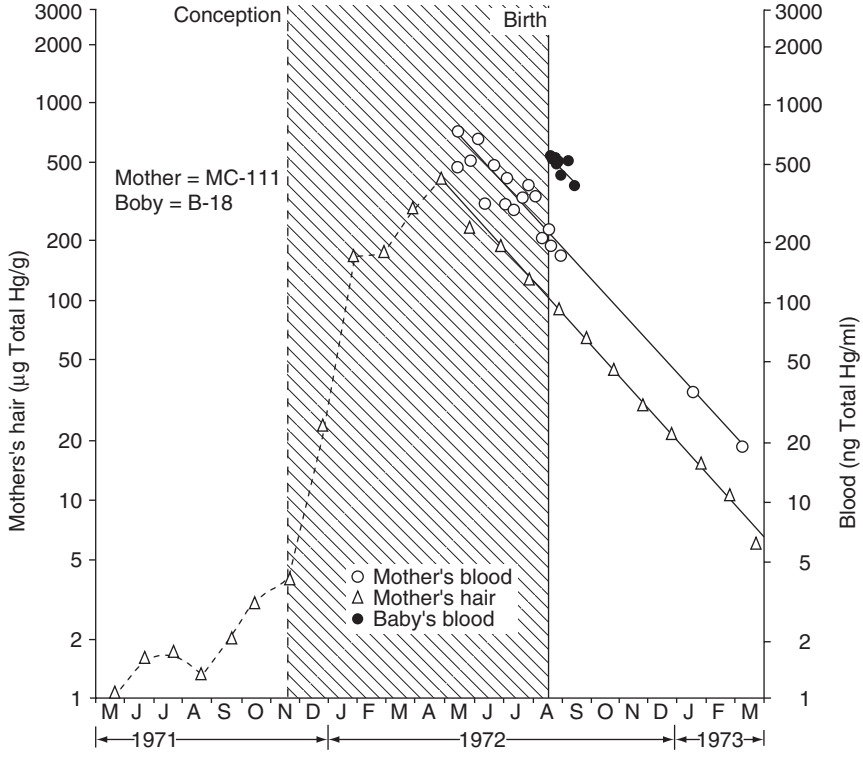

FIGURE 18 Concentration of total mercury in 1-cm segments of sample of mother's hair, whole blood, and baby's blood—prenatal exposure reproduced from Amin Zaki. (from Amin-Zaki *et al.*, 1976).

et al., 1976; Marsh *et al.*, 1981; Shahristani *et al.*, 1976). Methylmercury is incorporated into the newly forming hair in such a way that the concentration in hair is directly proportional to the simultaneous concentration in blood. Once incorporated into the hair strand, the concentration of mercury does not change. Thus, the concentration profile along the strands of hair represents a recapitulation of previous blood concentrations. The hair growth rate is approximately 1 cm/month so that the distance from the scalp in centimeters corresponds to the number of months of exposure before the time of collection of the sample. In the example given in Figure 18, it was possible to recapitulate, from the analysis of one hair sample, the past exposure over a period of almost 2 years, including the entire period of pregnancy. The close parallel between maternal hair and blood concentrations is obvious. It has been possible to use the approach of analyzing segments of hair to obtain an estimate of interindividual variability in biological half-times of a great number of individuals (Figure 13).

Caution must be observed when applying this approach. Hair samples are liable to external contamination, and satisfactory washing procedures have not been established. Furthermore, each metal should be tested to see whether hair concentration is proportional to blood concentration. Thus, it is difficult to find an indicator medium that is useful for estimation of exposure or concentration in critical organs for a wide range of metals. Blood is probably the single biological medium that has greatest general applicability. In some instances, blood values are not easily interpretable without detailed information on previous exposure history, etc. An example of this is cadmium, for which metal there is no direct relationship between blood concentration and the concentration in critical organs. Blood cadmium may thus be used to estimate exposure but does not necessarily reveal anything about risk of adverse effects if exposure has been highly variable over a period of years.

Because some biological samples (e.g., urine samples) are readily available, measurements performed on such samples may also be useful as a tool in the control of exposure (i.e., if analytical results obtained reflect recent exposure) but not concentration in critical organs. Considerations of biological monitoring for a number of metals and their compounds have been presented by Clarkson *et al.* (1988) and in Chapter 4.

When embarking on a program of biological monitoring, it is advisable to acquaint oneself with the toxicokinetic model and patterns of toxicity for the particular metal compound to be analyzed (see the specific chapters on individual metals). This is necessary to make the best possible choice.

Difficulties in the chemical analysis should also be given due consideration, because faulty chemical analysis can make an entire monitoring program entirely useless (WHO, 1979; see also Chapters 2 and 4).

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CHAPTER

4

Biological Monitoring and Biomarkers

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ABSTRACT

Biomonitoring was developed for the assessment of the health risks from exposure to metals at work, and the approaches and concepts of biomonitoring are derived from such exposures. At present, biomonitoring is increasingly used to assess exposure from the environment. Biomonitoring and assessment of external exposure are complementing activities, where the exposure assessments are much more widely applied, especially when the number of chemicals concerned is considered; environmental analysis also offers the distinct advantage of speciation analysis, which is very poorly developed for biomonitoring. Biomonitoring, on the other hand, provides information on exposure from all sources, and via all absorption routes, and also considers accumulation of the chemical in the body. Biomonitoring using exposure biomarkers thus considers interindividual differences in the absorption, whereas use of effect biomarkers also considers interindividual differences in sensitivity. Few effect biomarkers, however, have been validated. Biomarkers of susceptibility have so far not been adapted for use in metal toxicology. The major challenges of biomonitoring are the development of monitoring methods, which are inexpensive enough to be applied at a frequency that makes possible meaningful biomonitoring of metals with a short half-time; development of exposure biomarker guidance values specific to individual species of different metals; expansion of the repertoire of validated effect biomarkers; and validation and application to effect monitoring of the "omic" technologies.

1 INTRODUCTION

We approach the 80th anniversary of biological monitoring: in 1927, the first paper was published on the use of the concentration of lead in urine in exposed workers as means of diagnosing lead-induced industrial disease (Badham and Taylor, 1927). Throughout these 80 years, concepts and practices in biomonitoring have been developed mainly in relation to metals, and still today exposure to metals is the main area of application of biomonitoring.

Detailed description of the state of art of biomarkers for individual metals will be given in the respective chapters. This chapter will describe and discuss general principles and in some cases illustrate the principles by a few examples. In risk assessment of metal exposures, indicators of effects and indicators of exposure have been considered in several consensus documents published since the 1970s by the Scientific Committee on the Toxicology of Metals of the International Commission of Occupational Health (ICOH) (Belman and Nordberg, 1983; Clarkson *et al.*, 1988; Nordberg, 1976; Nordberg and Skerfving, 1993). Authoritative reviews on different aspects of biological monitoring, including analytical methods, reference values, and guidance values, have been published by IPCS, WHO, and others (Aitio, 1999; Albertini *et al.*, 2000; DFG, 1985–2003; 2006; IPCS, 1993; 2001; 2006; WHO, 1996 a,b). The term "biological monitoring" (synonym, biomonitoring) has been used for a long time and more widely during the past two decades (Berlin *et al.*, 1984; Clarkson *et al.*, 1988) to describe exposure and internal dose of metals by measurements in biological samples such as blood and urine and other human tissues and fluids.

The term has more recently been extended to also include other biomarkers, as will be described. The term "biomarker" was introduced during the past 20 years into the field of metal toxicology, environmental, and occupational health (IPCS 1993, 2001, IUPAC 2004; US NRC, 1989).

A *biomarker* is an indicator signaling an event or condition in a biological system or sample, giving a measure of exposure, effect, or susceptibility (IUPAC, 2004).

A *biomarker of exposure* relates exposure to a xenobiotic (i.e., a metal or metal compound) to the levels of this substance, or its metabolite, or of the product of an interaction between the substance and some target molecule or cell that can be measured in a compartment within an organism (after IUPAC, 2004, IPCS, 1993).

As seen from this definition, biomarkers of exposure include indicators of exposure, internal dose, and dose/concentration in critical organs, as in the classical concept of biological monitoring. It also encompasses measurements of interaction between the substance and macromolecules (e.g., DNA and protein adducts and enzyme complexes) as will be discussed in later sections of this chapter. The concept "biomarker of exposure" thus includes all aspects of exposure and internal dose from the contact with the external environment through internal dose to the dose at cellular level and at the molecular target site. The ultimate measurement of dose (i.e., interaction with target molecules) is also included.

Biomarkers of exposure are indirectly related to the occurrence of disease via information about the relationships between the exposure biomarker (the dose at the target) and the development of disease. Although relationships between exposure biomarkers and effects are indirect, biomarkers of effects or susceptibility have a direct relationship to the occurrence of disease or other adverse health effects. Definitions of the latter concepts are as follows:

A *biomarker of effect* is a biomarker that, depending on its magnitude, can be recognized as associated with an established or possible health impairment or disease (IUPAC, 2003; WHO, 1993).

A *biomarker of susceptibility* is a biomarker of an inherent or acquired ability of an organism to respond to exposure to a specific substance (IPCS, 1993; IUPAC, 2003).

In Figure 1, relationships have been outlined between exposure and dose, environmental monitoring,

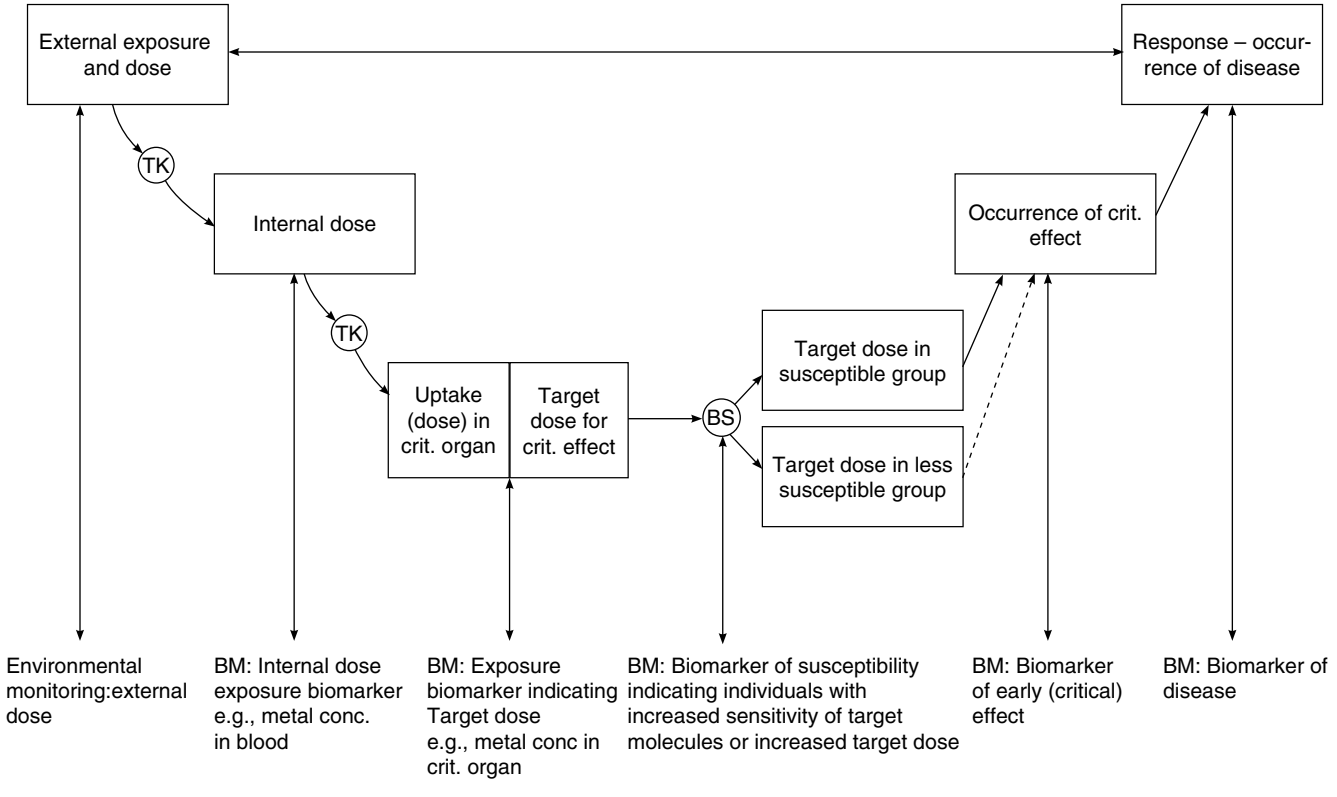

FIGURE 1 Relationships between external exposure and dose, environmental monitoring, biological monitoring (BM), and response. The importance of toxicokinetics a (TK) and susceptibility to adverse (critical) effects (as shown by biomarkers of susceptibility–BS) is indicated.

biological monitoring (BM), and response. External exposure can be measured by environmental monitoring as the concentration of a metal compound in air or food. The dose or intake per day can be estimated from the measured concentrations and estimated daily ventilation volume (inhaled dose) or amount of food consumed (oral dose or oral intake). A proportion of an oral or inhaled dose is taken up in the body, initially to blood. Metal concentration in blood, if monitored, is an *exposure biomarker of internal dose*. The proportion of the external dose absorbed into the body can be derived, and such information is an important aspect of the toxicokinetic or metabolic model of the metal compound in question and impacts on the adverse effects induced. Another important component of the toxicokinetic model describes the relationship between levels in indicator media (biomarkers), such as blood and urine, and accumulation (dose) of the metal in critical organs. The latter is closely related to the target dose. Accumulation of metals in critical organs can sometimes be monitored by external measurements such as Cd in kidney by *in vivo* neutron activation, thus providing *an exposure biomarker, indicating concentration in critical organ*. For effects occurring in internal organs, direct measurements of the interaction between the metal and target molecules are seldom possible. However, in some instances, there is a close relationship between effects in blood and in critical organs. An example is the interaction between lead and enzymes in the bone marrow, which can be monitored by measurements in blood, and they can, thus, be a useful biomarker of target dose. In some instances, it may be possible to use DNA adducts in blood cells as a biomarker of target dose causing carcinogenic effects in body organs. However, documentation of such relationships for metallic compounds is limited at present and may only be applicable for a few metal compounds, as pointed out in the Chapter 5.

In some instances, it may be possible to define population subgroups with particular sensitivity by studying polymorphism in enzymes and membrane molecules as *biomarkers of susceptibility*. However, actual examples of successful application of biomarkers of susceptibility in risk assessment of metal exposure so far are limited. Variation in binding of metals to sequestering proteins before interaction with target molecules may also form a basis for classifying subpopulations into susceptible and less susceptible groups. The observations of such a relationship using metallothionein gene expression in peripheral blood lymphocytes as a biomarker of renal susceptibility to damage caused by cadmium (Lu *et al.*, 2001) are interesting but need to be confirmed, refined, and developed.

Some examples of useful *biomarkers of early (critical) effects follow:* The detection of early damage to the kidney tubules by cadmium using urinary levels of low molecular weight proteins such as $beta_2$-microglobulin, protein HC, and the enzyme *N*-acetylglucosaminidase. *Biomarkers of disease* are monitoring markers used clinically in the diagnosis of disease. In the case of cadmium, damage to the kidney, excessive excretion of $beta_2$-microglobulin (>1 mg/g Crea), or decreased tubular reabsorption of phosphate are such markers for tubular damage.

In biological monitoring of toxic metals, knowledge about the toxicokinetics of the compound is of crucial importance. Because many metals display differentiated and specific patterns of disposition among tissues in the human body, and for several metals adequate knowledge is available on toxicokinetics and metabolic models, this situation can be seen as a special aspect of metal biomonitoring. Other aspects are that it has been possible for a long time to perform relatively precise determinations of metals in biological indicator media at a reasonable cost. The database and experience of biological monitoring of metals thus is considerable. However, the data are focused on a limited number of metals, whereas data on other metals are still incomplete or even lacking.

For cadmium and lead, biomarkers of effect have been available for a long time, and they are reasonably well validated. This effect of cadmium, renal tubular damage, is considered a critical effect.[1] However, in the case of lead, adverse effects on the central nervous system, notably in children, are considered to be the critical effect, and it is not known how closely the hematological effects of lead are related to the neurotoxicological effects. At present, available biomarkers of nonthreshold effect or target dose have not been validated and cannot be widely used or recommended for routine assessments. Considerable efforts are needed in validating recently developed effect and susceptibility biomarkers (Albertini *et al.*, 2000; IPCS, 2001).

2 SOURCES OF PREANALYTICAL AND ANALYTICAL ERROR

A number of errors may occur before the chemical analysis of the substance of interest, and analytical errors may also occur in the chemical analysis. When using exposure biomarkers in the classical sense of biological monitoring (i.e., analyses of metals in blood,

[1]For a discussion of critical effects in occupational and environmental health see Nordberg (1976), and Chapter 14.

urine, and other biological materials), such errors have been identified, and methods for their control have been developed. The errors include errors in design and sampling, such as poorly defined criteria, and biased selection of individuals for sampling. A number of errors can be grouped as physiological and kinetic, such as timing of specimen collection (in relation to the exposure), diurnal, meal- and exercise-induced variation, other variations in distribution (e.g., those induced by changes in body posture and by application of tourniquet). A further fundamental aspect important in the exposure biomonitoring of metals is contamination (and sometimes losses) during blood and urine specimen collection, storage, pretreatment, and analysis (Aitio, 1981; Aitio and Järvisalo, 1984; WHO, 1996). The importance of contamination depends on the level of interest so, for example, contamination is a much more important problem for the determination of plasma lead, where only a small fraction of whole blood lead is found, than with determination of lead in blood cells or whole blood. Vials used for collection and storage of samples should not contribute any significant amounts of the metal being analyzed. For example, glass tubes should not be used for aluminum analyses, Cd-softened plastic tubes should not be used for Cd analyses, etc. For metals constituting components of steel (e.g., Cr, Mo, Ni, Mn, V), considerable contamination can occur from steel needles during blood sampling, and special precautions are necessary to obtain meaningful results (see Chapter 2, Section 8). Smoking chemists performing the analyses can sometimes contaminate samples from metals present on their skin (e.g., cadmium). Improper calibration and standardization of instruments used for analyses can introduce analytical errors. Errors have been considerable in the past, as seen, for example, in the development of what is considered to be the reference values for several trace elements in blood or urine. Appropriate sampling, quality control, and descriptions fulfilling the recommendations by IUPAC (Cornelis *et al.*, 1995) have been provided in some articles, and such information has allowed the definition of reference values for some metals (Nordberg, 1996), but a continuous need exists for critical evaluation and continuous emphasis on quality control aspects for biomonitoring programs to generate good and useful information.

For biomarkers of target dose (e.g., metal–enzyme complexes, DNA and protein adducts) similar quality control aspects are warranted, but such biomarkers are still mainly a research tool at present, and very few, if any, have been validated, and quality control conditions have usually not been specified in detail.

Some biomarkers of effects have been validated according to principles developed in clinical chemistry. Further validation activities are warranted (Albertini *et al.*, 2000; IPCS, 2001).

3 QUALITY ASSURANCE; REFERENCE MATERIALS

The International Standardization Organization Guide ISO 17025 (ISO, 2000) general requirements for the competence of analytical laboratories and instructs on the main components of quality management, that is, quality system, audit, and review. Quality management has been proposed as a prerequisite for laboratory accreditation. An integral part of quality management is continuous assessment and control of quality; this can be divided into internal quality control and external quality assessment. A guideline has been published for the internal quality control for clinical chemistry laboratories (Büttner *et al.*, 1983). The IUPAC guideline for external quality assessment (proficiency testing) (Thompson and Wood, 1993) is being updated, with special emphasis on analyses for which the number of laboratories is small, which is the case for most biomonitoring analyses.

Internal quality control comprises the set of procedures undertaken by the laboratory for the continuous monitoring of operations and results to decide whether the results are reliable enough to be released. Internal quality control mostly depends on repetitive analysis of the same, homogeneous control specimens (reference materials). Together with replicate analyses of all samples, an estimate of the imprecision and changes in the systemic error, bias, may be obtained. By use of certified reference materials, accuracy may be ascertained (see Chapter 2, Section 12). Although the availability of reference materials, and notably of certified reference materials, has improved recently, for many elements certified reference materials (i.e., blood and urine) and concentration ranges relevant for biomonitoring are not available in matrices. At present, reference materials with specified concentrations of metals are presently available from the Institute for Reference Materials and Measurements, Reference materials unit, Geel, Belgium, web page: *www.irmm.jrc.be*; International Atomic Energy Agency, Vienna, Austria, web page: *www.iaea.org*; or National Institute of Standards and Technology, Gaithersburg, MD, USA web page: *www.nist.gov*

External quality assurance: The other main procedure to assist in achieving good quality in metals analyses are programs providing comparison among results from different laboratories. Such comparisons can be organized ad hoc by interested laboratories or more efficiently by joining an available external quality

assessment scheme. Guidance on the organization and running of such proficiency testing has been published and summarized (WHO, 1996). Examples of such schemes are those available from German Society for Occupational and Environmental Medicine, Erlangen, Germany *http://www.g-equas.de/*; United Kingdom National External Quality Assessment Service, *http://www.ukneqas.org.uk/*; Robens Institute of Industrial and Environmental Health, Guildford, UK *http://www.su rey.ac.uk/SBMS/eqas/index.html*; and Toxicology Centre, Québec, Canada *http://www.inspq.qc.ca/ctq/page/*

4 SPECIMENS IN USE; URINE SAMPLE STANDARDIZATION

It was mentioned previously that samples from a number of human tissues and fluids could constitute adequate materials for biological monitoring. However, the samples most often used are blood samples and urine samples.

4.1 Urine

The kidneys continuously produce urine. Blood is flowing through the renal glomeruli where fluid from blood plasma is filtered into the renal tubules. Such filtrate (i.e., primary urine) contains substantial amounts of glucose, salts, amino acids, and small proteins that are reabsorbed in the renal tubules together with most of the water. Determinations of metals in urine often can be used as an exposure biomarker, indicating internal dose of the metal, because a relationship often exists between levels in blood and other tissues and levels in urine. In steady-state conditions, excretion and absorption are balancing each other, and such conditions will make it possible to derive the absorbed dose from excretion. However, in practice, it is difficult to know whether steady-state conditions are at hand or not. Regardless of whether strict calculations are possible or not, knowledge about the toxicokinetic model of the metal under study is helpful when interpreting the results of BM. For metals that are accumulating in renal tubules (e.g., Cd, Hg), a relationship may also exist between renal and/or body burden and urinary excretion of the metal. This may be particularly useful for BM, because urinary excretion of the metal (e.g., Cd) may be a useful biomarker of dose in the kidney, which may be the critical organ, and the resulting damage constituting a critical effect.

Determinations of metal concentrations in urine for biological monitoring purposes and various means for their adjustment have been discussed (Aitio, *et al.*, 1994; Araki, 1980; Araki and Aono, 1989; Araki *et al.*, 1990; Diamond, 1988; Nieboer *et al.*, 1992). Analysis of metal concentration in urine in combination with measurement of the volume of urine excreted during a specified, not too short, time gives a measurement of *timed urinary excretion* of the metal, and this may be a useful exposure biomarker and also a biomarker of dose in the critical organ. The collection of such timed urine samples is usually preferred, not only for exposure biomarkers but also for effect biomarkers determined in urine (e.g., enzymes or other proteins). However, the total excretion, even during a longer interval of several hours, even 24 hours, may vary, depending on urine flow (Araki *et al.*, 1990), and correction for urine flow is necessary even for timed urine samples. The classical timed urine sample is the one obtained during 24 hours. Such a long time is advantageous, because it will include diurnal physiological variations. However, for routine biological monitoring, it is, in practice, not possible to collect all urine voided during such a long time, and shorter intervals may be used. Then imprecision in collection may be a problem, because it may be difficult to know whether a person has completely emptied the bladder or not. By hydrating the person, urine volume will be kept high, and the influence of this problem decreases. Collection of timed urine samples requires very detailed instructions and sometimes training programs and supervision.

In routine biomonitoring, the only available alternative is to use *spot samples.* When this is done, an adjustment for dilution can be done by various methods, the two most common ones are by creatinine and by relative density.

Creatinine (*CR*) is excreted in urine in relation to muscle mass. In persons with normal glomerular function, the amount excreted every day is relatively constant in the same person if the physical activity is constant and the same diet is consumed every day. However, creatinine clearance increases with increasing urine flow, and thus correction with creatinine is not an accurate correction for differences in the hydration state. Because of the dependence on muscular mass, creatinine correction gives a different estimate of the dose in men and women and in children. There is also a variation in the creatinine excretion in population groups on a variable diet. With variable physical activity, a considerable variation in CR excretion may occur even in the same individual. According to Alessio *et al.* (1985), the intraindividual coefficient of variation ranged from 10–50% of the mean. Adjustment by CR is done by analyzing the concentration of this substance in the same urine sample as the metal is analyzed. The concentration of metal is then expressed in relation to

CR (e.g., milligrams of metal per gram of CR or, preferably, micromoles of metal per moles CR).

The *relative density* of urine is the other commonly used measurement for adjustment of spot samples. In contrast to creatinine correction, relative density correction only concerns differences in urine density (i.e., changes in urine flow). The adjustment is usually by specific gravity increment in relation to a reference, most commonly a density of 1.018 or 1.024 using the formula:

$$C_s = C_o \times RD_s/(RD_o - 1.000), \text{ where:}$$

C_s = corrected concentration; C_o = observed concentration, RD_s = reference relative density increment (usually 1.024 or 1.018 − 1.000 = 0.024 or 0.018), and RD_o = observed relative density. It, thus, makes a big difference what the reference relative density is: using the value 1.024 results in corrected concentrations 33% higher than those when 1.018 is applied; the reference RD must be known when different studies are compared.

Araki (1980) has made extensive comparisons of the different corrections for metal excretion in urine and has derived a generic equation for the correction of concentrations in urine depending on the rate of urine flow:

$$C_s = C_o \times F^b$$

where C_s = concentration corrected to urine flow of 1 ml/min; C_o = observed concentration, F = observed urine flow ml/min; b = chemical-specific constant.

It can be seen from the equation (Aitio *et al.*, 1994) that when the chemical-specific constant approaches zero, no correction is needed (i.e., the urinary concentration is constant and independent of urine flow). This, however, does not seem to be true for any chemical. When b approaches 1, the corrected concentration is proportional to urine flow rate, and correction to relative density is accurate. This is the case for mercury (Araki *et al.*, 1990) and nickel in some circumstances (Nieboer *et al.*, 1992). At the value of b of 0.67, which is the constant for creatinine (Araki *et al.*, 1990), correction to creatinine excretion would seem most appropriate. Manganese and cadmium are candidates for this approach, whereas chromium and copper are intermediate and correction to relative density and creatinine are equally good (Aitio *et al.*, 1994). However, for several chemicals, interindividual variation of the constant b is very large (e.g., cadmium, manganese), and consequently, no general correction method will give accurate results. Also, the constant b may not be truly a constant but may depend on the concentration.

When the urine is very dilute or very concentrated, it is unlikely that any correction will give accurate results. Thus, when the relative density falls outside the range 1.010–1.030, a new specimen should be analyzed rather than a highly unreliable result reported. For creatinine, concentrations 3 and 30 mmol/L can be used as such limits.

4.2 Blood

Blood is composed of cells and plasma. Serum is obtained after clotting. All these blood components may be used for BM.

According to general concepts in toxicokinetics, the area under the plasma metal concentration curve (AUC) can be used to calculate absorbed dose after short-term exposure. However, for some metal species, this measurement is difficult and may not be possible in practice. It is, for example, very difficult to measure the relevant concentration of mercury vapor in blood plasma, because concentrations are low, and there is a rapid uptake in membranes of blood cells and subsequently by brain tissue (see further discussion in Chapter 33). However, plasma (total) mercury concentrations can be quite useful in BM of mixed methylmercury and inorganic mercury exposures, because they reflect predominantly inorganic Hg. Blood cell Hg, on the other hand, reflects mainly methylmercury exposure (but also partly inorganic Hg) as discussed in detail in Chapter 33. Because for several metals (e.g., Pb, Cd, MethylHg), plasma or serum levels are considerably lower than whole blood or blood cell levels, detection limits of chemical analyses have been a limiting factor. Serum or plasma values also are more prone to problems of contamination as mentioned previously. Even a limited degree of hemolysis will seriously influence (e.g., Pb) serum values because of the concentration gradient between blood cells and plasma for Pb. Whole blood metal determinations are widely used in BM. With the ongoing improvements of analytical methods and their detection limits, this may change in the future, but until then, a lot of the available databases are for whole blood values. In long-term exposures, like those prevailing in occupational and environmental situations, short-term fluctuations in plasma levels related to variations in hourly uptake may be of limited interest, because it is the uptake of the metal in the critical organ that is of interest, and this uptake and accumulation may be more closely related to the level in blood cells or urine. However, it is obvious that an understanding of the toxicokinetics of the metal compound in question (cf. Chapter 3 and the chapters on individual metals) is of fundamental importance for interpretation of levels of metals in various blood components.

Blood has a more constant composition than urine, and results of metal analyses are expressed as concentration values (i.e., mg/L, µg/L [or preferably, mmol/L, µmol/L], etc. Some metals (e.g., Pb, Cd) are bound mainly to blood cells, and it may be useful to adjust obtained values by hemoglobin concentration or hematocrit value (cf. Hense, 2003). Despite the obvious advantage of such adjustment, it is seldom practiced.

Another aspect when using metal analyses in blood for BM is the impact of hemolysis. Although some metal exposures may themselves induce hemolysis (e.g., mild hemolysis in occupational lead exposure and severe hemolysis in arsine poisoning), of more practical importance are other conditions giving rise to hemolysis such as glucose-6-phosphate dehydrogenase deficiency and hemolytic anemias.

5 REFERENCE VALUES

Although for many organic chemicals, the sole exposure may be at work, and thus any detected concentration in biomonitoring means that occupational exposure has taken place, this approach for the identification of the exposed people does not work for most metals: There is always an exposure from the environment (mostly from the diet). Thus, the identification of the exposed individuals in biomonitoring is on the basis of a comparison to reference values. IUPAC has given guidance on the development of reference values, reference intervals, and reference limits, and recommended the terminology to be used (IUPAC, 1993). For the biomonitoring of an occupationally exposed population, the reference limits are the highest (usually 95th percentile) concentrations of the element (reference values) observed in an occupationally nonexposed population that is similar in terms of ethnicity, sex, age, environmental exposure, and also smoking and other social habits to the occupationally exposed population studied (reference population). It is of fundamental importance that reference values are not influenced by contamination and other errors encountered in trace metal analyses (cf. foregoing sections). Reference values for a number of metals have been published from various countries. There is considerable variation among countries, depending on a combination of geological factors, dietary habits, and anthropogenic exposures that may affect an entire country (Iversen *et al.*, 2003). Examples are mercury levels in blood and hair that are much dependent on the fish consumption in the population studied (McDowell *et al.*, 2004; JECFA, 2004). Cadmium levels are higher in Japan than in other countries, probably because of a low-level contamination of rice that originates from a combination of geological factors and mobilization of Cd into the environment by human activities (Herber *et al.*, 1997). Substantial increases in the serum selenium concentration was observed in Finland, when selenium-enriched fertilizers were taken to wide use to counteract the low selenium intake caused by the low selenium content, high pH, and high iron content of the soil (Aro *et al.*, 1995).

6 ETHICAL CONSIDERATIONS

Biomonitoring produces data on an individual and, thus, information that is confidential. The results must not be used against the worker, thus, for example, compliance with legally binding blood lead values must not be achieved by sacking those who have high values. A worker's biomonitoring history must not be used to discriminate against him or her (e.g., at hiring—employers might wish to decide not to hire anyone for a job with exposure to lead with a previously elevated blood level or anyone with a documented previous nonexposure to lead). All BM activities require an informed consent from the persons monitored, and the samples may only be used for the stated purpose, thus, for example, ethanol, drugs or HIV, must not be analyzed). Results should be dealt with in the same way as other clinical chemistry data (i.e., they would not be disclosed on an individual basis to anybody except the person monitored and his or her doctor). It may, however, be necessary in some instances to make exceptions to this rule when there is legislation requiring biological monitoring of workers and it is required that results are communicated to the employer also. It is then important that the physician obtains informed consent before employment for such jobs. For further aspects, *cf.* Aitio (1999).

It is generally considered that ambient monitoring ensuring that no harmful exposures occur is preferred over BM, which can be seen as using the worker as a sample-collecting device. The problem is especially acute when the worker is exposed to genotoxic carcinogens, for which no exposure is considered to be safe.

7 BIOMARKERS OF EXPOSURE

7.1 Analytical Approaches

In-depth discussion of analytical methods for elements in different matrices, including those used in biomonitoring, is given in Chapter 2. The detection methods most widely used when analyzing metals in biological specimens are graphite furnace atomic absorption spectrometry and inductively coupled plasma mass spectrometry. For elements that can be converted to hydrides,

hydride generation atomic absorption spectrometry and atomic fluorescence spectrometry may be alternatives with good sensitivity. Atomic emission spectrometry is also a sensitive metal analysis method and offers the possibility of multielement analysis; however, in biomonitoring, this special feature is seldom very useful. For routine analysis, electrochemical methods are not very well suited for biomonitoring purposes.

7.2 Speciation in Biomonitoring

With few exceptions, routine biomonitoring depends on the analysis of the total content of an element as the biomarker rather than a speciation analysis. For some elements, this simplistic approach is sufficient; this is notably the case when the key effect of the element on the health is systemic and the relationship between the total element concentration and the effect is known. Thus, most of the time in occupational exposure, a reliable prediction of long-term health effects may be made from (total) lead in blood, (total) mercury in blood or urine, and total cadmium in blood or urine. However, in exposure to tetraethyl lead, or in exposure to methylmercury from diet, these analyses may be very misleading. This error may be even more marked when there is concomitant exposure to different species of the same metal. As is evident from element-specific chapters in this volume, the toxicity, including mutagenicity and carcinogenicity, of several metals very much depends on the identity of the elemental species or, for example, the oxidation state (see chromium, nickel, arsenic, mercury), inorganic versus organic compounds of the same element (see mercury, arsenic, tin). To overcome these problems, speciation analysis is an important aspect in biomonitoring now and will continue to be in the future. Speciation in biomonitoring may be approached by three different strategies: analysis of specific element species (as of now, practically limited to arsenic), fractionation by chemical analytical means to organic versus inorganic species (mercury, lead in blood mainly), or application to the analysis of information on the differences in the distribution of different species of an element (mercury in plasma, blood cells, urine; chromium in erythrocytes/plasma) (IPCS, 2007).

Even when the total concentration of an element is measured, exposure indices of the individual species to which the population is exposed should be developed and used when appropriate (IPCS, 2007).

7.3 Kinetics and Sampling: Timing and Frequency

The kinetics, especially kinetics of the disappearance, is a key element in the interpretation of the results from biomonitoring, and it also determines the timing and frequency of the sample collection.

When the half-time of the metal in the body fluid in which it is measured is long, the concentration reaches an equilibrium in continuous (or repetitive, e.g., daily) exposure and reflects long-term intake. The concentration thus forms a reliable basis for the assessment of long-term health effects, and the timing of sample collection during the working day or even working week is not important.

When the half-time is shorter, from a few hours to a day, there is a remarkable variation in the concentration over a working week and working day, and thus the concentration measured also reflects exposure over a short time and may not be representative of the average exposure over several days (the exposure also being variable, often with a shorter half-time than that of the concentration in blood/urine). A meaningful picture of the average exposure thus may only be obtained from several samples.

If the half-time is shorter than 6 hours, it may be impossible to do any quantitative biomonitoring. Concentration in the urine is related to the time-weighted average concentration in blood (or actually to the free concentration in plasma). Thus, for elements with a short half-time in the blood, it may be kinetically advantageous to analyze the urine rather than blood.

For many, if not all, metals, the disappearance from the blood exhibits several consecutive half-times, and thus by an appropriate sampling strategy, an idea of exposure over different time periods may be obtained (e.g., concentration of chromium in the urine of manual metal arc welders on Friday afternoon reflects mainly exposure over that day, that in a Monday morning urine specimen, exposure over the preceding week) (Aitio, 1988).

7.4 Interpretation of Results

Biomarkers of exposure may be used to identify exposed individuals or groups, quantify the exposure, assess the health risks, or assist in diagnosis of (environmental or) occupational disease. For the identification of the exposed, the analytical results are compared with the reference interval for the element in the matrix studied (see Section 5). Reference intervals are derived from reference values observed in a reference population, usually as the 95th percentile, meaning that 1 of 20 nonexposed will give a result outside the reference range. Because the reference values are different in different geographical locations (mainly because of variation in exposure to the element in question in the diet), reliable identification of exposure crucially depends on reference values determined in the area of analysis

and is up-to-date. For example, reference values for blood lead have changed drastically in many countries in recent years. A biomarker level above the reference limit does not tell anything of a possible health hazard; it simply means that the individual has been exposed to a greater extent than the reference population.

Reference values are intended to identify exposed individuals; if biomarker concentrations are available for several members of a group, a more appropriate assessment of the exposure situation of the group is obtained from a statistical comparison of the exposed group and the reference population.

Quantification of exposure relies on knowledge of the relationship between the exposure, usually measured as the time-weighted average concentration of the chemical in the air, and the concentration at a specified time of the biomarker in the blood or urine or, in rare cases, other biological media.

Assessment of health risk from biomonitoring may be achieved if the relationship between the effects and the biomarker concentrations is known. This is the case for lead in blood, cadmium in blood and urine, mercury in blood and urine, and, to a more limited extent, for arsenic in urine (see Chapter 19). For these elements, epidemiological studies area available, which have studied the relationship between the biomarker level and long-term health effects. Such studies are difficult to carry out, and often the study results are not very consistent.

For some chemicals (styrene, carbon disulfide), an assessment of health risks may also be obtained indirectly from the relationship between health effects and external exposure (concentrations in the air) and that between the biomarker concentration and the concentration in the inhaled air. For metals, however, this approach so far has not been used successfully for inhalation exposures. For oral exposures, however, a good correlation has been reported between cumulative intake of cadmium through food and health effects.

The interpretation of results obtained from biomarker studies, particularly molecular or "omic"-based biomarkers, requires correlative validation with other health outcome parameters such as more standard clinical chemistry markers and ultrastructural/histopathological evaluations for preclinical disease and ultimately overt clinical manifestations of disease (Fowler, 1980). These data are essential for interpreting and defining the predictive power of a given biomarker endpoint. Usually, such studies are conducted in experimental animal model systems, where it is possible to conduct all the necessary correlative studies and then extrapolate these findings to exposed human populations. Such extrapolations are usually possible because many biomarker systems (e.g., the heme pathway) are highly conserved across species.

Factors that may complicate interpretation of biomarker endpoints include species differences in metabolism of chemical agents, genetic susceptibility factors, nutritional status, and the presence of other compensatory molecular systems such as metallothionein induction, stress proteins, and glutathione synthesis. These protective systems, which are finite, may alter the expected shape of the dose-response curve for metals/metalloids with regard to biomarker responses as long as they are able to compensate for exposure to a given toxic element.

In addition, interpretation of results of biomarker studies for metals will also be influenced, particularly at low dose levels, by concomitant exposure to combinations of toxic elements (Fowler *et al.*, 2004; 2005; Mahaffey *et al.*, 1981). Interactions between toxic elements that are frequently additive and may also produce unexpected alterations in predictive responses to a given toxic element on an individual basis. Because humans are frequently exposed to mixtures of toxic elements (e.g., Superfund sites), it is critical to take such combined exposures into account for risk assessment purposes.

Chemically induced adverse health effects or diseases usually are not different from the same disease induced by other causes, and thus the causal diagnosis or etiognosis depends on the diagnosis proper plus a history of exposure considered sufficient to cause the disease. In the assessment of the history of exposure, biomonitoring is probably the best means, because it provides direct information of the exposure of the individual.

7.5 Biomarkers of Exposure as a Complement to Industrial Hygiene Measurements

Biomonitoring of metals is most widely applied in occupational health and thus has a similar objective as industrial hygiene measurements (i.e., mostly measurement of the concentration in the air). Although it is clear that for most chemicals, industrial hygiene measurements are the best and often the only way of assessing exposure, the two approaches complement each other because their scope and performance are not identical.

Air is a homogeneous and relatively simple matrix, and therefore, sample preparation is often simple and straightforward, and analytical methods relatively easy. When the metal compound is present in the air at low concentrations, the amount of air collected on the filter may be increased, and thus the sensitivity of the analysis is improved. Because air measurements also have a long tradition, established methods exist for practically every metallic element. For several

elements, fractionation and speciation analysis methods are available (see Chapter 2), and thus important qualitative information on the exposure may be gained from such analyses.

On the other hand, concentrations of chemicals in workplace air are seldom stable but fluctuate with time and are different in different locations. The amount of a chemical that reaches the alveolar region of the respiratory tract is directly related to the volume of respiration and thus to the workload. Exposure peaks often coincide with increased workloads caused by, for example, the malfunctioning of a closed process. Several chemicals, including some metal compounds, are absorbed also via the dermal route, and absorption through the skin is generally not related to the concentration in the air. Even when the exposure takes place mainly by inhalation, the bulk of the actual absorption may be through the gastrointestinal tract (notably aerosols with particle sizes too large to lead to deposition in the alveolar region). Personal working habits vary, and individuals may absorb different amounts of chemicals in apparently similar conditions. Protection afforded by masks varies, depending on the individual who wears it and on the condition of the mask. Furthermore, biomarker, in contrast to industrial hygiene measurements, reflects the accumulation of the chemical in the body. Biomarkers of exposure, which reflect all this variation in exposure and, at the same time, exposure from all sources, are thus closer than industrial hygiene measurement to the toxicologically important concentration of the chemical at the target site (Aitio, 1999).

Biomarkers of exposure do not consider interindividual or intraindividual differences in the toxicodynamics of the chemical, which are covered in an ideal case of effect biomarkers (see Section 8). Biomarkers do not differentiate between sources of exposure and to decrease the risk from the chemical, it may be necessary to consider (and analyze) separately, from where the exposure is derived, from work or from for example hobbies, or environment.

Biomarkers of exposure reflect the amount of the chemical in the systemic circulation, and models have been developed to predict concentrations in other compartments in the body. However, a major obstacle in the interpretation of biomonitoring data involves concentration and effects at the site of entry, such as the lungs after exposure to particulates containing metals; concentrations in the urine or even blood of nickel tell little of the concentrations or the health risks in the lungs after exposure to soluble or insoluble nickel. Similarly, irritation, a mainly concentration-derived effect, is not easily assessed from exposure biomonitoring data.

8 BIOMARKERS OF EFFECTS

Biomarkers of effects are molecular tools that can serve to identify changes or effects occurring in the organism as a result of exposure to any given toxicant. In the case of exposure to toxic metals, tests relying on effect biomarkers are usually only used when excessive metal levels are found in exposed populations or their environment. The combined use of biomarkers of effects and biomarkers of exposure is also important for a correct interpretation of the data, because a diagnosis of metal intoxication relying on biomarkers can be made only if the observed effects are, indeed, associated with an excessive exposure or body burden of the metal. To be applicable to the diagnosis of intoxication or to the monitoring of populations at risk, biomarkers must meet several criteria. First, they must be sensitive enough to detect effects at a stage when these are still reversible or at least not yet predictive of further organ degradation. They must also be specific of the target organ, and if this is not the case, sufficient information must be available on the possible confounding factors. Finally, biomarkers of effects must be measurable in the least invasive way possible by use of easily accessible biological media such as urine, blood, sputum, exhaled air, or exhaled breath condensate. Because of their noninvasiveness, some biomarkers can be used repeatedly without any major restriction related to exposure conditions, age, or health status of the examined subjects. These tests are particularly useful for monitoring susceptible populations such as children. Biomarkers meeting all these criteria and applicable to detect metal toxicity are relatively few, and among them the number of biomarkers that have undergone a complete validation are even less numerous.

8.1 Renal Toxicity Biomarkers

Metals, notably cadmium, lead, and mercury, have been known for a long time to be nephrotoxic, with numerous reports of tubulointerstitial nephritis possibly leading to renal failure, in most cases linked to high occupational or environmental exposures. Early signs of renal dysfunction have also been found at low environmental exposure levels, consisting for instance of an increased urinary loss of tubular enzymes and proteins. These effects have mainly been described in adults, but certain reports have also shown them to occur in children (de Burbure *et al.*, 2003; 2006). The early recognition of the nephrotoxicity of metals has led, since the 1960s, to intense research aimed at developing sensitive screening tests and deriving thresholds of toxicity. These studies have shown that the acute or chronic nephrotoxicity of most metals can be detected

by measuring a panel of a few specific urinary proteins or enzymes reflecting the integrity of the glomerulus or of the proximal tubule. Table 1 lists the biomarkers that have been the most validated in humans and animals for assessing the nephrotoxicity of heavy metals such as cadmium, lead, and mercury. These biomarkers are now routinely used in the health surveillance of industrial workers and also for screening metal-induced kidney damage in environmentally polluted areas (e.g., in Japan). To be reliable, these tests require the use of sensitive immunological methods with a detection limit lower than 1 mg/L for albumin and alpha$_1$-microglobulin and lower than 0.01 mg/L for retinol-binding protein and beta$_2$-microglobulin. Test packs based on radioimmunoassay, enzyme immunoassay, or fluoroimmunoassay are now commercially available. When applying these tests, it is advisable to avoid first morning samples and also extreme variations in urinary flow. Samples with urinary creatinine <3 mmol/L or >30 mmol/L (0.3/3 g/L) should be used with caution, because results obtained on these samples are no longer related to the diuresis (Bernard *et al.*, 1997).

In most cases, metal-induced nephrotoxicity develops in a dose-dependent manner after a prolonged exposure. This means that renal effects are likely to occur only above a certain accumulated dose. Depending on the susceptibility of the exposed population and the sensitivity of the renal biomarker, the threshold of urinary cadmium associated with the development of tubular proteinuria (e.g., increased urinary excretion of low molecular weight proteins) ranges from approximately 1–10 μg/g creatinine (Åkesson *et al.*, 2005; Bernard, 2004; Fowler and Norberg, 1996; Järup *et al.*, 2000; Uno *et al.*, 2005). Above these thresholds, the risk of tubular proteinuria increases almost linearly with the accumulated dose of the metal as reflected by the urinary cadmium level. In cadmium exposure, clearly increased excretion of low-molecular-weight proteins is predictive of clinical renal disease (Mueller *et al.*, 1998; Mutti, 2001). Renal effects of inorganic mercury resulting in an increased excretion of tubular enzymes or albumin are also likely to occur only from a threshold of urinary mercury that has been estimated to vary between 30 and 50 μg/g creatinine in occupationally exposed subjects.

8.2 Neurotoxicity Biomarkers

Neurotoxic effects of heavy metals are also well documented, especially for mercury or lead, with numerous reports of neurobehavioral changes after occupational exposure and of developmental effects in children with prenatal or early postnatal exposure. Much effort has been devoted during the past years to identify potential biomarkers of neurotoxicity. Serum prolactin and urinary homovanillic acid have been the most studied in experimental animals and in humans. These two markers that reflect the integrity of the dopaminergic system have been proposed for monitoring exposure to some metals such as lead, mercury, manganese, and aluminum. However, their sensitivity seems limited, because significant changes in these biomarkers have only been described at relatively high exposure levels such as those found in industry. At environmental exposure levels, these biomarkers show no or only weak associations with heavy metal exposure even in particularly sensitive populations such as children (de Burbure *et al.*, 2006).

8.3 Lung Toxicity Biomarkers

Current research in the field of biomarkers is opening new perspectives in the assessment of lung toxicity with the development of noninvasive molecular tools measuring the extent of lung inflammation or damage.

TABLE 1 Urinary Markers of Metal Nephrotoxicity

	Normal value	Significance	Precautions/confounders
Albumin (microalbuminuria)	<20 mg/g Cr	Glomerular damage	Very stable in urine Increases in orthostatic proteinuria, heavy exercise, pregnancy
β_2-Microglobulin (β_2-m)	<300 μg/g Cr	Proximal tubule damage/dysfunction	Unstable at urinary pH <5.6 Increases in some autoimmune or infectious diseases Increases in renal insufficiency (overflow proteinuria)
α_1-Microglobulin (protein HC)	<10 mg/g Cr	Proximal tubule damage/dysfunction	Very stable in urine Increases in renal insufficiency (overflow proteinuria)
Retinol-binding protein (RBP)	<300 μg/g Cr	Proximal tubule damage/dysfunction	Very stable in urine Increases in renal insufficiency (overflow proteinuria)
N-Acetyl-β_2-*D*-glucosaminidase	<5 UI/g Cr	Proximal tubule damage/dysfunction	Very stable in urine but sensitive to repeated freezing/thawing cycles Possibility to measure isoenzymes

Most promising current biomarkers of lung toxicity are those measurable in serum, sputum, exhaled air, and exhaled breath condensate. In exhaled air, one of the most useful biomarkers seems to be nitric oxide (NO), a marker of acute or chronic lung inflammation. Although few studies have applied the exhaled NO test to subjects exposed to heavy metals (Lund *et al.*, 2000), it is very likely that these tests could contribute to a better assessment or understanding of the effects of metals on the lung. Exhaled breath condensate (EBC), a fluid obtained by cooling exhaled air, also seems to be a promising tool for the assessment of the accumulated dose or the effects of inhaled chemicals, including toxic metals. EBC contains so little protein that it is unlikely that this material could serve for the dosage of lung-derived proteins. By contrast, it is possible to measure a variety of small molecules, including metals that could effectively reflect lung inflammation or oxidative stress and the metal doses retained or stored in the lung. Such a biomarker is, for instance, malondialdehyde, which has recently been found to be increased in workers exposed to a mixture of both transition and hard metals (Goldoni *et al.*, 2004). The assay of lung-specific proteins in serum ("pneumoproteins") represents another interesting approach in the detection of the effects of air pollutants, including their effect on the lung–epithelium barrier (Hermans and Bernard, 1999). One of the most studied lung-specific measurable proteins, called the Clara cell protein, is an indicator of the number of Clara cells lining the terminal airways. Surfactant-associated proteins A and B can also be used to assess the permeability of the alveolar–capillary barrier. However, tobacco smoking is a major confounder of these biomarkers, and experience with other lung toxicants suggests these biomarkers are mainly useful research tools for the refinement of risk assessment and the derivation of toxicity thresholds. These lung-specific proteins can also be measured in normal circumstances in serum at very low concentrations, which can only be determined with very sensitive immunoassays.

8.4 Biomarkers for Other Target Organs

Biomarkers of toxicity or disease exist for a variety of potential target organs of metals such as bone (urinary cross-linked N-telopeptide and C-telopeptide of type II collagen, bone-specific alkaline phosphatase, procollagen type 1-C peptide, osteocalcin, urinary calcium) or the male reproductive system (sperm count, morphology, and motility). Biomarkers of sperm quality seem quite sensitive to some metals (Telisman *et al.*, 2000), but difficulties in collecting the samples limit their application in practice. As to biomarkers of metal effects on the bone, very few studies, except those concerning cadmium effects on bone, have looked to the use of biomarkers for the assessment of the impact of metals on this target (Aoshima *et al.*, 2003). Effects of cadmium on the prostate have also been recently investigated by use of biomarkers (Zeng *et al.*, 2004).

8.5 Genotoxicity Biomarkers

Most potent carcinogens are genotoxic and can produce gene mutations. The search for alterations with appropriate biomarkers can, therefore, be used to assess the potential risk of cancer associated with exposure to organic chemicals but also some metals (Albertini *et al.*, 2000). Most biomarkers of genotoxicity require the collection of nucleated cells and are usually applied to blood lymphocytes. There are three types of genotoxicity assays that are most commonly used in the context of exposure to metals: the measurement of sister chromatid exchange (SCE) and in particular of cells with a high frequency of SCEs (HFCs); the measurement of micronuclei, which are fragments of DNA; and the analysis of chromosomal aberrations. However, as of now, these analyses are mainly used for research purposes. The available validation studies suggest that chromosomal aberrations are more closely related to individual susceptibility than to exposure to genotoxic chemicals; whereas sister chromatid exchanges are not related to cancer risk (Bonassi *et al.*, 2000; 2004; Hagmar *et al.*, 1998, 2004). DNA or protein adducts, or mutagenicity in urine, have not been successfully applied in the biomonitoring of metals.

9 FUTURE TRENDS

There is an ever-increasing capacity for speed, sensitivity, and understanding the importance of chemical species for both analytical techniques for metals/metalloids and biotechnology innovations for applying molecular or "omic" technologies for biomarkers. In particular, the incorporation of automated or robotic analytical systems has greatly improved the speed with which analyses for metals may be conducted. Linkages between complementary analytical systems (e.g., GC mass spectrometry or HPLC mass spectrometry) have also increased the ability of the analyst to rapidly conduct chemical speciation studies that provide valuable information on the chemical species that are present.

The advent of the "omic" molecular technologies (genomic, proteomic and metabolomic/metabonomic) has also greatly increased the speed and precision of biomarker endpoint measurements. These technologies are also being automated and developed with

computerized interfaces. As noted elsewhere (Fowler, 2005a), there are a number of challenges facing the validation of "omic" technologies, but there is also great promise if the nature of these early molecular responses can be understood in relation to other toxicological endpoints. A recently published symposium on "Molecular Biomarkers" (Fowler, 2005b) provides a useful review of studies from a number of countries with a particular emphasis on toxic metal/metalloid exposures.

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CHAPTER

5

Selected Molecular Mechanisms of Metal Toxicity and Carcinogenicity

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ABSTRACT

This chapter will summarize some of the molecular responses exhibited by cells that come into contact with toxic metals. We will consider the transport of toxic metals into cells and how this interferes with the transport of essential metals. Toxic metals also interfere with the intracellular action of essential metals and may cause toxicity and cancer by this mechanism. Some metals have very specialized effects on enzymes, and there are proteins that can bind toxic metals such as metallothionein, which will also be discussed. Some metals are also slightly mutagenic and genotoxic, and these effects will be discussed. Because most metals that are carcinogenic, such as nickel (Ni), arsenic (As), beryllium (Be), and cadmium (Cd), with the exception of chromium (Cr), do not interact with DNA and are not mutagenic, we discuss other mechanisms such as epigenetic effects to account for their carcinogenic activity, as well as how they affect the expression of genes. Metals also interfere with cell signaling, and, in particular, we will discuss the hypoxic signaling pathway as well as those involving PI3K, AKT, ROS, MAPK, NFκB, NFAT, and AP1. Finally, the basis of the interaction of toxic metals with all cellular constituents involves their coordination with biological ligands, which will be addressed throughout the chapter.

1 TRANSPORT OF TOXIC METALS BY MOLECULAR/IONIC MIMICRY OF ESSENTIAL COMPOUNDS

1.1 Introduction

Metals are found throughout the environment, and although certain metals are highly toxic, others are essential to living systems. Essential metals are needed in the diet to maintain proper human health, because they cannot be synthesized by the body and are required for enzyme reactions or other physiological processes. The cellular uptake and export of these essential metals is tightly regulated by various homeostatic transport mechanisms, because an excess of, or lack of, an essential metal, can also be toxic. Some of these processes are very specific, whereas others are more promiscuous and are able to transport a multitude of metals that are potentially toxic. Transport proteins may recognize nonessential metals because they resemble essential metals or other compounds, effectively acting as molecular mimics. Unregulated transport of nonessential metals or deregulation of essential metal transport is a major factor in determining the potential toxicity or carcinogenicity of metallic compounds. In fact, various diseases are caused by alterations in metal transport and metabolism. The transport of a number of metals is discussed here, but more detailed reviews are available (Ballatori, 2002; Clarkson, 1993; Goyer, 1997).

1.2 Iron

Iron (Fe) is an essential metal whose regulation is controlled by both uptake and export proteins. Both iron deficiency and excess iron can be potentially toxic to cells, so its transport is stringently controlled. One of the major proteins involved in the cellular uptake of iron is transferrin. Transferrin is capable of binding two molecules of ferric iron. In addition to binding ferric iron, transferrin is also able to bind nickel, vanadium, and other metals (Boffi *et al.*, 2003; Harris, 1986; Sun *et al.*, 1999). One may speculate that other metals may interfere with iron transport by occupying sites on transferrin molecules. Diferric transferrin gets internalized into an acidic endosome via transferrin receptor–mediated endocytosis. Iron is subsequently released from the transferrin receptor, reduced, and transported out of the endosome into the cell via the divalent metal transporter-1 (DMT1/DCT1/NRAMP2). DMT1 is also involved in the transferrin receptor's independent transport of iron. Interestingly, DMT1 is able to transport a multitude of other metals including nickel (Ni), manganese (Mn), cobalt (Co), copper (Cu), and zinc (Zn) (Chen *et al.*, 2005; Garrick *et al.*, 2003). The transport of metals into the cell by DMT1 is one example of how nonspecific transport of toxic metals like Ni may interfere with the transport of essential metals. Indeed, it has been shown that Ni and other toxic metals can compete with iron for entry into the cell at DMT1 (Chen *et al.*, 2005; Garrick *et al.*, 2003).

1.3 Zinc

Zinc is an essential component of many metalloenzymes and transcription factors that are involved in various cellular processes such as gene expression, signal transduction, transcription, and replication (Berg and Shi, 1996). The movement of zinc across cellular membranes in humans involves various zinc transporters, including the ZnT family of proteins and the Zip proteins, both members of the transporter super family known as the ZIP (ZRT1, IRT1-like protein) family. The ZnT family of transporters is involved in the sequestration and release of zinc, whereas DMT1 and Zip proteins are believed to be responsible for importing Zn into the cells (Harris, 2002). Similarly to DMT1, the ZIP proteins are not specific for Zn transport and can also likely transport other transition metals into the cell. It has been shown that Mn, Cd, and Cu can compete with Zn for uptake by hZIP2, a human zinc transporter, suggesting that these metals are also substrates for the transporter (Gaither and Eide, 2000).

1.4 Phosphate and Sulfate Mimics

Phosphate and sulfate are carried across the cell membrane by various transport proteins (Clarkson, 1993). Some metal oxyanions resemble these compounds and compete for transport with phosphate and sulfate through their respective carriers. For example, selenate, molybdate, and chromate resemble sulfate and can be transported by sulfate transporters. The transport of sulfate has been shown to be inhibited by these mimics (Cardin and Mason, 1975; Mason and Cardin, 1977; Shennan, 1988). Likewise, vanadate and arsenate are structurally similar to phosphate, and, as a result, they are substrates for phophate carriers. Arsenate has been shown to compete with phosphate transport in a number of cell lines and tissue systems (Clarkson, 1993). Vanadate has also been shown to inhibit the uptake of phosphate at the NaPi-3 cotransporter (Timmer and Gunn, 1998). In addition, phosphate can be mimicked in nontransport-related reactions like the synthesis of ATP (arsenate) or inhibition of ATPase (vanadate) (DeMaster and Mitchell, 1973; Karlish *et al.*, 1979).

1.5 Organic Complexes

Toxic metals are often transported into cells complexed to organic solutes. Methionine is an amino acid that can be transported into the cell via the L-type neural amino acid carrier (Ballatori, 2002). Unfortunately, the toxic metal methylmercury can bind to the amino acid cysteine, forming a complex that resembles the amino acid methionine, which can then be transported into the cell by this amino acid carrier (Aschner and Clarkson, 1988; Kerper *et al.*, 1992). In addition, glutathione carriers can transport methylmercury complexed with glutathione (Ballatori and Clarkson, 1984a; 1984b; Dutczak and Ballatori, 1994). Other metals (Cu, Zn, Cd, Cr, and Pb) have been reported to form complexes that mimic glutathione and are transported by glutathione transporters (Aaseth *et al.*, 1982; Alexander *et al.*, 1981; Ballatori and Clarkson, 1985; Cherian and Vostal, 1977; Gyurasics *et al.*, 1991; Norseth *et al.*, 1982). In addition, uptake of zinc–histidine and copper–histidine complexes by amino acid transporters has been reported (Aiken *et al.*, 1992; Horn *et al.*, 1995; Horn and Thomas, 1996; Oakley *et al.*, 2004). Arsenite can be transported by glucose transporters and by the water transporter, aquaglyceroporin (Liu *et al.*, 2002). The ability to directly bear a resemblance to organic compounds or form complexes that mimic endogenous compounds is an important mechanism by which toxic metals can be transported by cells.

1.6 Metal–Anion Complexes

Metal–anion complexes are also capable of mimicking endogenous anions. For example, it has been suggested that various metals may be transported on the HCO_3^-/Cl^- anion exchange carrier as a carbonate complex by mimicking the monovalent anions that are usually transported. Zinc has been shown to be taken up by the anion exchanger as the anionic complex ($[Zn(HCO_3)_2\ Cl]^-$) (Torrubia and Garay, 1989). Cadmium and lead have also been shown to be transported into red blood cells through the anion exchanger (Lou *et al.*, 1991; Simons, 1986b; 1986c).

1.7 Calcium Channels

Voltage-dependent calcium channels are other sites where toxic metals may gain access to the cell. It is well known that lead can mimic calcium in many cellular processes. Indeed, it has been shown that lead is able to enter voltage-activated L-type calcium channels in place of calcium. Further research has shown that these channels will actually be blocked by and may transport a wide variety of divalent and trivalent metal cations, including cadmium, nickel, and other toxic metals (Schafer, 2000).

1.8 Summary

Various mechanisms exist for the transport of toxic metals into the cell. Most of them involve molecular mimicry, in which the toxic metal either directly or as a complex resembles an endogenous compound. The transport of toxic metals is then carried out, because the transporters for the endogenous compounds have low substrate selectivity, allowing them to transport toxic metals into the cell.

2 INTERFERENCE WITH THE FUNCTIONS OF ESSENTIAL METALS BY TOXIC METALS

2.1 Introduction

Essential metals have numerous functions inside the cell. Perhaps one of the most important, although not the only, role is to act as a cofactor/component in enzymes and various molecules. There are hundreds of enzymes that contain essential metals, and it would be nearly impossible to discuss all of them and their possible interactions with toxic metals, but a few representative examples are discussed. It is important to realize that even though many enzymes contain the metal iron, for example, not all of the enzymes have iron bound in the same manner or even the same form. The manner by which the metal is bound and the form of the essential metal in a given enzyme, likely play a major role in its susceptibility to interference by toxic metals.

2.2 Calcium

Proper calcium metabolism is crucial at both an intracellular and an extracellular level. Toxic metals are able to interfere at both levels by an assortment of mechanisms. The toxic metal cadmium interferes with calcium metabolism in both the kidney and in bones. Hypercalciuria is observed in the kidneys of people exposed to cadmium, indicating an interference with calcium regulation (Buchet *et al.*, 1990; Lauwerys *et al.*, 1990; Scott *et al.*, 1978). This may be one of the causes of Itai-Itai disease. In addition, cadmium can be deposited in the bone and may interfere with calcification, decalcification, and bone remodeling (Berglund *et al.*, 2000; Tsuritani and Honda, 2004). Aluminum has also been shown to replace calcium in the soft tissue part of the bone and affect bone structure, although this may be related to cellular toxicity (Severson *et al.*, 1992). Lead, another toxic metal, also interferes with calcium metabolism in a variety of ways. Evidence has shown that a diet low in calcium can result in higher levels of lead in tissues, demonstrating an important metabolic link between the two (Barton *et al.*, 1978; Fullmer, 1992; Mahaffey *et al.*, 1973). Lead also affects calcium at the cellular and molecular levels. In the nervous system, lead is able to disturb proper neurotransmitter kinetics, block the uptake of calcium by nerve terminals, and compete with calcium for uptake by calcium channels (Barton *et al.*, 1978; Fullmer, 1992; Mahaffey *et al.*, 1973; Minnema *et al.*, 1988; Simons, 1986a; Simons and Pocock, 1987). In addition, lead can replace calcium in calcium/sodium ATP pumps, compete with calcium for binding sites on calcium-binding proteins, compete for uptake into mitochondria, and bind to or interfere with secondary messenger calcium receptors such as calmodulin or protein kinase C (Fullmer, 1992; Goldstein, 1977, 1990; Habermann *et al.*, 1983; Simons, 1986a). It has also been reported that nickel, another potentially toxic metal, results in an elevation of free intracellular calcium (Salnikow *et al.*, 1999).

2.3 Zinc

Cadmium, lead, and As have all been shown to affect the proper regulation of zinc, an essential metal found in more than 300 enzymes and numerous transcription factors. Cadmium can occupy the same sites as zinc on metallothionein (MT), and whereas MT-bound cadmium is nontoxic in the liver and kidney, it is toxic to the renal tubules when it is excreted in the

urine (Chan and Cherian, 1993; Nordberg *et al.*, 1971a; 1971b). Lead has wide-ranging effects on processes that use zinc. Lead is known to increase zinc excretion in rats (Victery *et al.*, 1987). The activity of zinc-containing heme enzymes like δ-aminolevulinic acid dehydratase can be decreased by lead, suggesting that lead may substitute for zinc in some zinc-containing heme enzymes (Meredith *et al.*, 1977; Tomokuni *et al.*, 1991). In addition, lead, arsenite, and selenium may disrupt zinc finger proteins. By disturbing zinc finger proteins (approximately 3% of identified genes in the human genome code for proteins with zinc finger domains), these metals may have widespread effects on transcription factors, DNA damage signaling, and DNA repair proteins (Hartwig *et al.*, 2003; Maret, 2004).

2.4 Magnesium

Magnesium can be found attached to phosphate on the backbone of DNA. One model suggests that nickel can replace magnesium, leading to increased chromatin condensation and subsequent DNA methylation (Lee *et al.*, 1995). This model provides an interesting link between the interaction of toxic metals with essential metals and epigenetics.

2.5 Iron

Iron (Fe) is an important essential metal used in many enzymes and cellular redox reactions. Low cellular iron levels can be detrimental to a healthy cell, whereas excess free iron can lead to the production of reactive oxygen species (ROS) via the Fenton reaction. Cellular iron levels are tightly regulated by homeostatic mechanisms to maintain the appropriate amount of iron in the cell. Disruption of iron homeostasis by toxic metals is a newly emerging area, with many aspects currently under investigation. We previously mentioned that nickel and other divalent metals could compete with iron for entry into the cell at DMT1. One reason why nickel may be able to compete for iron-binding sites is because they have similar ionic radii. Metals can interfere with many other iron-dependent processes as well. Iron homeostasis is maintained by the iron regulatory protein-1 (IRP1)/iron response element (IRE) system. When iron levels are low, the 4Fe–4S cluster protein aconitase loses its cluster and is converted into IRP1. IRP1 can alter the regulation of mRNAs that have IREs in their sequence. These IREs can be found in the 3′ or 5′ untranslated region of mRNAs that encodes for proteins involved in iron uptake, storage, and use (For a complete review of eukaryotic iron regulation systems, see Aisen *et al.* (2001). Nickel has been shown to increase the binding of IRP1 to IRE by lowering cellular iron levels (Chen *et al.*, 2005). Aluminum has been reported to increase the stability of IRP2, another iron regulatory protein (Ward *et al.*, 2001).

Toxic metals also affect iron-dependent enzymes. The 4Fe–4S enzyme aconitase loses its enzymatic activity when cells are exposed to nickel or cobalt. It has also been shown that toxic metals can affect various iron-dependent enzymes involved in the hypoxic response. The hypoxia inducible factor (HIF)-1α is controlled by two enzyme groups that are members of the iron and 2-oxoglutarate–dependent dioxygenase family of enzymes, the HIF-prolyl hydroxylases and an asparagine hydroxylase. It has been reported that the activity of both of these enzymes can be inhibited by toxic transition metals such as cobalt and nickel (Ivan *et al.*, 2001; Hewitson *et al.*, 2002; Hewitson *et al.*, 2003; Jaakkola *et al.*, 2001; Schofield and Ratcliffe, 2004; Ward *et al.*, 2001).

2.6 Copper

Copper (Cu) is an essential metal for living systems and is found in an assortment of enzymes, including superoxide dismutase (SOD), ferroxidases, and cytochrome oxidase. Its transport is highly regulated, and various metals, including zinc, cadmium, and molybdenum, have been reported to interfere with its transport or availability in biological systems. It has also been shown in rodents that lead can alter copper metabolism, leading to decreases in plasma copper levels (Klauder and Petering, 1979; Skoczynska *et al.*, 1994).

3 TOXIC METAL–BINDING MOLECULES

3.1 Introduction

Because metals are generally indestructible, the accessibility of metals in biological solutions plays a crucial role in determining their potential toxicity. Metals can bind to many types of macromolecules in the cell, although this discussion will be limited to molecules involved in the transport and/or storage of toxic metals. Frequently, the binding of metals to proteins is a defense to reduce toxicity by preventing availability of the metals, although there are instances when a metal bound to a protein can be more toxic than the metal alone. The ability of low-molecular-weight thiols and protein sulfhydryl groups to bind metals plays a major part in determining which proteins/peptides will bind and transport toxic metals. We have limited consideration to two important metal-binding molecules, MT and glutathione.

3.2 Metallothioneins (MTs)

MTs, which were originally isolated from equine kidney, are low-molecular-weight, cysteine-rich, intracellular proteins that have the capability to bind toxic metals (Margoshes and Valee, 1957). MTs are rich in cysteine, and therefore, thiol groups, which gives them avid metal-binding characteristics (Waalkes and Perez-Olle, 2000). They can bind essential metals such as Cu and Zn, as well as toxic metals such as Cd, Pb, and Hg (Nordberg and Nordberg, 2000). MT can integrate as many as 7 divalent metal atoms (e.g., Zn^{2+}) or 12 monovalent atoms (e.g., Cu^{+}) (Binz and Kagi, 1999). The metal content of MTs can vary, depending on the stability constants of different metals, and does not have to contain only one type of metal. Thus, MTs can exist as mixed metal proteins, carrying multiple metal species. It has been reported that up to 18 different metals can associate with MTs, although the stability constant of the different metals varies (Kagi and Kojima, 1987; Nath *et al.*, 1988). For example, the toxic metals Cd, Pb, and Hg can displace the essential metal Zn. In addition to binding toxic metals, MTs can also donate metal ions to ligands that have higher affinity constants. It is believed that MTs play an integral role in the detoxification of toxic metals, although they also play a major role in essential metal metabolism.

With respect to toxic metal detoxification, MTs are regulated in an interesting manner. *In vivo*, MTs are mainly bound to Zn. Based on the affinity constants for MT, a number of toxic metals, including Hg^{2+}, Ag^{+}, Cu^{+}, Cd^{2+}, Pb^{2+}, and Bi^{2+}, would be able to displace Zn^{2+} from MT (Kagi and Kojima, 1987; Nath *et al.*, 1988). The free zinc is able to bind the metal transcription factor (MTF-1) and induce the synthesis of MT by binding to metal response elements in the promoter region. The newly synthesized MT molecules would be available to bind free toxic metal ions. In addition to Zn^{2+}, other metals such as Cd^{2+}, Hg^{2+}, Cu^{+}, Bi^{2+}, Pb^{2+}, Ni^{2+}, and Mn^{2+} have been shown to induce MT synthesis by diverse mechanisms, indicating the general nature of MTs in responding to toxic metal stress with a detoxification response (Waalkes and Goering, 1990; Waalkes and Klaassen, 1985).

One of the most extensively studied properties of MTs relates to the hypothesis that they protect the cell against cadmium toxicity (Nordberg *et al.*, 1994). Studies in which the primary isoforms of MT (MT-1 and MT-II) are knocked out in mice have shown that these mice were more susceptible to cadmium toxicity than control mice (Masters *et al.*, 1994; Michalska and Choo, 1993; Zheng *et al.*, 1996). In addition, when MT-1 was overexpressed in transgenic mice, cadmium was less lethal and produced less nephrotoxicity at levels of exposure to cadmium that injured control mice expressing normal amounts of MT-1 (Liu *et al.*, 1995). Other studies have cautioned that MTs are not the sole factor in determining Cd toxicity, because only a subset of the adverse effects of Cd can be prevented by MT. Testicular necrosis in mice exposed to Cd could not be blocked by the overexpression of MT-1 (Dalton *et al.*, 1996), but this may be because Cd was injected, and there was not enough time to induce MT as there would be if exposure occurred by ingestion. In addition, progesterone-induced synthesis of MT actually increased cadmium cytotoxicity in rat liver cells (Shimada *et al.*, 1997). MT can also increase the residence times of toxic metals in the body, which may lead to chronic effects when protective mechanisms are overwhelmed (Satoh *et al.*, 1997).

Protection against metal toxicity was probably not the intended purpose for MTs, because many of the toxic metals that they bind have only in recent times been concentrated in the biosphere through anthropogenic releases. The more likely explanation is that the ability of MTs to detoxify toxic metals is an accidental occurrence related to the ability of toxic metals to mimic physiological metals such as zinc that MTs are known to bind and perhaps regulate its homeostasis in cells (Richards and Cousins, 1975; 1976). MT affects Zn absorption and excretion by the intestine, as well as the supply of Zn to cellular transport proteins like ZnT-1 and DMT1, and the supply of Zn to Zn-containing enzymes and zinc finger proteins (Gunshin *et al.*, 1997; Palmiter and Findley, 1995; Palmiter *et al.*, 1996a; 1996b; Richards and Cousins, 1975; 1976). Although it seems MT can play a role in sequestering various metals, the precise physiological role is still unknown and the subject of current investigations.

3.3 Glutathione

Glutathione (GSH) is responsible for carrying out a variety of physiological and metabolic functions, including, but not limited to, the detoxification of electrophilic compounds, free radicals, and metals. It is the most abundant nonprotein thiol in many species and contains six possible metal binding sites (Wang and Ballatori, 1998). As summarized by Ballatori, GSH can affect the transport, disposition, and overt toxicity of metals in four ways: (1) it functions in the mobilization and delivery of metals between ligands; (2) it functions to transport metals across cellular membranes; (3) it serves as a source of cysteine, and (4) it serves as a cofactor for redox reactions (Ballatori, 1994). Although GSH is able to bind a large number of endogenous compounds, here we will be concerned only with its ability

to bind toxic metals. GSH is able to bind, transport, and store several metals affecting metal homeostasis in biological systems. In addition, glutathione-bound metals are more easily eliminated from the cell and the entire organism (Wang and Ballatori, 1998). Metals that can reportedly bind to glutathione are copper, selenium, zinc, chromium, mercury, cadmium, arsenic, silver, and lead (Aaseth *et al.*, 1982; Alexander *et al.*, 1981; Ballatori, 1994; Ballatori and Clarkson, 1985; Cherian and Vostal, 1977; Gyurasics *et al.*, 1991; Norseth *et al.*, 1982; Wang and Ballatori, 1998). The metal–sulfhydryl complexes are kinetically labile, and GSH-bound metals can exchange with other ligands leading to a rapid redistribution of metals in biological systems.

When GSH binds to a metal, two main outcomes are possible. The metal can be stabilized in a nonreactive form, or metal compounds with different speciation can be formed by redox reactions. Under the right conditions (when thiol groups dissociate to their thiolate anion), the latter can actually result in the formation of reactive oxygen species (ROS), although this event is usually quenched by other amino acid groups on GSH (Pompella *et al.*, 2003). More often, GSH can bind to metals and prevent the toxic effects of those metals. It has been reported that 0.5 m*M* GSH could prevent the inhibition of ethoxyresorufin O-deethylase activity by Hg^{2+}, Cu^{2+}, and Zn^{2+} but not Cr(VI) or Fe^{2+} (Oliveira *et al.*, 2004). The toxic effects of copper have been inversely associated with GSH concentration (Freedman *et al.*, 1989). The addition of GSH has been shown to protect against Hg^{2+}-mediated toxicity in *in vitro* preparations of renal tissue, isolated proximal tubule fragments from rabbits, and proximal tubular cells from rats (Burton *et al.*, 1995; Lash and Zalups, 1996; Zalups and Barfuss, 1993). Other beneficial effects of GSH–metal complexes are that they can serve as sources of metals during the synthesis of metalloproteins. GSH has been reported to deliver copper to apometallothionein, aposuperoxide dismutase, and apohemocyanin (Wang and Ballatori, 1998). Not all metal binding to GSH is beneficial. GSH can affect the transport of metals across cellular membranes (see Section 2). Metals like Hg^{2+} bound to GSH can accumulate in target organs, leading to increased toxicity. It has been shown that excess GSH can increase the toxicity of Hg^{2+} (Zalups and Barfuss, 1993). Thus, the precise role of GSH in metal toxicity remains unclear, and there may be species-, organ-, metal- and cell-specific effects.

Overall, GSH is an important mediator of metal toxicity because of the relatively high affinity of metals for thiol groups. It can affect metal transport, deposition, and overall exchangeability. Although GSH can chelate metals and reduce their overall availability, thus decreasing toxicity, it can also facilitate their transport across biological membranes and intensify toxicity in target organs.

3.4 Summary

MT and GSH are not the only metal-binding proteins in biological systems. In fact, many more exist and play a role in overall metal metabolism. Other thiol-containing proteins, such as bovine serum albumin (BSA), are important in binding toxic metals. In addition, proteins like transferrin and ferritin ensure that the redox-active metal Fe does not create oxidative stress. In plants, phytochelatins are important metal-binding proteins (Cobbett, 2000). The ability to sequester potentially toxic metals in an unreactive form is an essential trait in all organisms.

4 MUTAGENIC AND GENOTOXIC EFFECTS OF METALS

4.1 Introduction

Most of the toxic metals have very poor direct interactions with DNA and in general are weakly mutagenic. Even chromate, which can yield trivalent Cr DNA adducts is not highly mutagenic in many systems (Landolph, 1989; Patierno and Landolph, 1989). However, it is of interest that carcinogenic metals can interact with other environmental carcinogens. For example, metals can inhibit the repair of benzo[a]pyrene (BaP) or UV-induced DNA adducts and potentiate the mutagenicity of BaP or UV (Hartwig, 1998; Hu *et al.*, 2004a; 2004b). It is also of interest to note that there are poor animal models for metal-induced tumors, and many of the cancers that arise in humans occupationally exposed to carcinogenic metals, such as Ni compounds and chromate, are associated with exposure to other environmental carcinogens such as cigarette smoking (Gibb *et al.*, 2000; Grimsrud *et al.*, 2002; 2003). Recent success in inducing cancers with arsenite and chromate in mice was associated with the use of hairless mice exposed to both UV and these carcinogenic metals through their drinking water (Davidson *et al.*, 2004; Rossman *et al.*, 2001; 2004). Thus, in general, carcinogenic metals have strong interactions with carcinogens, such as PAH, UV, and the many carcinogens that are present in cigarette smoke. Some carcinogenic metals such as Ni compounds are able to induce cancers in animals exposed to these metals alone, although Ni also has a strong interaction with other organic carcinogens such as BaP and UV (Schwerdtle *et al.*, 2002; Waalkes *et al.*, 2004; Wozniak and Blasiak, 2004).

4.2 Mutagenicity and Genotoxicity of Nickel Compounds

Ni compounds have been deemed human carcinogens by the International Agency for Research on Cancer (IARC) (International Agency for Research on Cancer, 1990). Interestingly, these compounds are not very mutagenic in most of the tested assay systems (Coogan *et al.*, 1989; Costa, 1991b; Costa *et al.*, 2002). Although, Ni compounds are not mutagenic in cultured murine or human fibroblasts, they do induce strong cell transformation, particularly in murine systems (Biedermann and Landolph, 1987; Miura *et al.*, 1989). Other studies have shown that Ni compounds induce oxidative stress, genomic instability, and chromosome damage (Biggart and Costa, 1986; Conway and Costa, 1989; Costa *et al.*, 2002; Kargacin *et al.*, 1993; Kasprzak *et al.*, 2003; Lin *et al.*, 1991; Sen and Costa, 1985). Ni compounds have also been shown to cause cell transformation accompanied by global deregulation of gene expression (Landolph *et al.*, 2002; Verma *et al.*, 2004). Perhaps one of the most important effects of Ni in terms of affecting DNA is its ability to silence genes through DNA methylation and other epigenetic effects (Klein and Costa, 1997; Klein *et al.*, 1991). The epigenetic effects of nickel compounds are more potent with the water-insoluble forms of Ni compounds such as Ni_3S_2, but this effect is also evident when cells are exposed to soluble Ni compounds for long periods of time (Costa, 1995; Lee *et al.*, 1995; Oller *et al.*, 1997). Ni compounds can also induce oxidative stress *in vivo* (Coogan *et al.*, 1989; Doreswamy *et al.*, 2004; Kasprzak, 1995).

4.3 Mutagenicity and Genotoxicity of Chromium Compounds

IARC (1990) concluded that hexavalent Cr compounds are likely human carcinogens based on the available data. Water-soluble chromate compounds were tested for cell transformation, mutagenicity, and chromosomal damage and were found to be very active (Bianchi *et al.*, 1983; Biederman and Landolph, 1987; 1990; Patierno *et al.*, 1988). Analysis of the nature of mutations in a 104-base pair segment of the HPGRT gene exon 3 showed a selectivity in substitutions for GC base pairs (Chen and Thilly, 1994). Treatment of a shuttle plasmid *in vitro* with a Cr (VI) reduction system with glutathione or cells containing the shuttle plasmid yielded mutations predominantly at GC sites (Liu *et al.*, 1999). Cr is known to have a preference for binding to the phosphate backbone of DNA and with positively charged N7 of guanine (Voitkun *et al.*, 1998; Zhitkovich, 2005; Zhitkovich *et al.*, 1995). Chromate is also synergistic with other organic carcinogens, such as those present in cigarette smoke. One possible mechanism for these effects involves the inhibition of the repair of BaP DNA adducts (Feng *et al.*, 2003; Hu *et al.*, 2004a). Chromate exposure has been shown to induce oxidative stress in cells, and these effects are greater with some insoluble Cr compounds (Leonard *et al.*, 2004b; Martin *et al.*, 1998; Shi and Dalal, 1994). Oxidative stress, the reduction of Cr (VI) to Cr (III), and the binding of Cr (III) to DNA are thought to be involved in its mutagenesis and genotoxicity. The binding of Cr (III) to the DNA can yield a wide variety of DNA lesions, including ternary complexes of Cr (III) with its reducer (ascorbic acid or GSH), as well as DNA–DNA crosslinks, DNA–protein crosslinks, single-strand breaks, and formation of alkaline labile sites (Cantoni and Costa, 1984; Coogan *et al.*, 1991; Costa, 1990; 1991a; Miller and Costa, 1989a; 1989b; Miller *et al.*, 1991; Sugiyama *et al.*, 1986; 1987; Zhitkovich, 2005; Zhitkovich *et al.*, 1995).

4.4 Mutagenicity and Genotoxicity of Arsenic

IARC has determined that As and As compounds are carcinogenic to humans. In general, As compounds show poor genotoxicity and mutagenicity. Some studies have found evidence of DNA damage by arsenite such as micronuclei formation (Liu and Huang, 1997; Schaumloffel and Gebel, 1998) and DNA protein crosslinks (Gebel, 1998); however, others did not find DNA protein crosslink formation by arsenite (Costa *et al.*, 1997). As has not been found to be mutagenic in bacteria and cultured mammalian cells (Rossman *et al.*, 1980). However, a very consistent effect of As compounds is being a comutagen, possibly by inhibiting the repair of DNA lesions produced by genotoxic agents such as UV (Rossman, 1981a; 1981b; Rossman *et al.*, 1977; 1986). Additional studies that support the comutagenic effects of As are those studies showing that it is also a cocarcinogen (Rossman *et al.*, 2001; 2004). In addition, As has been shown to inhibit the ligation step of DNA repair and, thus, can interact with many genotoxic insults (Hu *et al.*, 1998).

4.5 Mutagenicity and Genotoxicity of Cd

IARC has determined that there is sufficient scientific evidence to classify cadmium and cadmium compounds carcinogenic in humans. In general, Cd compounds are weakly mutagenic in most assay systems (Filipic and Hei, 2004), but have been shown to be genotoxic to Leydig cells of the testes (Yang *et al.*, 2003). The mechanism by which Cd may be genotoxic is by indirectly inducing oxidative stress in cells as a result of its inhibition of antioxidant enzymes and depletion of antioxidant molecules such as GSH (Stohs *et al.*, 2000). Cd has been reported to be very active in

inducing apoptosis in a wide variety of cells, because of its induction of oxidative stress (Aydin *et al.*, 2003; Bagchi *et al.*, 2000; Jimi *et al.*, 2004; Poliandri *et al.*, 2003; Shin *et al.*, 2004; Tzirogiannis *et al.*, 2003; Watjen *et al.*, 2002). Cd has also been shown to inhibit DNA repair (Buchko *et al.*, 2000; Hartwig *et al.*, 2002a; 2002b; Waisberg *et al.*, 2003; Zharkov and Rosenquist, 2002).

5 EPIGENETIC EFFECTS OF METAL COMPOUNDS

5.1 Introduction

Epigenetics involves a heritable change in gene expression that does not result in a change of DNA sequence (Riddihough and Pennisi, 2001). Recent research has established that epigenetic changes play an important role in tumorigenesis (Feinberg, 2004). The major epigenetic changes taking place during the development of cancer include aberrant DNA methylation and histone modifications (Momparler, 2003). These epigenetic events can act in concert to silence the expression of genes that suppress tumorigenesis or activate oncogenes (Feinberg, 2004). Metal compounds, such as As, Cd, and Ni, have been shown to affect levels of DNA methylation and/or histone modifications at both global and gene-specific levels. In the first part of this section, DNA methylation and specific histone modifications will be introduced. The effects of metal compounds, mainly, As, Cd, and Ni, on these epigenetic parameters will be reviewed, and at the end of this section, we will include a summary table of epigenetic events affected by metals for easy future reference (Table 1). Because this is an emerging field of study, we have spent considerable time on this part of the chapter.

5.2 Epigenetic Events in the Development of Cancer

5.2.1 DNA Methylation

DNA methylation occurs at the cytosine residue of CpG dinucleotides of DNA, in which a methyl group is transferred from S-adenosyl-methionine (SAM) and covalently attached to the C-5 position of cytosine by a family of DNA-methyltransferases (DNMTs). DNA methylation plays an important role in the regulation of gene expression and the silencing of repeat elements in the genome (Feinberg, 2004). The deregulation of DNA methylation mainly includes hypomethylation, which leads to oncogene activation and chromosomal instability, and hypermethylation, which leads to the silencing of tumor suppressor genes (Jones and Laird, 1999).

TABLE 1 Summary of the Epigenetic Events Affected by Metals

Epigenetic events	Classification	Metal inducers
DNA methylation	Hypomethylation	As, Cd, Ni
	Hypermethylation	As, Cd, Ni
Histone modifications	Acetylation	As, Ni
	Methylation	Ni
	Phosphorylation	As

5.2.2 Histone Modifications

Histone modifications occur at the N-terminal and the C-terminal of histone tails, and include, but are not limited to, acetylation, methylation, phosphorylation, ubiquitination, sumoylation, and ADP-ribosylation. These modifications play a pivotal role in regulating gene transcription and other chromatin-associated processes (Jenuwein and Allis, 2001).

5.2.2.1 Histone Acetylation

All four-core histones can be reversibly acetylated at defined lysine residues on their N-terminal tails by histone acetyltransferases (HATs) and deacetylated by histone deacetylases (HDACs) (Klochendler-Yeivin and Yaniv, 2001). In general, hyperacetylation contributes to the formation of an "open" chromatin state and permits access of transcription factors to DNA. In contrast, hypoacetylation contributes to a "closed" chromatin and transcription repression (Turner, 2000). Lysine acetylation is believed to neutralize part of the positively charged histone tails, which may weaken the interactions between histone–DNA (Grant *et al.*, 1998), histone–histone (Fletcher and Hansen, 1996), nucleosome–nucleosome (Lutter *et al.*, 1992; Norton *et al.*, 1989; 1990), or the interaction between nucleosomes and other nonhistone chromosomal proteins (Hecht *et al.*, 1995). As a result, it may destabilize higher order chromatin organization and facilitate the access of transcription factors to nucleosomal DNA, resulting in enhanced DNA transcription. In addition, proteins with histone acetyltransferase (HAT) activity have been shown to be coactivators of transcription, whereas those with histone deacetyltransferase (HDAC) activity are corepressors of transcription (Lehrmann *et al.*, 2002). The balance between acetylation and deacetylation of histones plays an important regulatory role in gene transcription. Deregulation of this balance has been linked to the progression of cancers and diverse human disorders (Lehrmann *et al.*, 2002; Timmermann *et al.*, 2001).

5.2.2.2 Histone Methylation

Histone methylation occurs predominantly on histones H3 and H4. There are two types of histone

methylation, targeting either arginine (R) or lysine (K) residues. Generally, arginine methylation is involved in gene activation, and histone methyltransferases (HMTs) are recruited to promoters as coactivators. By comparison, methylation of the lysine residue can have multiple effects on chromatin function, depending on the specific lysine and the level of modification (mono-, di-, or trimethylation of a single lysine). For instance, H3-K9 dimethylation and H3-K27 trimethylation are both largely associated with gene silencing and heterochromatin formation, whereas methylation of H3-K4, H3-K36, or H3-K79 is associated with active chromatin (Maison and Almouzni, 2004; Peterson and Laniel, 2004).

5.2.2.3 Histone Phosphorylation

The correlation between mitotic chromosome condensation and phosphorylation of histones H1 and H3 is well established, although the specific function of this modification in chromosomes remains to be determined. Physical studies have shown that phosphorylation disrupted histone–DNA interactions and destabilized chromatin structure (Chadee *et al.*, 1995; Hendzel *et al.*, 1997). Phosphorylation of a histone H2A variant, H2A.X at serine (S) 139, increases during the early stages of DNA fragmentation in apoptosis and correlates with double-stranded DNA breaks (Gurley *et al.*, 1973). Recently, it has been reported that phosphorylation of histone H2B at S14 correlates with cells undergoing apoptosis in vertebrates, which may serve as a marker for apoptosis (Cheung *et al.*, 2003).

5.2.2.4 Histone Ubiquitination

The most prevalent ubiquitinated histones are monoubiquitinated-H2A and H2B (Nickel and Davie, 1989). Approximately 10% of total H2A and to a lesser extent, 1–1.5% of H2B is ubiquitinated in eukaryotic cells, although ubiquitinated H2A (uH2A) has not been reported in the budding yeast *Saccharomyces cerevisiae* (Goldknopf *et al.*, 1977). In higher eukaryotic organisms, the ubiquitin molecule is linked to K119 of H2A and K120 of H2B (K123 in yeast). Physiological observations suggest that ubiquitinated histones may have multiple functions and structural effects rather than playing a role in the degradation by the 26S proteasome (Nickel and Davie, 1989). Reports on the roles of histone ubiquitination in chromatin fractions are controversial and, in many cases, contradictory. Nevertheless, the current view favors that the ubiquitination of H2B is associated with gene transcription (Davie and Murphy, 1990; Krogan *et al.*, 2003). It is also required for methylation of H3 K4 and K79 in yeast (Dover *et al.*, 2002). In contrast, the ubiquitination of H2A has been linked to polycomb gene silencing (Wang *et al.*, 2004a). It has been shown that the levels of uH2A were higher in transformed human fibroblasts and keratinocytes than in their normal counterparts (Vassilev *et al.*, 1995).

5.3 Impacts of Metal Compounds on Epigenetics

5.3.1 As

Arsenite (As) has been shown to reduce global levels of DNA methylation, as well as cause both DNA hypomethylation and hypermethylation of specific genes or DNA fragments. The status of histone acetylation and phosphorylation is also changed on As exposure and has been associated with the activation of genes.

It has been shown that chronic exposure of TRL 1215 (a rat liver epithelial cell line) to As (0.125–0.500 μ*M* for more than 18 weeks) resulted in the depletion of S-adenosyl-methionine (SAM), global DNA hypomethylation, and malignant transformation. As-induced DNA hypomethylation was a function of dose and exposure duration and remained constant even after withdrawal of As (Zhao *et al.*, 1997). Overexpression of several oncogenes and genes regulating cell proliferation were detected in the As-transformed cells, including the protooncogene *c-myc* (Chen *et al.*, 2001). Although the DNA methylation status on the promoter of the *c-myc* gene was not examined, the transcription of *c-myc* gene was increased by As in a dose- and time-dependent manner and strongly correlated with levels of global genomic DNA hypomethylation. Because As is detoxified by methylation using a methyltransferase and SAM as the methyl donor, it is conceivable that As metabolism may deplete intracellular methyl group storage and thereby lead to DNA hypomethylation. This, in turn, may facilitate aberrant gene expression and play a role in As carcinogenesis (Chen *et al.*, 2001). However, the data concerning DNA methylation alterations after As exposure are ambiguous. By using quantitative PCR/*Hpa*II restriction site analysis to examine DNA methylation status, it has been shown that exposure of human lung adenocarcinoma A549 cells to sodium arsenite (As III) (0.08–2 μ*M*) or sodium arsenate (As VI) (30–300 μ*M*) for 2 weeks produced significant dose-dependent DNA hypermethylation within the promoter region of p53 (Mass and Wang, 1997). In addition, by use of methylation-sensitive arbitrarily primed PCR, both hypomethylation and hypermethylation of genomic DNA fragments (300–500 bp) were detected with As treatment in both human kidney cells (UOK) and in A549 cells. Although the affected genes have not yet been identified, this result suggested that

the As-induced imbalance of DNA methylation could disrupt normal gene expression and may have a potential role in the As-induced cellular dysfunctions and cancer development (Zhong and Mass, 2001).

As-induced gene expression changes have been associated with alterations in histone modifications and signaling pathways. As has been shown to activate the stress-inducible gene Hsp70 through the p38 MAPK pathway (Thomson *et al.*, 2004). The activation of this gene was accompanied by histone H4 acetylation and H3 phosphorylation, whereas histone H3 remained markedly hypoacetylated at Hsp70 chromatin (Thomson *et al.*, 2004). As could also upregulate protooncogenes *c-jun* and *c-fos*, which would require the action of ERK. Chromatin immunoprecipitation (ChIP) assays revealed that As treatment dramatically induced the phosphorylation and acetylation of H3 associated with the *c-fos* and *c-jun* genes through an ERK-dependent pathway (Li *et al.*, 2003). In addition, it has been shown that As induced phosphorylation of histone H3 at serine 10 in a time- and dose-dependent manner in JB6 Cl 41 cells (He *et al.*, 2003). As also dramatically increased phosphorylation-acetylation of histone H3 at a global level, which preceded the induction of mRNA levels of MAPK phosphatase 1 (MKP-1). ChIP revealed that As-induced phosphorylation-acetylation of histone H3 associated with the MKP-1 gene and enhanced the binding of RNA polymerase II to MKP-1 chromatin (Li *et al.*, 2001). MKP-1 has been shown to play a critical role in mediating the feedback control of MAPK cascades in a variety of cellular processes, including proliferation and stress responsiveness. Therefore, it has been suggested that As contributes to the carcinogenic process by triggering alterations in global chromatin structure, as well as perturbing the transcription of specific genes, including *c-jun*, *c-fos*, and MKP-1 (Li *et al.*, 2001; 2003).

5.3.2 Cd

Cadmium (Cd) exposure can result in DNA hypomethylation initially, but prolonged exposure can lead to DNA hypermethylation (Takiguchi *et al.*, 2003). In TRL1215 rat liver cells, after 1 week of exposure to cadmium (<2.5 μ*M*), DNMT activity and genomic DNA methylation were reduced. However, significant increases in DNMT activity and DNA methylation were detected after 10 weeks of Cd exposure. These cells exhibited properties of a transformed phenotype, including hyperproliferation, increased invasiveness, and decreased serum dependence (Takiguchi *et al.*, 2003).

5.3.3 Ni

Nickel (Ni) can cause global hypermethylation and gene specific hypermethylation, both of which have been linked to gene silencing. Ni exposure can also lead to global and gene-specific decreases of histone acetylation.

It has been shown that Ni inhibits the activity of DNMTs both *in vivo* and *in vitro* (Lee *et al.*, 1998). In cells, Ni reduced DNMTs activity and the levels of genomic DNA methylation; however, this inhibition was transient. After a recovery period after Ni treatment, DNMT activity rebounded slightly and genomic DNA methylation levels increased to above basal levels (Lee *et al.*, 1998). In Chinese hamster ovary (CHO) cells, Ni bound selectively to heterochromatin and caused chromosomal damage in heterochromatic regions (Klein and Costa, 1997; Klein *et al.*, 1994a; 1994b). The binding of Ni to heterochromatin may play a key role in Ni-induced cell transformation and loss of senescence. Senescence in Ni-transformed cells was restored after treatment with the DNA methylation inhibitor 5-azacytidine (5-AzaC), suggesting that DNA hypermethylation played an important role in the silencing of the senescence gene(s) during Ni-induced cell transformation (Klein *et al.*, 1991). Further studies in transgenic CHO cell lines provided additional evidence linking Ni with DNA hypermethylation and inactivation of genes positioned near heterochromatin. Ni was found to silence a transgene that was stably transfected adjacent to a heterochromatic region in CHO cells without causing mutations or deletions of the transgene (Lee *et al.*, 1993). Instead, increased DNA methylation and chromatin condensation were observed at the Ni-silenced transgene locus. The demethylating agent 5-AzaC reversed this silencing, indicating that DNA hypermethylation was involved in the Ni-induced transgene inactivation (Lee *et al.*, 1995).

In addition to affecting DNA methylation, Ni has been shown to decrease histone acetylation globally in yeast, as well as a variety of mammalian cells (Broday *et al.*, 2000; Kang *et al.*, 2003). ROS generation has been shown to play a role in the inhibition of histone acetylation by Ni (Kang *et al.*, 2003). Ni was also found to inhibit the acetylation of histones *in vitro* using purified recombinant histone acetyltransferase (Broday *et al.*, 2000; Kang *et al.*, 2003). The decrease of histone H4 acetylation in chromatin associated with the Bcl-2 promoter region was verified by ChIP assays, indicating the involvement of histone hypoacetylation in Ni-induced Bcl-2 downregulation. This suggested that histone hypoacetylation might play a role in Ni-induced cell apoptosis, where Bcl-2 is one of the targets (Kang *et al.*, 2004). Hypoacetylation of H4 induced by Ni was observed at lysine's 12 and 16 in the N-terminal tail of histone H4 (Broday *et al.*, 2000). Because a histidine residue is present at

position 18 on histone H4, it has been suggested that this may be an anchoring site for Ni coordination. It was proposed that Ni might inhibit the acetylation of histone H4 through binding with its N-terminal histidine-18, rendering the neighboring lysine residues less accessible to histone acetyltransferases (Zoroddu *et al.*, 2000; 2002). Besides histidine 18 of histone H4, Ni-binding sites in H3, H2A, and H2B have also been identified *in vitro* (Kasprzak *et al.*, 2003). By using ^{63}Ni autoradiography of proteins on nitrocellulose membranes, Ni was found to bind to histone H1 and core histones (Huang *et al.*, 1995). In cells, such as CHO, NRK-52 (rat renal tubular epithelium), and HPL1D (human lung epithelium) cells, cleavage of C-terminal tail H2A by Ni was also found. Although the mechanisms and the consequences of this event remain unknown, Ni-induced truncation of H2A is likely to alter the chromatin structure and affect gene expression (Kasprzak *et al.*, 2003).

Histone modifications also participated in the Ni-induced transgene silencing and cell transformation. In yeast, changes in histone acetylation patterns, but not DNA methylation, are important regulators of gene transcription. Ni silenced a URA3 marker gene when it was located near (1.7 kb) a heterochromatic telomeric silencing element but not when it was placed 2.0 kb from this same element. Pretreatment of yeast cells with the histone deacetylase inhibitor trichostatin A (TSA) resulted in reduced levels of URA3 gene silencing after Ni exposure, indicating that histone acetylation was involved in this silencing (Broday *et al.*, 1999). Similarly, in mammalian cells, TSA pretreatment prevented Ni-induced transformation of PW or HOS cells. In addition, the exposure of Ni-transformed cells to TSA reversed the transformed phenotype as indicated by their inability to grow in soft agar (Zhang *et al.*, 2003). Results from ChIP assays revealed a loss of acetylation at histone H3 and H4, as well as increased methylation of H3 K9 at the Ni-silenced transgene locus in the transgenic Chinese hamster cells (Yan *et al.*, 2003). In a similar manner, treatment of the transgenic Chinese hamster cells with TSA during or after Ni exposure reduced Ni-induced inactivation of the transgene. Moreover, although exposure of Ni-silenced cell clones to 5-AzaC or TSA alone could partially reactivate the transgene expression, combining 5-AzaC with TSA was more efficacious in reactivating the transgene. These data suggested that in mammalian cells, both DNA hypermethylation and histone deacetylation participated in Ni-induced transgene silencing (Sutherland *et al.*, 2001). Table 1 summarizes the epigenetic events caused by metal compounds discussed previously.

6 EFFECTS OF METALS ON CELL SIGNALING PATHWAYS AND GENE EXPRESSION

6.1 Introduction

Epidemiological studies have shown that occupational and environmental exposure to metals and metalloid species, such as arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), nickel (Ni), and vanadium (V), was associated with increased risk of various cancers and adverse health effects. Although the underlying mechanisms through which metals cause cancers remains unclear, recent studies have shown that activation of a variety of signaling pathways caused by metal exposure can affect the expression of numerous genes that play important roles in carcinogenesis. The metal-induced cellular signal transduction pathways mainly include ROS, the mitogen-activated protein kinases (MAPKs), the phosphoinositide 3-phosphate kinase (PI3K), hypoxia-inducible factor 1 (HIF-1), nuclear transcription factor κB (NF-κB), nuclear factor of activated T cells (NFAT), and activator protein 1 (AP-1). In the first part of this section, each pathway will be introduced briefly, and the impact of individual metals on these pathways will then be reviewed, with particular attention given to changes in gene expression. At the end of this section, we will summarize these effects in Table 2. It should be noted that the effects of metals on other pathways or transcription factors have been previously reviewed elsewhere (Chen and Shi, 2002; Leonard *et al.*, 2004a; Salnikow *et al.*, 2000).

TABLE 2 Summary of Signaling Pathways and Transcription Factors Affected by Metals

Factor	Description	Major components	Metal inducers
ROS	Reactive oxygen species	$^{-}$OH, H_2O_2, O^{-}	As, Cd, Cr, Co, Ni
MAPKs	The mitogen-activated protein kinases	ERK, JNK, p38	As, Cd, Cr, Co, Ni
PI3K	The phosphatidylinositol-3 kinase	p85, p110	As, Ni, Cu
HIF-1	Hypoxia-inducible factor 1	HIF-1alpha, HIF-1beta	As, Cr, Co, Ni, V
NF-κB	The nuclear transcription factor kappa B	p50, p65	As, Cd, Cr, Co, Ni
NFAT	The nuclear factor of activated T cells	NFATC1-C4	Ni, V, Fe
AP-1	Activator protein 1	c-fos, c-jun	As, Cd, Cr, Ni, Fe, V

6.2 Signal Transduction Pathways Affected by Metal Compounds

6.2.1 ROS

Reactive oxygen species (ROS) are highly reactive molecules with unpaired electrons formed during oxidative metabolism. They include the superoxide anion (O_2^-), the hydroxyl radical (OH), and hydrogen peroxide (H_2O_2) (Halliwell, 1981; Halliwell and Gutteridge, 1984). They are continuously generated and eliminated in biological systems and play important roles in the regulation of cell proliferation, apoptosis, transformation, and senescence. Cells are able to defend against ROS stress with antioxidant scavengers (i.e., ascorbate, glutathione, and thioredoxin), and antioxidant enzymes (i.e., superoxide dismutase [SOD], catalase, glutathione peroxidase, and thioredoxin reductase). With their antioxidant defense systems, cells can adapt to low physiological concentration of ROS (Storey, 1996). However, ROS can cause oxidative damage to cellular macromolecules, including DNA, proteins, and lipids, when generation of these potentially harmful species exceeds cellular antioxidant defenses. ROS play important roles in the initiation of cellular injury that can lead to the development of cancer (Toyokuni, 1998; Toyokuni *et al.*, 1995). One of the most important ways that ROS may cause cancer is by acting as signal transduction messengers to activate signaling pathways, such as MAPK, PI3K, NF-κB, and NFAT (Leonard *et al.*, 2004a). Therefore, we have included ROS in the discussion of the effects of metal compounds on signaling pathways. Studies have shown that exposure of cells to As, Cd, Co, Cr, Cu, Ni, as well as many other metal compounds, can generate excessive ROS in cells by Fenton-type reaction, Haber–Weiss reaction, or by reacting directly with cellular molecules.

6.2.2 MAPK

The mitogen-activated protein kinase (MAPK) signaling pathway plays an important role in controlling gene expression programs in response to extracellular signals in eukaryotic cells. There are four major groups of MAPK family in mammalian cells, and they are serine/threonine kinases: extracellular signal regulated kinase (ERK), c-jun N terminal kinase (JNK), p38, and extracellular signal regulated kinase-5 (ERK5)/big MAPK-1 (BMK1) (Lewis *et al.*, 1998). Each group also contains a number of isoforms. MAPK signaling pathways feature a series of phosphorylation cascades. On receiving extracellular signals such as growth factors, hormones, and stress stimuli, the MAPKs are activated by phosphorylation on threonine and tyrosine by the MAPK kinases (MAPKK), which are activated by serine/threonine phosphorylation by MAPK kinase kinases (MAPKKK). Additional protein kinases and members of the Ras and Rho families of small GTPases may also participate upstream of the MAPKKKs. Once activated, MAPKs can alter the activity of target transcription factors and transcriptional coregulators by phosphorylation, resulting in changes in the expression of genes that mediate cell growth, proliferation, differentiation, apoptosis, and transformation (Schaeffer and Weber, 1999). Studies have shown that metals, such as As, Cd, Co, Cr, and Ni, can activate MAPK pathways.

6.2.3 PI3K/Akt

The phosphatidylinositol-3 kinase (PI3K) pathways are involved in regulating cell growth, differentiation, and apoptosis (Fresno Vara *et al.*, 2004). PI3Ks constitute a lipid kinase family characterized by their abilities to phosphorylate phosphoinositides (PIPs) at the 3′-OH of the inositol ring in the cell membrane (Toker and Cantley, 1997). The best-known class of PI3Ks is a heterodimer composed of a regulatory subunit, p85, and a catalytic subunit, p110. On activation by growth factor receptor protein tyrosine kinases, PI3K is recruited to the cell membrane, where it phosphorylates PIPs to generate the second messenger phosphatidylinositol-3,4,5-triphosphate (PIP3). PIP3 then recruits a subset of signaling proteins to the membrane, including protein serine/threonine kinase 3′-phosphoinositide–dependent kinase 1 (PDK1) and Akt/PKB. Akt is also a serine/threonine kinase and is activated by phosphorylation by PDK1 (Fresno Vara *et al.*, 2004). Activation of the PI3K/Akt pathway induces cell growth and inhibits apoptosis. As, Cd, Co, Ni, and V have been shown to be able to activate the PI3K/Akt pathway.

6.2.4 HIF-1

The transcription factor hypoxia-inducible factor 1 (HIF-1) plays an essential role in cellular oxygen homeostasis (Wang and Semenza, 1993). It is a heterodimer composed of α- and β-subunits (Wang *et al.*, 1995). The β subunit, HIF-1β/ARNT, is constitutively expressed; whereas the α subunit (HIF-1α) is highly oxygen sensitive. It is rarely detectable under normal oxygen tension but is dramatically induced in response to hypoxia. Under reduced oxygen tension, HIF-1α is stabilized and translocates to the nucleus, where it dimerizes with HIF-1β and HIF-1 becomes activated (Wang *et al.*, 1995). As a result, the activated HIF-1 stimulates the transcription of various cancer-related genes involved in angiogenesis, cell survival, glucose transport, and metabolism (Semenza, 2003). For example, HIF-1 upregulates the vascular endothelial growth

factor (VEGF), which plays a key role in tumorigenesis and angiogenesis. HIF-1α is regulated by a reduced oxygen level largely by its posttranslational modifications, resulting in stabilization, nuclear translocation, DNA binding activity, and transcriptional activity of the protein (Ferrara and Davis-Smyth, 1997). The posttranslational modifications of HIF-1α include prolyl hydroxylation at proline 402 and 564 within the oxygen-dependent degradation (ODD) domain by HIF-prolyl hydroxylases (HPHs) (Epstein *et al.*, 2001), asparaginyl hydroxylation at asparagine 803 in the C-terminal activation domain (C-TAD) by factor inhibiting HIF-1 (FIH-1) (Lando *et al.*, 2002), acetylation of lysine 532 in the ODD domain by an acetyltransferase (ARD-1) (Jeong *et al.*, 2002), phosphorylation induced by ERK activity (Richard *et al.*, 1999), as well as ubiquitination by the Von Hippel–Lindau (pVHL) complex (Maxwell *et al.*, 1999; Salceda and Caro, 1997). Co and Ni are well-known agents that mimic hypoxia and, in turn, activate HIF-1. Some other metals such as As, Cd, Cr, and V have also been reported to activate the HIF-1 pathway.

6.2.5 NF-κB

Initially discovered as a factor in the nucleus of B cells that binds to the enhancer of the kappa light chain of immunoglobulin, the nuclear transcription factor kappa B (NF-κB) was later found to be present in all cell types. In resting cells, NF-κB is inactive in the cytoplasm and composed of a heterodimer of p50 and p65 bound to the inhibitor of NF-κB (IκB). In response to inflammatory stimuli, tumor promoters, carcinogens, viral proteins, or mitogens, IκB is phosphorylated and ubiquitinated, leading to its degradation. Degradation of IκB releases NF-κB to translocate into the nucleus, where it regulates the transcription of specific genes that mediate various cellular functions, such as inflammation, transformation, proliferation, invasion, angiogenesis, metastasis, and apoptosis (Shishodia and Aggarwal, 2004). Although NF-κB is required for the normal function of the immune system, its deregulation has been implicated in a variety of cancers in which NF-κB is overexpressed. Metals such as As, Cd, Co, Cr, and Ni have been found to affect NF-κB.

6.2.6 NFAT

The nuclear factor of activated T cell (NFAT) proteins are a family of transcription factors whose activation is controlled by Ca and the Ca/calmodulin-dependent serine phosphatase calcineurin. Like NF-κB, originally identified in T cells as an inducer of cytokine gene expression, NFAT was later found to play various roles in cells outside of the immune system, including differentiation, angiogenesis, chondrogenesis, and adipogenesis. The best-characterized transcription factors of NFAT family are NFATC1-C4 (Rao *et al.*, 1997). In resting cells, NFAT proteins are phosphorylated and reside in the cytoplasm; on stimulation, during a sustained elevation of calcium, they are dephosphorylated by calcineurin and translocated to the nucleus. Once in the nucleus, NFAT proteins become transcriptionally active and bind to consensus DNA sites, therein controlling the transcription of target genes. On cessation of Ca signaling, NFAT proteins are rephosphorylated and translocated back to the cytoplasm, resulting in the termination of their activities. NFAT can interact with NF-κB and AP-1 (Macian *et al.*, 2000; 2001). Their activities are further modulated by diverse signal transduction pathways, which affect NFAT kinases and nuclear partner proteins. Ni, V, and Fe have been shown to activate NFAT.

6.2.7 AP-1

The transcription factor activator protein 1 (AP-1) is a dimeric complex that is composed of members of the JUN, FOS, ATF (activating transcription factor), and MAF (musculoaponeurotic fibrosarcoma) families (Eferl and Wagner, 2003). The predominant form of AP-1 in most cells is Fos/Jun heterodimer, which has a high affinity for binding to the TPA-responsive element (TRE) (also called AP-1 site). C-jun and c-fos are mostly considered to be oncogenic and associated with carcinogenesis (Angel and Karin, 1991). Extracellular stimuli such as cytokines, UV radiation, growth factors, oxidative stress, and carcinogens can induce AP-1 and promote AP-1 binding to the TRE of its target genes that are involved in cell growth, inflammatory responses, and repair processes. As, Cd, Cr, Co, Ni, and V have been shown to activate AP-1.

6.3 Impacts of Metal Compounds on Signal Transduction Pathways and Gene Expression

6.3.1 As

Arsenite (As III) has been shown to generate ROS, activate MAPK, PI3K/Akt, HIF-1, and AP-1. It can also activate or inhibit NF-κB depending on the doses, cell types, and/or exposure time.

As can induce ROS formation by activating radical-producing systems in cells. As (>1 μ*M*) activated NADH oxidase to produce superoxide, which then caused oxidative DNA damage during a 4-hour treatment in human vascular smooth muscle cells (Lynn *et al.*, 2000). Similarly, As (5 μ*M*) stimulated a twofold increase in the activity of plasma membrane NADH oxidase by 1 hour and generated excessive superoxide

in PAEC porcine aortic endothelial cells (Smith *et al.*, 2001). As also induced a dose-dependent increase of up to threefold in intracellular oxygen radical production in A (L) human–hamster hybrid cells within 5 minutes. This As-induced ROS, particularly hydroxyl radicals, played an important causal role in its genotoxicity (Liu *et al.*, 2001b).

It has been shown that both As (III) (arsenite) and As (VI) (arsenate) activated the MAPK kinases, ERK, JNK, and p38 in NHEK normal human epidermal keratinocytes (Tanaka-Kagawa *et al.*, 2003). As (III) also activated ERK, JNK, and p38 in BEAS human bronchial epithelial cells (Samet *et al.*, 1998), resulting in the activation of c-jun (a major phosphorylation target of JNK) and transcription factor 2 (ATF-2) (a substrate for p38 and JNK), as well as, increased interleukin-8 (IL-8) protein levels. In addition, activation of JNK by As was involved in As-induced p21Cip1/Waf1 upregulation and endothelial apoptosis in human umbilical vein endothelial cells (HUVEC) (Nuntharatanapong *et al.*, 2005). In JB6 Cl 41 mouse epidermal cells, activation of JNK was required for As-induced p53-independent apoptosis (Huang *et al.*, 2001b), whereas activation of ERK, but not JNK, was required for As-induced cell transformation (Huang *et al.*, 1999). Moreover, in LMH chicken hepatoma cells, As induced heme oxygenase 1(HO-1), which is overexpressed in various cancers, in an ERK- and p38-dependent manner (Elbirt *et al.*, 1998). The ability of As to activate different MAPKs and cellular signals depends on its concentration. In rat lung epithelial cells, a low level of As (2 μ*M*) stimulated the ERK signaling pathway and enhanced cell proliferation. In comparison, a high level of As (40 μ*M*) stimulated JNK signaling pathway and induced cell apoptosis. This result indicated that a high concentration of arsenic exposure caused apoptosis, whereas a low concentration of arsenic exposure was carcinogenic and might result in accumulation of aberrant cells (Lau *et al.*, 2004).

Studies have indicated that As activated Akt and its downstream effector eNOS via PI3K and p38 pathways in HaCat human keratinocytes (Souza *et al.*, 2001). In human Jurkat cells (a T cell leukemia cell line), As induced the activation of Akt and its downstream glycogen synthase 3β (GSK3β) via PI3K, and this activation promoted the As-triggered caspase activation (Hossain *et al.*, 2003).

It has been shown that As induced HIF-1α protein levels and increased the expression of VEGF through HIF-1 in DU145 human prostate carcinoma cells. As-activated PI3K/Akt pathway and As-generated H_2O_2, but not MAPK pathway, were required for this induction (Gao *et al.*, 2004). Upregulation of VEGF by As has also been shown to be independent of HIF-1, but dependent on p38, in OVCAR-3, human ovarian cancer cells (Duyndam *et al.*, 2003).

As could be an inducer or inhibitor of NF-κB, depending on the cell type, the concentration of As, and the duration of treatment. In human foreskin keratinocytes, arsenic induced keratinocyte proliferation and enhanced both NF-κB and AP-1 activity at lower concentrations (≤1 μ*M*), whereas at higher concentrations (≥5 μ*M*), it induced keratinocyte apoptosis by the Fas/Fas ligand (Fas/FasL) pathway, in which NF-κB activity was not enhanced while AP-1 was activated. These results indicated that upregulation of NF-κB at lower As concentrations was correlated with keratinocyte proliferation. In contrast, higher concentrations of As enhanced AP-1 and induced Fas/FasL-associated apoptosis (Liao *et al.*, 2004). At low concentrations, As activated NF-κB and NF-κB–dependent transcription in PAEC cells, although it was insufficient to activate MAPKs with these concentrations. However, the MAPKs, ERK and p38, were activated in response to levels of As that caused cell death (Barchowsky *et al.*, 1996; 1999). As induced transactivation of NF-βB in a dose- and time-dependent manner in C141 mouse skin epidermal cells through ERK- and JNK-pathways (Huang *et al.*, 2001b). At high concentrations, As has been shown to activate NF-κB, resulting in p53-independent apoptosis in JB6 mouse epidermal cells. Conversely, high levels of As (500 μ*M*) have also been shown to inhibit NF-κB–mediated gene transcription by blocking IκB kinase activity and IκBa phosphorylation and degradation in BEAS/2B and HEK293 human embryonic kidney cells (Roussel and Barchowsky, 2000). In human GM847 fibroblasts, As increased the DNA binding of NF-κB after 24 hours of treatment, whereas it decreased this inflammatory mediator with longer treatment (10–20 weeks) of As (Hu *et al.*, 2002).

As is an efficient inducer of *c-fos* and *c-jun* gene expression and a potent stimulator of AP-1 transcriptional activity. Induction of c-jun and c-fos transcription by As correlated with activation of JNK and p38 in human keratinocytes. Phosphorylated JNK and p38 transcription factors in turn activated the *c-jun* and *c-fos* genes (Cavigelli *et al.*, 1996).

6.3.2 Cd

Cadmium (Cd II) exposure of cells can generate ROS, activate MAPKs, NF-κB, and AP-1 but is known to suppress HIF-1 levels.

Cd caused a significant increase of ROS production, especially H_2O_2, in MRC-5 human fetal lung fibroblasts (Yang *et al.*, 1997). A concentration-dependent increase in ROS and loss of activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase, glutathione

reductase, and glutathione peroxidase, were observed when CRL-1439 normal liver cells were exposed to Cd (100–300 μ*M*) (Ikediobi *et al.*, 2004). In mouse neuronal cells, Cd induced ROS, which in turn, activated JNK and p38, as well as their substrates, such as activating transcription factor 2 (ATF-2), CRE-binding protein (CREB), and c-Jun. This response was accompanied by the induction of HO-1, poly (ADP-ribose) polymerase cleavage, and a caspase-independent cell death (Rockwell *et al.*, 2004).

MAPKs (i.e., ERK, JNK, and p38) are activated with differing sensitivity to Cd exposure. In CL3 human non-small cell lung carcinoma cells, the kinase activity of JNK was induced dose dependently by Cd (30–160 μ*M*) treatment. High doses of Cd (130–160 μ*M*) markedly activated p38, but low Cd doses did not. Conversely, the activity of ERK was decreased by doses of Cd (≤80 μ*M*) and moderately activated by high Cd doses. Low doses of Cd transiently activated JNK and simultaneously reduced ERK activity, whereas high doses of Cd persistently activated JNK and p38. JNK and p38 cooperatively participated in apoptosis induced by Cd. In addition, the decreased amount of ERK signaling induced by low Cd doses contributed to growth inhibition or apoptosis (Chuang *et al.*, 2000). In CCRF-CEM cells, a human T cell line, ERK and p38 were phosphorylated by 1 μ*M* of Cd, whereas levels greater than 20 μ*M* of Cd were required for the phosphorylation of JNK. In the time-course study, ERK and p38 were phosphorylated earlier than JNK after Cd exposure (Iryo *et al.*, 2000). It has also been shown that exposure of human breast cancer cells (MCF-7) to 10 μ*M* Cd stimulated phosphorylation of ERK, JNK, and p38, and activation of p38 pathway was required for Cd-induced HO-1 gene expression (Alam *et al.*, 2000). Pulse treatment of U-937 monocytic cells with Cd (2 hours at 200 μ*M*) induced a rapid phosphorylation of p38, as well as a late phosphorylation of ERK. P38 activation was an early and specific regulatory event for the Cd-induced apoptosis (Galan *et al.*, 2000). Cd persistently activated the JNK pathway in LLC-PK1, a renal epithelial cell line, and an elevation of intracellular Ca was required for this activation (Matsuoka and Igisu, 1998). P38 and ERK can be simultaneously or independently activated, depending on the concentrations of Cd ranging from 40–100 μ*M*. These signaling pathways have been shown to participate in the induction of heat shock proteins (HSPs) by Cd in 9L rat brain tumor cells (Hung *et al.*, 1998).

Cd has been shown to increase p53 protein level and induce phosphorylation of p53 at Ser 15 in MCF-7 cells, and this effect depends on PI3K related kinases, but not on MAPKs (Igisu, 2001).

Cd reduced the amount of HIF-1α protein in hypoxia and inhibited HIF-1α accumulation induced by Co and desferrioxamine (DFO), although it did not affect the levels of HIF-1α mRNA in Hep3B human hepatoma cells. Antioxidants and a proteasome inhibitor prevented the HIF-1α degradation caused by Cd, indicating that Cd triggered a redox/proteasome-dependent degradation of HIF-1α protein, reducing HIF-1 activity, and in turn, suppressing the induction of hypoxia-inducible genes (Chun *et al.*, 2000).

Cd induced significant activation of AP-1 and all three members of the MAPK family in mouse epidermal JB6 cells, and activated ERK was involved in Cd-induced AP-1 activity (Huang *et al.*, 2001c). Exposure of mesangial cells to Cd caused an increase of protooncogene *c-fos* mRNA level. Activation of ERK and JNK acted in concert during the Cd-induced *c-fos* increase (Ding and Templeton, 2000a; 2000b; Wang and Templeton, 1998). JNK activity and c-jun mRNA levels, as well as AP-1 DNA binding activity, were significantly enhanced by Cd in primary rat hepatocytes (Hsiao and Stapleton, 2004).

In mouse brain microvessel endothelial cells (bEnd.3), Cd induced the translocation of NF-κB and increased its DNA binding activity. This activation was required for Cd-induced intercellular adhesion molecule-1 (ICAM-1) expression (Jeong *et al.*, 2004).

6.3.3 Cr

Chromium (Cr (VI)) can generate ROS, activate MAPKs, NF-κB, and HIF-1 in cells. Cr mediates free radical generation by Fenton-type reaction, Haber–Weiss reaction, or by reacting directly with cellular molecules (Leonard *et al.*, 2004b). It has been shown that Cr (VI) was able to enter A549 cells at low concentrations (<10 μ*M*), resulting in an elevation of ROS in cells (Liu *et al.*, 2001a). In the same cell line, Cr-induced ROS generation was shown to be responsible for the early stage of Cr-induced apoptosis, whereas Cr-activated p53, mostly by ROS-mediated free radical reactions, contributed to a late stage induction of apoptosis (Wang and Shi, 2001; Ye *et al.*, 1999). Cr (VI)-induced ROS production, as well as oxidative damage to tissue and to DNA, was also observed in a number of cell lines, such as human peripheral blood mononuclear cells, chronic myelogenous leukemic K562 cells, and J774A.1 murine macrophage cells. Moreover, p53 has been shown to play a major role in Cr (VI)-induced oxidative stress and toxicity (Bagchi *et al.*, 2001). ROS acted as a second messenger mediating Cr (III)-induced apoptosis in lymphocytes. It activated the Src-family tyrosine kinases, which, in turn, led to the activation of caspase-3 in Cr-induced apoptotic cell death (Balamurugan *et al.*, 2002; 2004).

Exposure of CL3 human non-small cell lung carcinoma cells to Cr (VI) markedly activated JNK and p38 and moderately activated ERK in a dose- (10–80 μ*M*) and time- (1–12 hours) dependent manner. Activation of ERK, JNK, and p38 by Cr (VI) were through diverse redox mechanisms. When Cr (VI) was removed from the medium, the activated p38 decreased rapidly, whereas the activated JNK decreased gradually (Chuang *et al.*, 2000). In A549 human lung epithelial cells, Cr (VI) (10 μ*M*) increased ROS and selectively activated JNK, relative to ERK or p38 MAPK (O'Hara *et al.*, 2003). Moreover, it has been shown that ERK acted as the upstream kinase for the phosphorylation of the p53 at Ser15 in response to Cr (VI) in the same cell line (Wang and Shi, 2001).

Cr (VI) has been shown to activate HIF-1 activity by inducing HIF-1α but not HIF-1β and increased the level of VEGF expression in DU145 human prostate carcinoma cells. P38, but not PI3K nor ERK, was required for Cr-induced HIF-1α expression (Gao *et al.*, 2002).

Exposure of cultured Jurkat cells to Cr (VI) (2 μ*M*) resulted in activation of DNA binding activity of NF-κB via Cr (VI)-mediated free radical reactions (Ye *et al.*, 1995). Cr (<10 μ*M*) also activated NF-κB in A549 cells (Liu *et al.*, 2001a). By use of two cell lines that were developed from BEAS-2B human bronchial epithelial cells, IKK cells, in which a IκBα expression vector with normal NF-κB activity was stably transfected; and KM cells, in which a mutated IκBα kinase expression vector exhibiting very little NF-κB activity was stably transfected, it has been shown that with Cr (VI) stimulation, KM cells, but not IKK cells, exhibited pronounced cell death. This result indicated that NF-κB activation was required to prevent cells from undergoing Cr (VI)-induced apoptosis (Wang *et al.*, 2004b). Cr (VI) activated NF-κB and AP-1 in a dose-dependent manner, but both activations were substantially inhibited by antioxidants. Furthermore, Cr-induced AP-1 was mediated by p38, and Cr-induced NF-κB involved the IKK pathway (Chen *et al.*, 2000).

6.3.4 Co

Cobalt (Co II) can produce ROS, activate MAPKs, NF-κB, and HIF-1 in cells.

Co can generate ROS by Fenton-type or Haber–Weiss reactions. It has been shown that exposure of a variety of cell lines to soluble salts of Co, such as pulmonary artery smooth muscle cells (PASMC), A549, neuronal PC12 cells, human hepatoma cells Hep3B and HepG2, resulted in increased ROS production (BelAiba *et al.*, 2004; Chandel *et al.*, 1998; Porwol *et al.*, 1998; Salnikow *et al.*, 2000). Co-mediated ROS production in neuronal PC12 cells was believed to mediate Co-induced apoptosis (Zou *et al.*, 2002). Co (400 μ*M*, IC50) has been shown to activate ERK in C6 glioma cells, as well as in HeLa human cervical cancer cells, and this pathway is believed to mediate Co-induced apoptosis (Kim *et al.*, 2003; Yang *et al.*, 2004).

Co has been known to mimic hypoxia by inducing HIF-1 and hypoxic-regulated genes, such as erythropoietin (EPO) and VEGF in various cell lines (Semenza *et al.*, 1994; Wang and Semenza, 1993). Co also induced Cap43, a hypoxia-regulated gene in a HIF-dependent manner in A549 cells (Salnikow *et al.*, 2000). Activation of HIF-1α by Co involved the PI3K pathway and production of ROS in airway smooth muscle (ASM) cells (Chachami *et al.*, 2004). However, it has also been shown that the formation of ROS was not involved in Co-induced HIF-1 activation or upregulation of hypoxia-dependent genes in A549 cells (Salnikow *et al.*, 2000). The ability of Co to mimic hypoxia occurs because Co can affect a variety of genes/enzymes that are responsible for the posttranslational modification of HIF-1α. Co has been shown to inhibit prolyl hydroxylase activity, alter the levels of factor inhibiting HIF (FIH1) and ARD1, and occupy the VHL-binding domain of HIF-1α. These changes prevent HIF-1α degradation, lead to increased stability of HIF-1α, and increase the transcription of HIF-1–dependent genes.

Co induced the DNA-binding activity of NF-κB in HUVEC, which could induce the transcription of adhesion molecules ICAM-1, VCAM-1, and E-selectin in endothelial cells (Goebeler *et al.*, 1995; Sultana *et al.*, 1999).

6.3.5 Ni

Nickel (Ni II) can generate ROS, activate MAPKs, PI3K, HIF-1, and NF-κB, although its impact on AP-1 is equivocal.

Ni compounds, such as NiS, Ni3S2, NiO (black and green), and NiCl2, have been shown to be active inducers of ROS in CHO cells (Huang *et al.*, 1993; 1994a; 1994b). Intracellular glutathione was also depleted by Ni exposure (Andrew *et al.*, 2001b). The Ni-induced ROS played a role in oxidative base damage, measured by the formation of 7,8-dihydro-8-oxo-2′-deoxyguanosine (8-oxo-dG) (Huang *et al.*, 1995). An involvement of ROS has also been shown to participate in the inhibition of DNA repair by Ni^{+2} (Lynn *et al.*, 1997). Increase of ROS generation by Ni exposure was suggested to increase Ca-dependent activation of NFAT, possibly contributing to cell transformation (Huang *et al.*, 2002b). However, it has been shown that the formation of ROS was not involved in the activation of other transcription factors and their downstream genes. For example, activation of HIF-1 and HIF-1–dependent

genes by Ni seemed to be independent of ROS generation (Salnikow *et al.*, 2000).

Ni induced the phosphorylation of ERK, JNK, and p38 in human monocyte–derived dendritic cells. These MAPKs were involved in Ni-induced CD83 and the interleukin-12 p40 production (Aiba *et al.*, 2003). It has also been shown that ERK, JNK, and p38 participated in the expression of CD83, CD86, and CCR7, therefore regulating maturation induced by Ni in human CD34(+)-derived DC dendritic cells (Boisleve *et al.*, 2005).

Exposure of Cl 41 mouse epidermal cells to Ni^{+2} resulted in activation of PI3K, Akt, and p70 S6 kinase (p70(S6k)). PI-3K/Akt pathway, but not p70(S6k), was required for Ni-induced HIF-1 transactivation and Cap43 induction (Li *et al.*, 2004).

It is well documented that Ni is able to mimic the hypoxic response. Ni can stabilize HIF-1α protein, and activate HIF-1, which in turn upregulates a battery of hypoxia-inducible genes (Semenza *et al.*, 1994; Wang and Semenza, 1993). By using the Affymetrix gene chip combined with Northern blots, a variety of genes have been shown to be upregulated or downregulated in an HIF-dependent manner when cells were exposed to Ni compounds (Salnikow *et al.*, 2002; 2003a; 2003b). For example, it has been shown that 1,4-alpha-glucan branching enzyme 1 (GBE1) was one of the most upregulated genes by Ni (Zhao *et al.*, 2004a). This gene was also upregulated by Co, hypoxia, the iron chelator (deferoxamine, or DFO), and the prolyl hydroxylase (PH) inhibitor (dimethyloxalylglycine, DMOG). Moreover, it was upregulated by these treatments only in HIF-$1\alpha^{+/+}$, but not HIF-$1\alpha^{-/-}$ mouse cells, suggesting that this gene was induced through a HIF-dependent hypoxic signaling pathway (Zhao *et al.*, 2004a). Similarly, Bcl-2-binding protein Nip3 (BNIP3) was upregulated by Ni^{+2} in a HIF-dependent manner (Salnikow *et al.*, 2003b). Serpina3g, a member of the mouse serpin family, was downregulated by Ni treatment through the HIF-1 pathway (Zhao *et al.*, 2004b). Although the mechanism of HIF-1 activation by Ni is not clear yet, several hypotheses have been proposed. For instance, it has been reported that the ability of Ni to stabilize HIF-1α protein is likely by inhibiting the posttranslational modifications of HIF-1α. The enzymatic activity of prolyl hydroxylases requires Fe as the activating metal, 2-oxoglutarate as a cosubstrate, and ascorbic acid as a cofactor. Ni may impair the activity of prolyl hydroxylases by depleting cellular Fe through its competition of Fe entry into cells through the DMT-1 transporter and/or by substituting for Fe in the HIF-prolyl hydroxylase (Bunn *et al.*, 1998; Chen *et al.*, 2005; Huang *et al.*, 1997). Ni also down regulated the transcription of the asparaginyl hydroxylase FIH-1 and acetyltransferase ARD-1 in A549 cells (Ke *et al.*, In Press).

Ni^{+2} activated NF-κB DNA binding and induced gene transcription of adhesion molecules ICAM-1, VCAM-1, and E-selectin in HUVEC (Goebeler *et al.*, 1995). It also induced mRNA production and protein secretion of the NF-κB–controlled proinflammatory cytokine IL-6 in a dose-dependent manner. Ni-induced activation of NF-κB and adhesion molecule expression was inhibited by the antioxidant pyrrolidine dithiocarbamate, indicating some involvement of redox-dependent mechanisms (Goebeler *et al.*, 1995). It has also been shown that Ni phosphorylated IκB and NF-κB in human monocyte–derived dendritic cells (Aiba *et al.*, 2003). NF-κB activity was also induced by Ni exposure in 3T3 and BEAS-2B cells, where NF-κB played a role in the induction of Cap43 by Ni. Conversely, similar treatment of cells with soluble Ni salts did not induce AP-1 activity (Huang *et al.*, 2002b). The effect of Ni on AP-1 is controversial though. It has also been shown that Ni-induced *c-jun* and *c-fos* mRNA levels, as well as increased phospho- and total c-Jun protein levels in BEAS/2B cells. This Ni-dependent activation of AP-1 was through an oxidant-independent pathway, and AP-1 was necessary for Ni-induced expression of plasminogen activator inhibitor (PAI-1) (Andrew *et al.*, 2001a). The Ni-induced NFAT activation seemed to be mediated by the generation of H_2O_2 by Ni, because the scavenging of Ni-induced H_2O_2 with N-acetyl-L-cysteine (a general antioxidant) or catalase (a H_2O_2 scavenger) abolished this activation. In contrast, pretreatment of cells with sodium formate (an OH radical scavenger) or SOD (an O_2^- radical scavenger) did not show any inhibitory effects (Huang *et al.*, 2001a).

6.3.6 Other Metals

In addition, some other metal compounds, such as vanadate (V), copper (Cu (II)), and iron (Fe (II)), have also been shown to impact signal transduction pathways. For example, V induced HIF-1α and VEGF by a PI3K/Akt-dependent, but a MAPK-independent, pathway in DU145 cells (Gao *et al.*, 2002). It also affected the expression of NFAT through H_2O_2 in JB6 mouse epidermal cells (Huang *et al.*, 2001a). V exposure activated AP-1 dose dependently, and O_2^-/H_2O_2 were involved in this activation (Ding *et al.*, 1999). It has been shown that Cu generated ROS in cells, but it activated PI3K independent of ROS (Ostrakhovitch *et al.*, 2002). Cu has also been shown to induce HIF-1α and VEGF levels (Martin *et al.*, 2005). NFAT and AP-1 could be induced in JB6 cells exposed with coal containing high levels of bioavailable iron or ferrous sulfate (Huang *et al.*, 2002a). Table 2 summarizes the signaling pathways and transcription factors induced by all of the metals discussed in this section.

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CHAPTER

6

General Considerations of Dose-Effect and Dose-Response Relationships*

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ABSTRACT

In 2003, the International Union of Pure and Applied Chemistry (IUPAC) issued a glossary of terms that included the definition of dose-effect and dose-response relationships (Nordberg *et al.*, 2004). Dose-effect relationship is defined as an association between dose and the resulting magnitude of a continuously graded change, either in an individual or in a population. Dose-response relationship is an association between dose and the incidence of a defined biological effect in an exposed population usually expressed as a percentage.

In this chapter, we introduce the concepts of dose, response, effect, and the relationships between them and the curves that illustrate them. Modeling the dose-response relationships, comparing the shapes of the curves, and modeling the data are also addressed. Thoughtful discussion is given to species extrapolations, including the complexities of gaining statistical power with limited-sized animal experiments. Benchmark dose is also introduced as an alternative metric to NOAEL (no observed adverse effect level) to determine an allowable exposure to toxic chemicals.

*This chapter is based on the chapter with the same title by Emil A. Pfitzer and Velimir Vouk in the 2nd edition of the Handbook.

Disclaimer: The findings and conclusions in this chapter are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention/Agency for Toxic Substances and Disease Registry or the Environmental Protection Agency.

1 GENERAL ASPECTS OF DOSE-RESPONSE RELATIONSHIPS

A dose-effect relationship exists if a change in dose of a chemical causes a quantifiable change in the effect observed. When the observed effect does not have a variable level within an individual, but is either present or not present (often called all-or-none effect), a dose-response relationship exists if the percentage of a population responding with that effect depends on the dose of a chemical. These statements are basic concepts of pharmacology and toxicology. Whether we are concerned with beneficial effects, as in pharmacology, or with adverse effects, as in toxicology, an increased dose of a chemical is expected to cause either an increased *effect* in an individual or an increased *response* in a population.

1.1 Use of the Terms Effect and Response

It is useful to apply the terms *effect* and *response* in the manner just specified, although semantic considerations could allow the two terms to be used as synonyms. This specialized use should not cause confusion but rather should provide a focus on a relationship with dose that is given either (1) by a graduated change

in a continuum of biological changes that can be quantitatively measured to provide an "effect" or (2) by the presence of a specified change in a certain proportion of the individuals in a population providing "response."

This distinction in terminology is needed for a clear and precise description of changes resulting from a dose or doses of a chemical. When a population of individuals receives a dose of a chemical, both effect and response occur simultaneously (i.e., some individuals respond with an effect of a certain magnitude, whereas other individuals respond with a greater or lesser effect). Furthermore, more than one kind of effect may occur at the same time. The need to differentiate the variations in magnitude and kind of effect from the variations in the number of individuals responding makes this use of the terms *effect* and *response* valuable. A set of hypothetical data is used to illustrate the interrelationships between effect and response.

1.2 Interrelationships Among Dose, Effect, and Response

First, consider the simplest situation for the observation of a dose-effect relationship. This would be for an individual subject in which a series of doses of a chemical would be administered; after each dose, a single kind of effect would be quantitatively determined. One could then plot the magnitude of the dose against the magnitude of the effect. This situation assumes that each successive dose is independent (i.e., the subject returns completely to the original state between doses). However, in most situations the effect has some residue that may change the magnitude, and possibly the kind, of effect resulting from the next dose. Therefore, it is generally necessary to use populations of individual subjects to quantity changes due to individual doses where each time the subject is naive.

Next, consider a study in which a population of 40 subjects is randomly distributed into four groups of 10 each, and each group of 10 is given a different dose of a chemical. The effect on each subject is then measured on a quantitative scale, and the values are plotted with dose as in Figure 1.

With such data as in Figure 1, it may be useful to make general statements (either graphical or mathematical) about the relationship with dose. Specifically, it is useful to interpolate between doses. Thus, one may plot (see Figure 2) a curvilinear relationship using the median effect at each dose (the term "median effect" meaning that 50% of the population responded with this or a lesser effect). Alternately, one could determine the percent responding at each dose by a defined level of effect and plot the relationships as shown in Figure 3. In addition, one could plot the distribution of effect for the population at a specific dose level, as shown in Figure 4.

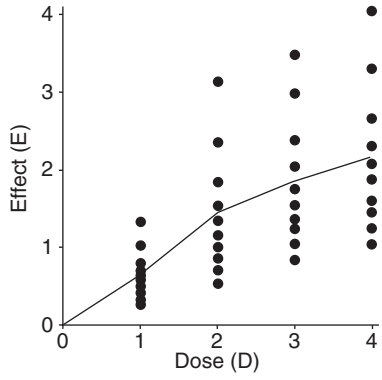

FIGURE 1 Hypothetical observations of effect on 10 subjects at each dose. The line is point-to-point based on the arithmetic mean of the values for effect.

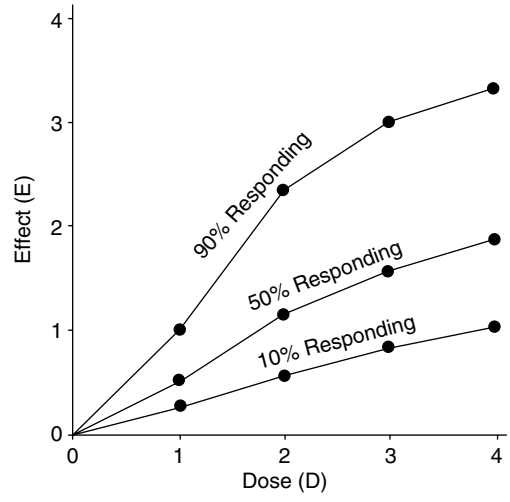

FIGURE 2 Point-to-point plots for the dose-effect relationships at equi-response based on data from Figure 1.

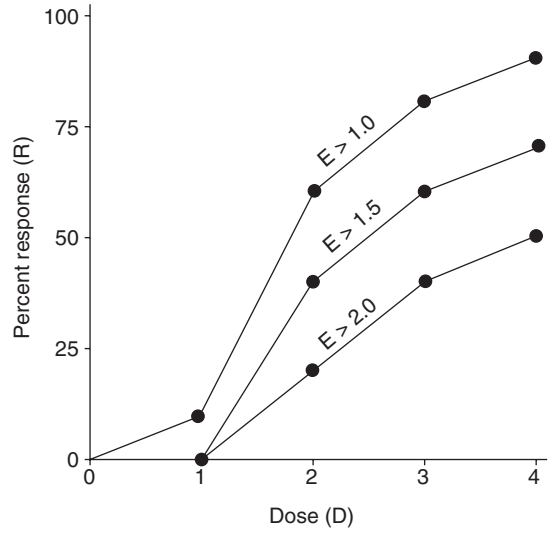

FIGURE 3 Point-to-point plots for the dose-response relationships at equi-effect based on data from Figure 1.

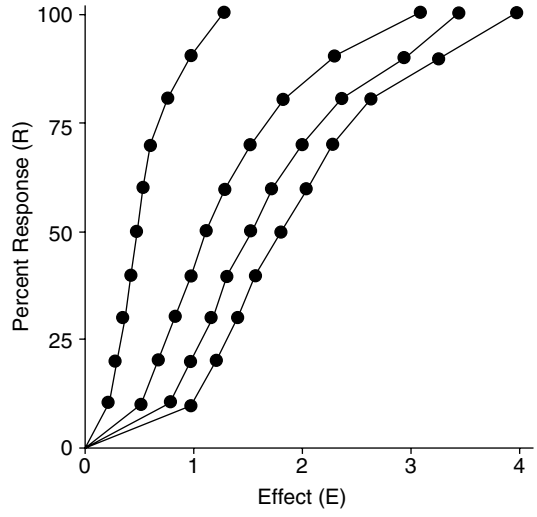

FIGURE 4 Point-to-point plots for the relationships between effect and percent response at equi-dose levels based on data from Figure 1, using dose levels of D = 1, 2, 3, and 4.

The interrelationship among dose, effect, and response has been presented as a three-dimensional model by Loewe (1959) and Hatch (1968). Although three-dimensional models are conceptually useful, the data necessary to construct them are not usually available, and such models are not considered further here.

Quantitatively, the generated relationship can then be used, after the application of several uncertainty factors, in estimating risk guidance values to humans when exposed to the same chemical. For example, the animal-derived dose-response relationship can be used to estimate the dose level at which no significant increase in response over background occurs. This dose level is referred to as the "No Observed Adverse Effect Level" (NOAEL) that is fundamental in the application of risk assessment to hazardous chemicals. Dose-response modeling in occupational epidemiology is usually motivated by questions of causal inference (e.g., Is there a monotonic increase of risk with increasing exposure?) or risk assessment (e.g., How much excess risk exists at any given level of exposure?).

Qualitatively, the shape of the dose-response curve can be indicative of underlying mechanisms. Usually, the shape of the curve varies along the dose ranges applied. In some instances, no response is observed until a dose large enough is applied to elicit a significant response. This dose is referred to as a "threshold." In other cases, there may be a gradual increase in the response as the dose increases. This increase may follow a linear or curvilinear relationship at a low dose. The magnitude of this increase may shrink as the dose range is elevated. At high enough doses, a further increase of response is observed as the dose gets larger. This describes "saturable" phenomena at higher doses, typical of many chemicals that require bioactivation by metabolism. Whether we assume that a dose-response has a threshold or follows a linear relationship at a low dose usually follows from our understanding of mechanism of toxicity. For example, low-dose linear responses are assumed when a chemical is found to be mutagenic. Instances of a "threshold" effect at a low dose are assumed whenever a chemical depletes the body's natural capacity to fight toxicity, perhaps by the inactivation of critical proteins as the chemical dose increases.

1.3 Definitions of Dose and Response

Determination of the dose-response relationship, which is the association between dose and the incidence of a defined biological effect in an exposed population usually expressed as percentage (Nordberg et al, 2004), depends on the numerical evaluation of both dose and response. Dose is defined as the total quantity of a substance administered to, taken up, or absorbed by an organism, organ, or tissue (Nordberg *et al.*, 2004) and can be measured with *in vitro* or *in vivo* experiments. When measured *in vivo*, doses are usually expressed as milligrams per kilogram of body weight in the case of oral exposure, as parts per million or billion (ppm/ppb*) in cases of inhalation exposure, or milligrams per square meter in cases of dermal exposures. *In vitro* dosing units will depend on the experiment that is usually described as concentrations (e.g., ppm or μg/L) in the medium where the experiment is conducted.

Effect can be defined as a graduated biological change in a continuum of changes that can be quantitatively measured. For biological effect to be useful in dose-response relationships it has to be readily quantifiable. It is common for a chemical to cause multiple effects, some effects occurring simultaneously in time, and some effects interacting with each other. The measured effect in the dose-response relationship has to also be independent from other exposures. This assumption of independence is important because it ensures the direct relationship between the dose and the measured response. If response depends on other responses caused by the chemical, it will be difficult to associate the response with the delivered dose.

The measured effect can quantitatively fall within one of the following:

*The SI unit for concentration of a substance in air is mg/m^3, which is ppm multiplied by molecular weight in grams divided by 24.45 (at 25°C and 1 atmosphere).

• Dichotomous (Quantal)—A dichotomous effect may be reported as either the presence or absence of an effect or change.

• Continuous—A continuous effect may be reported as an actual measurement or as a contrast (absolute change from control or relative change from control). In the case of continuous data, when individual data are not available, the number of subjects, mean of the response variable, and a measure of response variability (e.g., standard deviation [SD], standard error [SE], or variance) are needed for each group.

• Categorical—For categorical data, the responses in the treatment groups are often characterized in terms of the severity of effect (e.g., mild, moderate, or severe histological change).

Categorical variables represent data that may be divided into groups. The grouping is either nonordered (nominal) such as gender or race or ordered (ordinal) such as high, medium, or low temperature. Most effects of acute chemical poisoning can be cast into a categorical classification. This obviously applies to binary response data (e.g., mortality: dead vs. alive subjects; irreversible vs. reversible toxicity: presence or absence of congenital abnormalities, infertility, blindness, severe burns, necrosis). Categorical variables also applies to graded responses (e.g., a toxicological effect might have four levels of severity: no effect, mild effects, severe effects, and death).

2 MODELING OF DOSE-RESPONSE RELATIONSHIPS

The interrelationship among dose, time of exposure, and response is fundamental to the quantitative analysis of the dose-response relationship. Standard dose-response models are generally based on Haber's law or its generalizations. Dose (or concentration) and time of exposure are both important in determining the intensity of response to a toxic agent. For a given response intensity, Haber's rule ($c \times t = K$) has been proposed as a law of toxicology, but this rule is just one special case of a more general relationship $c \times t^m = K$, where *c* is the concentration, *t* equals time, and the exponent *m* is quite variable. For non-carcinogens, *m* generally has a value between 0 and 1, whereas for carcinogens, *m* is usually between 1 and 5. The absence of a universal value for *m*, or one that is generally applicable to different classes of toxicants, makes it not yet possible to develop a Haber-type rule with which to extrapolate successfully between exposure scenarios (Bunce and Remillard, 2003).

A common type of dose-effect relationship is one in which successive equal increments of dose cause a smaller and smaller increment of effect. Three examples of this type of relationship are illustrated by the logarithmic model, the power function model, and the equilibrium model (Figures 5, 6, and 7).

The logarithmic model is illustrated in Figure 5. There are several features of interest in this curvilinear relationship. First, the value for *b*, the base of the logarithm, must be greater than one and is most commonly equal to 10, as in the common (or Briggsian) system, or e (= 2.71828…), as in the natural (Napierian) system. Another feature is that for the simple logarithmic expression represented by curve 5A—whenever the dose is equal to one—the effect will always be equal to zero. If the model requires that the effect is zero when the dose is zero, the model can be amended by adding the constant 1 to the dose as illustrated by curve 5B. It may also be noted that curve 5A, but not curve 5B, will be a straight line when dose is plotted on the log scale of semilogarithmic graph paper.

The power-function model is also commonly used for dose-effect relationships. Figure 6 illustrates the power-function model for relationships in which the value for *n* is less than 1 but greater than zero (i.e., a positive fraction less than 1). Several features of the power-function model are noteworthy. First, the simple model described by $E = K(D)^n$ will always show a zero effect with a zero dose. A shift away from the origin may be accomplished by altering the model to $E = K(D + a)^n$, where *a* is some constant. Second, the power function model may be plotted as a straight line when both dose and effect are on a log scale or when they are plotted on a log–log scale. Thus, $E = K(D)^n$ becomes $\log E = \log K + n(\log D)$, where *n* can be seen to be the slope of the straight line and *E* is equal to *K* when the dose is equal to 1. Note that the zero dose–zero effect

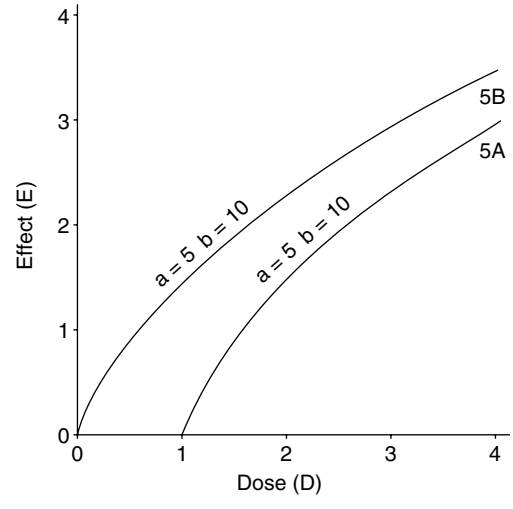

FIGURE 5 Examples of logarithmic models. Curve 5A, $E = \log_b D$. Curve 5B, $E = a\log_b(D + 1)$.

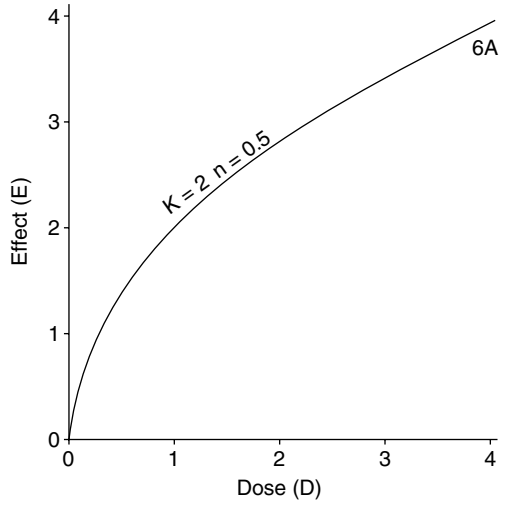

FIGURE 6 Example of a power-function model when 0<n<1. $E = KD^n$.

point cannot be plotted on log–log paper but nevertheless exists as a condition of the model. When = 0.5 as in curve 6A, the expression becomes $E = K(D)^{0.5}$.

Both the logarithmic and the power-function model share the feature that as the dose becomes infinitely large, the effect also becomes infinitely large. Many, if not all, biological systems have some maximum effect that they are capable of demonstrating and the maximum response in any population can be no more than 100%. Figure 7 illustrates two curvilinear relationships in which a maximum effect is approached asymptotically with increasing dose. These relationships may be described as steady-state models, because the effect approaches a steady state with increasing dose.

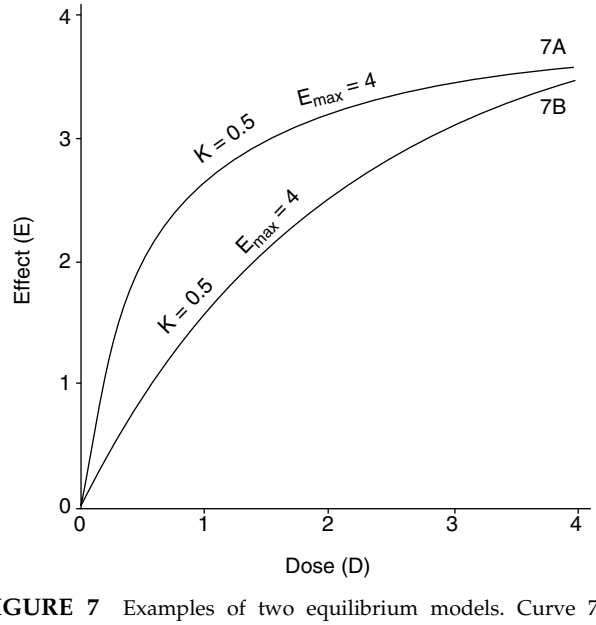

FIGURE 7 Examples of two equilibrium models. Curve 7A, $E = E_{max}[D/(D + K)]$. Curve 7B, $E = E_{max}[1-\exp(-KD)]$.

Curves 7A and 7B have three factors in common; they both originate from the origin (i.e., the zero dose–zero effect point) they both approach a maximum effect of four units asymptotically, and they both contain a single constant, *K*, with a value of 0.5. The difference between the two curves is obvious from their mathematical expressions. The derivation of the mathematical expressions is discussed in Section 3 dealing with possible biological bases for the relationships. It is of interest that curve 7A can be constructed as a straight line by plotting 1/D against 1/E. This is because $E = E_{max}[D/(D + K)]$ may be rewritten as $1/E = 1/E_{max} + [K/E_{max}][1/D]$. This plot allows the experimental determination of E_{max} from the y-intercept and subsequently also the value for *K* from the slope. This expression and procedure is identical to the Lineweaver–Burk plot used in the study of enzyme-substrate kinetics. Curve 7B may also be plotted as a straight line by plotting dose on a linear scale against the value of $E_{max}/[E_{max} - E]$ on a logarithmic scale. However, to do this, E_{max} must be known, and the value is often difficult to determine experimentally.

An additional important feature of curves 7A and 7B is that if dose is plotted on a logarithmic scale, the curves become sigmoid in shape, being asymptotic to zero effect and to the maximum effect. Additionally, the effect axis may be presented as a percent value. Thus, in Figure 7, effect values of 0, 1, 2, 3, and 4 would become 0, 25, 50, 75, and 100% effect values.

2.1 The Shape of Dose-Response Curves: S, Hormesis, U-Shaped

When successive equal increments of dose cause greater and greater increment in effect, the curvilinear relationship swings upward instead of downward. The exponential model and the power-function model are examples of such relationships.

The exponential model is illustrated in Figure 8. As with the logarithmic curves in Figure 5, the value for b must be greater than 1. At zero dose, the effect will always have a value of 1 for the simple exponential model described by $E = b^{a(D)}$ or $\log_b E = a(D)$ as seen with curve 8A. Curve 8A, but not curve 8B, will be a straight line when plotted on semilogarithmic graph paper with effect on the log scale.

The power-function model represented in Figure 9 requires that the value of *n* be greater than 1, whereas the previous power-function model illustrated in Figure 6 requires that the value of *n* be less than 1 but greater than 0. Curve 9, as well as curve 6A, will become a straight line when the data for dose and effect are plotted on log–log scale.

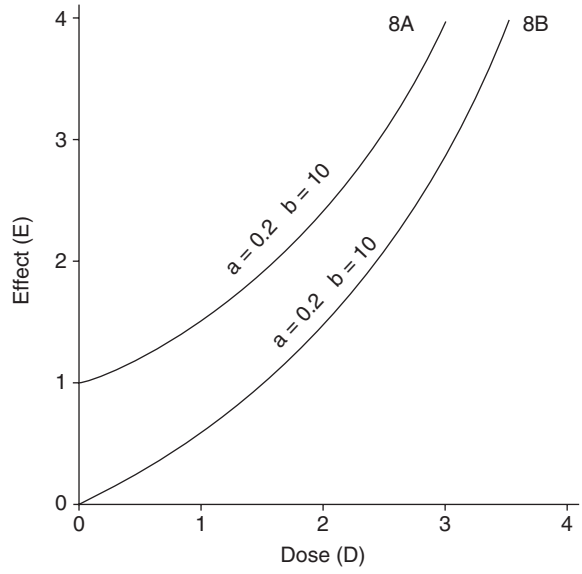

FIGURE 8 Examples of exponential models when b>1. Curve 8A, $\log_b E = a(D)$. Curve 8B, $\log_b (E + 1) = a(D)$.

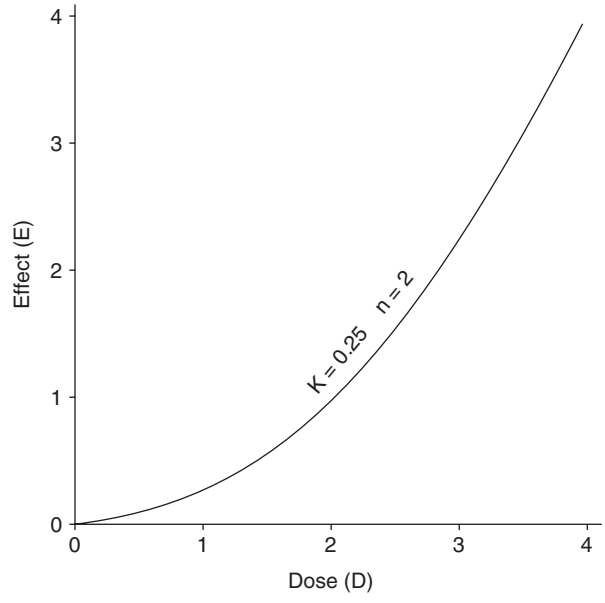

FIGURE 9 Example of a power-function model when n>1. $E = KD^n$.

2.2 The Sigmoid Curve

The sigmoid (or ogive) curvilinear relationship is often observed with dose-effect data and is almost always observed with dose-response data. This model as illustrated in Figure 10 has to have an upward swing (curvature) at the lower dose levels and a downward swing (curvature) at the higher dose levels. The middle range seems to be close to linearity and these curves may or may not be symmetrical.

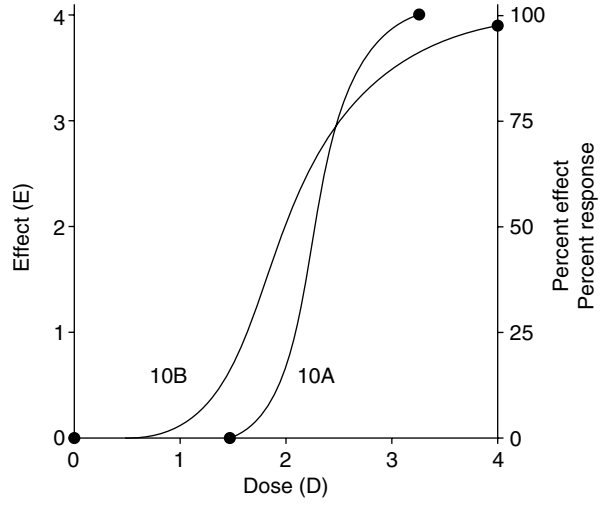

FIGURE 10 Examples of sigmoid models.

Two types of sigmoid curves are illustrated in Figure 10. The nature of the model is that there is an initial slow increase in effect with increasing dose, followed by a relatively linear or directly proportional increase in effect with dose, and finally another slow increase in effect with increasing dose, up to the maximum effect that the system can demonstrate. In curve l0A, the effect is zero until the value of the dose becomes greater than 1.5, and the maximum effect of 4 units is observed at a dose of 3.25; further increases in dose will produce only the same maximum effect of 4 units.

In contrast, curve l0B has characteristics similar to curve l0A, except that the curve begins at the origin (zero dose–zero effect) and is asymptotic to the maximum effect of 4 units. The graphical representation of Figure 10 does not provide a clear picture of a quantifiable value for effect at the lower dose levels, but the model for curve 10B requires that there is an extremely small, but finite, value for effect at infinitely small values for dose. The significance of this feature and the consequence of this contrast with curve 10A are discussed in later sections.

One of the most common explanations of the sigmoid or ogive curve is that a value on the effect scale includes the summation of all of the effects occurring at dose levels up to and including that dose. This explanation is particularly appropriate for the dose-response relationship. It was noted earlier that models with a maximum effect provide the possibility of converting the effect scale with graded units to a percent effect scale as illustrated in the right hand margin (Figure 10). The percent scale may alternately be defined as a percent response scale that would be necessary if the curves l0A and l0B are to be considered as dose-

response relationships rather than dose-effect relationships. For the dose-response relationship the sigmoid model, therefore, represents the cumulative frequency distribution of subjects showing an effect as a function of dose. A graphical representation of this statement is given in Figure 11. It is obvious that biological systems may include complexities that prevent any simple generalization by a graph or mathematical expression. On the other hand, understanding and communication of scientific observations are greatly enhanced by the presentation of general principles and models or mechanisms for observations.

2.3 Hormesis—Inverted U- or J-Shaped Curves

There is evidence to suggest that some toxic substances may also impart beneficial or stimulatory effects at low doses, but that at higher doses they produce adverse effects. This term "hormesis" (meaning to excite) was first coined in 1943 by Southam and Ehrlich (1943) in studies dealing with the effects of red cedar extracts on fungal metabolism and was later used more extensively to describe radiation effects. Numerous articles have been published that indicate that the hormetic dose-reponse model, which is characterized by a low-dose stimulation and a high-dose inhibition and is seen as either an inverted U- or J-shaped curve is common in the biomedical/toxicological literature, and is highly generalizable according to biological model tested, endpoint measured, and chemical class/physical agent used (Calabrese, 2002).

The form of this dose-response curve may be either of an inverted U or a J shape, depending on the endpoint measured. In cases of endpoints such as growth, longevity, fecundity, and cognitive function, the response would be seen as an inverted U shape. In the case of disease incidence, it would be seen as a J shape (Figure 12) (Calabrese, 2005).

In some situations, hormetic dose response is a normal component of the array of traditional dose-response relationships. Above NO(A)EL doses the hormetic dose response and the traditional sigmoidal dose response are the same. At below NO(A)EL doses the threshold response model assumes there is no real treatment-related difference between the control group response and the exposed group response below the NO(A)EL treatment.

2.4 U-Shaped Curves and Essentiality

The dose-response relationship for an essential substance such as vitamin or essential trace element

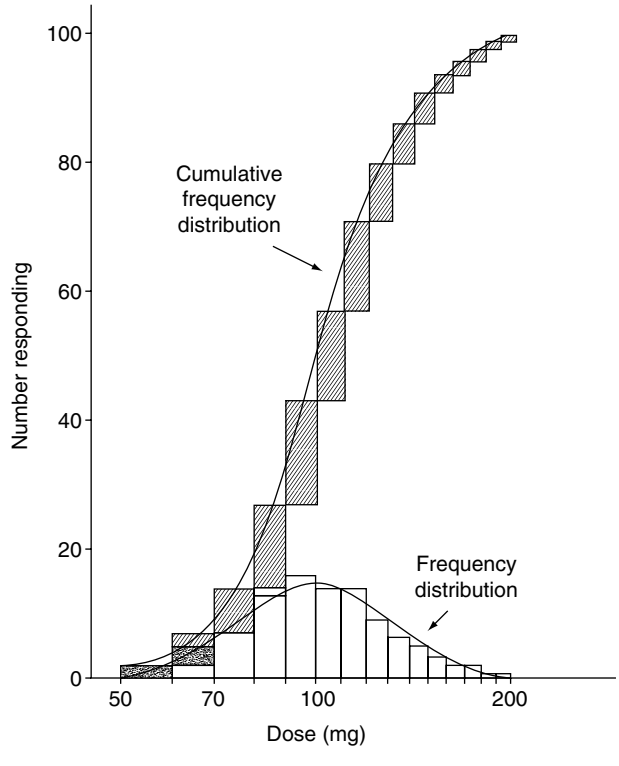

FIGURE 11 The relationship between the frequency distribution curve and the S-shaped cumulative frequency distribution curve (from Goodman and Gilman, 1975).

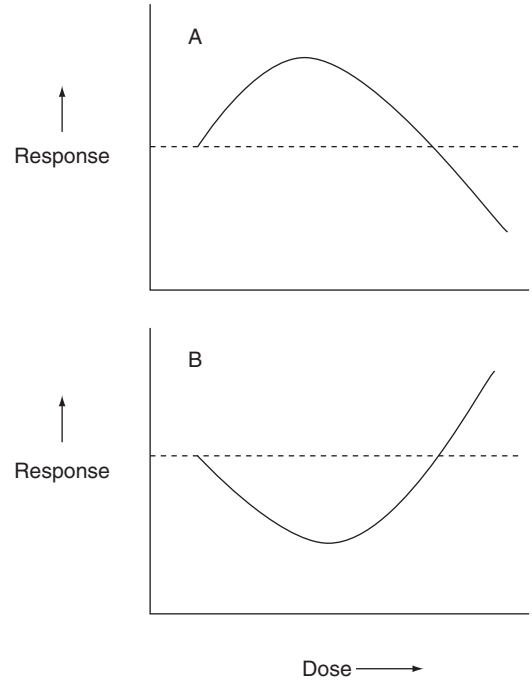

FIGURE 12 (A) The most common form of the hormetic dose-response curve depicting low-dose stimulatory and high-dose inhibitory response, the inverted U-shaped curve. Endpoints displaying this curve include growth, fecundity, and longevity. (B) The hormetic dose-response curve depicting low-dose reduction and high-dose enhancement of adverse effects. Endpoints displaying this curve include carcinogenesis, mutagenesis, and disease incidence.

is U shaped. At very low doses, there is a high level of adverse effect, which decreases with an increasing dose and similarly at very high doses there is a high level adverse effect, which decreases with decreasing doses. The region of the dose-response relationship at very low doses for essential nutrients is commonly referred to as a "deficiency" (Eaton and Klaasen, 2001, Chapter 9). As the dose is increased, the deficiency no longer exists, and no adverse response is detected, the organism reaches a state of homeostasis.

There are eight metals generally accepted as essential: cobalt, copper, iron, magnesium, manganese, molybdenum, selenium, and zinc. The dose-response relationships for these substances are all U shaped; however, the point at which deficiencies and contrarily poisoning occurs is specific to each metal (Nordberg *et al.*, 2000).

3 MODELING THE DATA

The strength of a scientifically based model is that it provides some understanding of possible mechanisms for the observed relationships. This provides confidence for extrapolations to other experimental conditions. In addition, one can intelligently design experiments to verify or to refine the proposed mechanism for the observed relationships. Difficulties in the use of models are: (1) inherent assumptions in the model may be false for the specific data; (2) the model may fit only a narrow range of values for either variable; (3) the data may actually fit one or more models equally well and; (4) the complexities of some biological systems may not be currently amenable systematic description.

When a model has been selected as appropriate for a given set of data, it is necessary to obtain the best fit of the data to the chosen curvilinear relationship. Best fit, in general, means that the variation of the actual data points from the curve will be at a minimum. There is also the implication that, because the data points do not usually fall exactly on the curve, some estimate of the variability, such as confidence limits, should be made.

The standard procedure for describing a set of data is the use of statistical descriptors such as mean and standard deviation (SD) or standard error. From these calculated quantities, additional statistical measures may be estimated such as the 95% confidence limits. The method for these procedures is well described in standard texts. Specifically, these statistical descriptors help in predicting events for a "large" population from a small sample, or subset, of the population. In the case of a simple linear model where one requires the curve to originate from the zero dose–zero effect point, a hypothetical set of data might provide the basis for the following statement: From the experimental set of data, the estimated slope of the dose-effect relationship for the true population is 1.0, and the true slope of the dose-effect relationship should be between 0.8 and 1.2, unless the experimental set of data was a random sample, which would not be expected more than once in 20 times.

It is important for the presentation of a dose-effect relationship to show the best fit, if a model is assumed, and to show the variation of the data in statistical terms. The preceding example expresses the variation over the full dose range in terms of the slope. When one has made multiple observations at the same dose, it is also possible to illustrate the statistical variation in effect at that dose. This is usually expressed as a mean and standard error and is often shown graphically as a vertical line on either side of the mean at the appropriate dose value. This is particularly useful for empirical curvilinear relationships and for visualization of the comparison of differences between several curvilinear relationships.

A frequently used metric for fitting a straight line to a series of data points is the least squares error function. Use of this metric leads to calculation of a slope that will make the sum of the square of the differences between observed effect and predicted effect a minimum. This square is used to simplify calculations by avoiding negative values for those points falling below the line. The simplicity of this method and the ease of calculations for a straight line rather than for a curved line provide the reasons why emphasis has been given to the ability to plot the various models as straight line relationships.

The previously described relationship between the sigmoid model and the probability curve has provided a basis for converting the sigmoid curve into a straight line. The SD, or normal deviate, of the dose (or log dose) provides an arithmetic progression on the dose scale relative to the mean ($\bar{x}$), i.e., $\bar{x}$ – 2 SD, $\bar{x}$ – 1 SD, $\bar{x}$, $\bar{x}$ + 1 SD, $\bar{x}$ + 2 SD, etc.

The cumulative frequency is a percentage that increases as the dose increases. Specifically, for a normal probability distribution, the approximate cumulative number of responders at a given dose will be 2.3% at $\bar{x}$ – 2 SD, 15.9% at $\bar{x}$ – 1 SD, 50% at $\bar{x}$, 84.1% at $\bar{x}$ + 1 SD, and 97.7% at $\bar{x}$ +2 SD, where $\bar{x}$ is the mean response at that dose.

For convenience in calculations, a unit called the *probit* was invented. The probit has a value of 5 $\bar{x}$, where the normal deviate is zero and the number of responders is 50%, a value of 6 at $\bar{x}$ +1 SD, where there are 84.1 % of the responders, a value of 4 at $\bar{x}$ – 1 SD, where there are 15.9% responders, etc. The probability or probit graph may have either a linear scale on the x-axis for normal frequency distributions or a logarithmic scale for

log-normal frequency distributions. Thus, the probit or log probit model is identical to the sigmoid model but has been transformed to a straight line (Finney, 1971).

In the earlier example of making the best fit of data to a model, it was assumed that variations at different doses had equal importance or weight. In the probit model, however, the observations are precise at the 50% response point and lose precision as response either increases or decreases. The least squares method has, therefore, been modified to provide a variable weight to the deviation as a function of the level of response and is called the "maximum likelihood" method. This method allows similar statistical statements of variation for the sigmoid or probit models, as was described for the linear model.

The logistic model is very similar to the probit model except that the frequency distribution follows the logistic curve rather than the probability curve. Further reference to the logistic model is made in the next section. The equation of the logistic function reads $P = 1/[1 + \exp(-(a + bx))]$.

With some of the simple curvilinear relationships, it was noted that simple adaptations could amend the model to fit a given experimental requirement. For example, the simple logarithmic model may be amended by adding the value of one to the dose so that the curve will include the zero dose–zero effect point. In a similar manner, the power-function model could be amended to account for a baseline effect by simply adding the value for the baseline effect as a constant to the experimentally expected effect. Models may also be amended to account for a threshold dose if the simple model required the zero dose–zero effect point.

It is also possible that a dose-effect relationship will not fit the same model over the entire dose range. There could be many biological reasons for the need to adapt the model at one dose range to a different model at another dose range, or at least to amend the constants of the model as a function of dose. For example, the homeostatic mechanism that alters effect may be exhausted and inoperable at higher dose levels. Enzymes may be inducible over certain dose ranges, leading to altered metabolism and altered effects. The chemical may be stored in depots with differing affinities for the chemical and different elimination rates, causing a multiphase change with time and/or dose.

Heterogeneity in the population being studied may cause an abnormal frequency distribution. For example, two populations differing in susceptibility may overlap in such a way that a composite of the two dose-response relationships would be required. Clearly, the dose-effect or dose-response relationship may be based on complex mechanisms that are not adaptable to modifications in a practical biological model. The experimenter must be alert to conditions that should not be forced into a simple model.

The problem of adapting a model to provide either for a threshold or for a zero dose–zero effect point requires particular care. In many cases, the experimental data itself will not be adequate to provide support for either possibility. In such cases, it is necessary to rely on the theory underlying the model or to refuse to extrapolate to conditions that cannot be experimentally validated.

The problem of the baseline effect is in large part the problem of the "normal" range. An individual may exhibit some range of effect values when tested repeatedly in the absence of a dose. The normal range may be considerably expanded when the experiment includes many different individuals. For most clinical laboratory measurements, the normal range is considered to be the 95% confidence limits around the mean value for a population sample that is considered to be either "healthy" or representative of the total population. The gray area between "normal" and "abnormal" may be subjective at times, and the limits of normality require careful definition.

The variation and magnitude of effect or response at zero dose can greatly confound interpretations of experiments. For example, an incremental increase of 5% response on top of a 0% response at zero dose may be relatively easily validated on statistical grounds. However, the same 5% incremental increase on top of an existing baseline response of 50% may be very difficult to differentiate from the "normal" variation around the 50% baseline value.

Figure 11 illustrates the relationships between the frequency distribution and the sigmoid model of a dose-response relationship. The biological basis for this model is the observed variability among individuals within a given population. The frequency distribution shows that a given effect will be demonstrated by a few individuals in the population at low doses and that a few individuals will be more resistant and will require very high doses before demonstrating the same effect. Most individuals will, however, demonstrate the effect in the middle range of doses as illustrated in Figure 13. The relative susceptibility or resistance is considered to be related to genetic differences, health status, differences in detoxification or elimination rates, differences in absorption rates, different volumes of distribution, or other biological variables that make one individual different from another. These differences are not sufficiently great to consider any individual as not belonging to the population, and the variation in a large population will approximate that predicted by a probability curve.

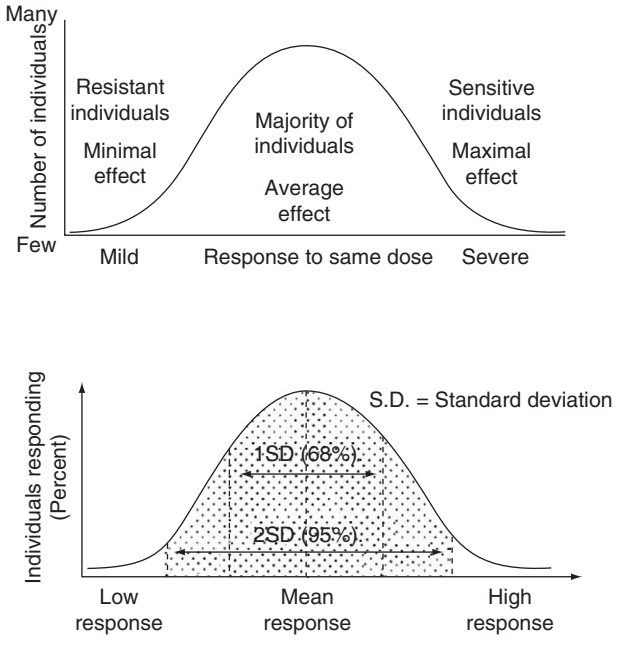

FIGURE 13 The top graph illustrates that within a population, most responses to a toxicant are similar; however, a wide variance of responses may be encountered, some individuals are susceptible and others are resistant. As demonstrated above, a graph of individual responses can be depicted as a bell-shaped standard distribution curve. As illustrated in the bottom curve, dose responses are commonly presented as mean + 1 SD (standard deviation), which incorporates 68% of the individuals. The variance may also be presented as two standard deviations, which incorporates 95% of the responses. A large standard deviation indicates great variability of response.

The frequency distribution may fit a symmetrical curve given by the equation, $y = \exp(-x^2)$, and is then known as a normal distribution. However, many frequency distributions in nature are observed to have a distribution skewed in the direction of higher doses. This skewness is particularly evident for effects caused by a mean dose that is close to zero. The restriction that zero dose is limiting for zero effect creates an inherent skewness to the frequency distribution. When dose is plotted on a log scale, however, the restriction of the zero dose is removed from the dose scale, and it is observed that many distribution frequencies in nature become normalized (i.e., more symmetrical) when log dose is used. Such frequency distributions are often called log-normal distributions.

Thus, the sigmoid model of the dose-response relationship has a biological basis depending on a normal or log-normal distribution of susceptibility of individuals to a given effect at different doses. It is important to remember that the effect in this case is an all-or-none event (i.e., the individual is either showing the effect or not: there is no gradation of the effect under consideration).

When a dose-effect relationship is observed to fit the sigmoid model, it might be postulated that the effect results from the cumulative reaction of many individual receptors having a frequency distribution of susceptibility. In other words, individual receptors, cells, or fibers may each demonstrate an all-or-none reaction at a given dose. If the cumulative reactions produce a type of effect that can be graded in a continuum, a dose-effect relationship of the sigmoid type may be constructed. If, however, the cumulative responses produce an effect in the individual that is also all-or-none, a population of individuals will be required to produce a dose-response relationship for the effect.

A feature of the probability curve, and consequently the corresponding frequency distribution, is that the response is asymptotic to the dose scale (x-axis). That is, by definition, a small fraction of the population will respond to even the smallest of doses, and, conversely, a small fraction that will never respond, even to the highest of doses. In practice, such observations may not exist, or they may not be seen because of limitations in the size of the population. This feature is illustrated in curves 10A and 10B. In curve 10A, there is a finite dose, often called the threshold dose, that must be exceeded before any response is observed, whereas curve l0B begins at zero dose–zero response.

3.1 Biological Basis for Modeling

The biological basis for the threshold is usually considered to be one or more of the following mechanisms:

1. The specific biological mechanism is not capable of producing the effect at or below the threshold dose.
2. Homeostatic, defense, and/or repair mechanisms are capable of countering the effect at the threshold dose in even the most susceptible subject.
3. The biological factors of absorption, transport, metabolism, and elimination do not allow a sufficient dose to reach the site of effect for the most susceptible subject of the population.
4. Any quantitative effect smaller than the effect of the threshold dose will not subsequently progress to that effect.

The biological basis for the no-threshold model is that the smallest possible dose (i.e., one molecule or one particle) may interact with a cell or cellular component, initiating a chain of events that ultimately produces the effect of concern. Pathogenetic mechanisms involving such a chain of events, usually involving self-replication, have been described for carcinogenesis and mutagenesis.

The basic problem of low-dose extrapolation is the selection of a mathematical model that is biologically

appropriate, so that we can have some assurance of its applicability both within and outside the range of measured dose-response values. The models currently used for low-dose extrapolation of data on carcinogenicity are of two kinds: dichotomous models and "time-to-occurrence" models. The dichotomous models relate dose to the probability of the occurrence (expected response) of a particular effect. The second group of models relates "time-to-occurrence" of an effect (i.e., duration of latency period) to dose.

The simplest dichotomous model that has found practical application and that has a biological basis for some effects is the linear or one-hit model. This model is based on the concept "that the effect can be induced after a single susceptible target has been hit by a single biologically effective unit of dose" (Hoel *et al.*, 1975). This concept leads to the following equation linking the probability (P) of an effect to the dose (D):

$$P(D) = 1 - \exp(-yD)$$

where y is an unknown parameter that has to be determined from experimental data, if possible. For small values of yD (i.e., for low doses) the equation reduces to a simple expression;

$$P(D) \approx yD$$

because exp(−x) can be expanded into a series $\exp(-x) = 1 - x + x^2/2! - x^3/3! + \ldots$, and when x is small, all terms involving x can be neglected except the first (i.e., x). A procedure for low-dose extrapolation based on this model has been described, for example, by Hoel *et al.* (1975).

A closely related model is based on the multievent theory of carcinogenesis proposed by Armitage and Doll (1961) and leads to the following equation relating the probability of effect (P_D) to the dose (D):

$$P(D) = 1 - e^{-(\lambda_0 + \lambda_1 D + \lambda_2 D_2 + \ldots \lambda_k D!}$$

where k is the number of transitional events in the carcinogenic process and λ_0, λ_1,…are unknown positive parameters. At low doses, the higher order terms in D^2, D^3, etc. may be neglected, and P(D) reduces to

$$P(D) \cong 1 - e^{-(\lambda_0 + \lambda_1 D)} \cong \lambda_0 + \lambda_1 D$$

where λ_o now represents the background response. A procedure for low-dose extrapolation based on this model has been suggested in an International Symposium on General Air Pollution and Human Health with Special Reference to Long-Term Effects, held in Stockholm, 8–11 March 1977 (Task Group on Air Pollution and Cancer, 1978) and was also used by the Safe Drinking Water Committee (NAS, 1977).

There are several other dichotomous models that have been proposed for low-dose extrapolation, the best known being the log-probit model of Mantel and Bryan (1961), the logistic model, and the model based on the Weibull distribution (see, e.g., Chand and Hoel, 1974). These models have, however, little biological basis, which is a serious limitation.

The use of such models involves a high degree of uncertainty, as is well illustrated by a comparison made by the FDA Advisory Committee on Protocols for Safety Evaluation (1971) (see Table 1). Three models, the probit, logit, and one-hit model, were compared under conditions of essentially equally good fit to experimental data. All three models gave identical responses of 50 and 16% at doses of 1 and 0.25, respectively; however, when extrapolation was made to responses as low as 1 in 10,000 or 1 in 1 million, the predicted doses differed by factors of approximately 10^3 and 3×10^4. Of all the models of this type, the one-hit model is the most conservative and will give the lowest doses corresponding to predetermined low responses. This may give rise to great practical problems when using the one-hit model, particularly if the substance considered has not been well established as a carcinogen.

The "time-to-occurrence of effect" models are based on the observation that the median latency period for some effects, for example, cancer, induced by some chemicals depends on the dose and increases with decreasing dose, whereas the individual latency

TABLE 1 Comparison of Three Models for Expected Response as a Function of Dose

Dose (in multiples of the Dose)	Probit model (%)	Logistic model (%)	One-particle model (%)
16	98	96	100
8	93	92	>99
4	84	84	94
2	69	70	75
I	50	50	50
0.5	31	30	29
0.25	16	16	16
0.125	7	8	8
0.0625	2	4	4
0.040	I		
0.022			
0.0155	0.1		
0.0144			
0.00315		0.1	
0.00144			0.1
0.00136	0.0001		
0.000412	0.000001		
0.0000098		0.0001	
0.00000144			0.0001
0.00000016		0.000001	
0.0000000144			0.000001

Adapted from FDA (1971).

periods are statistically distributed, either log normally (Blum, 1959; Druckrey, 1967) or according to the Weibull distribution function (see, for example, Pike, 1966). Albert and Altshuller (1973) have applied the Blum–Druckrey model to a variety of carcinogenesis data assuming that the dose (D) is related to the median "time-to-occurrence" of cancer (t) by the equation $t^nD = C$, where n is a positive parameter greater than 1, and C is a constant. The model based on the Weibull distribution seems to be better justified than the log-normal model used by Albert and Altshuller because of the theoretical basis given by Pike (1966). However, it seems that different data on cancer incidence would require different models and that there is no single model that could be applied to all types of data. The low-dose extrapolation should not be attempted unless the experimental data are biologically appropriate for such extrapolation. The following aspects should be given particular attention: the types of animal models used, the number of animals used both in the experimental and in the control group, the route of administration, the toxicity of the agent and side effects, the survival of animals in the course of the experiment, the availability of detailed pathological analysis of individual animals, and the possible presence of synergistic factors that can greatly modify the outcome of the experiment. The quality standards for long-term bioassays have been discussed in several publications (FDA, 1971; Health and Welfare, Canada, 1973; NAS, 1961; WHO, 1978).

4 SPECIES-TO-SPECIES EXTRAPOLATIONS

The relevance of experimental data to the assessment of the carcinogenic risk to man was discussed by an *ad hoc* working group of the International Agency for Research on Cancer (IARC, 1977). Information in the first 16 IARC monographs on the evaluation of the carcinogenic risk of chemicals to man showed that of approximately 25 chemicals or manufacturing processes generally accepted as being causally related to cancer in man, all of those that have been adequately tested produced cancer in at least one animal species. At present, 95 agents, mixtures, and exposures have been evaluated as being in Group 1: Carcinogenic to humans (IARC, 2005). For several chemicals the evidence for carcinogenicity was first obtained in experimental animals (IARC, 1977). Thus, there seems to be adequate evidence that chemicals carcinogenic in laboratory animals are carcinogenic in man. There is also tentative evidence for a quantitative relationship between the dose of a chemical that is carcinogenic in animals and the dose that is carcinogenic in man (Rall, 1979). There is no reason to assume that the same is not true for other toxic effects produced by chemicals. Thus, it seems justifiable to use animal data for predicting health risk in man.

The difficulties in quantitatively translating the toxicological information obtained from one species to another are many. The first problem is the large number of experimental animals that can be used. Normally, 100–1000 experimental animals are used in one experiment; the size of the human population to be protected may be as large as 10^6–10^8. Also, animal populations used in experiments are usually homogeneous, whereas human populations are largely heterogeneous. Laboratory animals are healthy, whereas the human populations to be protected include subpopulations that are ill and weak and that may, therefore, be particularly susceptible to the effects of a chemical. There are also differences regarding nutrition and the presence of other factors in the human environment that can modify the response to toxic chemicals. Furthermore, there may be differences in receptor sites. The size of different animals is also of concern, because it determines the number of susceptible cells and the residence time of the chemical in the organism. Thus, for the same dose in terms of mg/kg body weight, human tissues may be exposed much longer than mouse tissues. For this reason, it was recommended (Hoel *et al.*, 1975) the dose be expressed per unit body surface rather than body weight. This conversion of doses reduces the species differences considerably (NAS, 1977). The length of life is another variable that may largely influence the response. Man lives 35 times longer than a mouse, and, therefore, in man there are many more cell divisions and a better chance for neoplastic change. Thus, generally it seems that man is more susceptible to the effects of chemicals than the small rodents routinely used in the laboratory, although exceptions for some toxic effects exist.

Despite these difficulties in applying the information obtained in laboratory experiments to man, there seems at present no alternative to the current practice of using laboratory test results as a basis for human safety evaluation of chemicals, particularly as regards new products. Also, there are at present no generally valid criteria on which to base the interpretation of laboratory experiments in terms of human health risk, and we have to continue to rely on the experience and the expert judgment of trained toxicologists.

In addition to animal experiments, it is important to include the evidence from epidemiological studies in the evaluation of chemicals and their affects on man. Clear epidemiological evidence is often difficult to obtain because of questions of exposure amounts, duration, chemical mixtures, etc. In the past, most

epidemiological studies were limited to early worker exposure studies, which were cited as foundational for most toxicological and epidemiological studies for assessing dose-effect and dose-response relationships. More recently, however, community-based epidemiological studies have become more available and have yielded revealing results (i.e., Trasande *et al.*'s 2005 study of mercury emissions and blood mercury levels). A detailed discussion of epidemiological methods is found in Chapter 8.

Recent cancer risk assessment guidelines (USEPA, 2005) recommend that different approaches be adopted for determining an allowable exposure level for a carcinogen depending on whether it is thought to exert its carcinogenic effects in animals by a mode of action that has a threshold or nonlinear dose-response in the low-dose region. Part of that weight-of-evidence narrative includes consideration of the results from genetic toxicity studies. Although some of the reactions leading to genetic toxicity and tumor induction have biological thresholds (e.g., because of DNA repair or metabolic activation) or are nonlinear in the low-dose region, it has been generally assumed that the carcinogenicity dose-response is linear and without a threshold for chemicals testing positive in genetic toxicity studies. Furthermore, such a mechanistic assumption is predicated on having data to answer the question of genetic toxicity; however, discordant outcomes from genetic toxicity tests complicate the interpretation. The "positive/negative" interpretation of these tests must be considered in conjunction with the fundamental toxicological concept of dose-response and all additional mechanistic research to better determine relevance to human health. Moreover, USEPA guidelines for human cancer risk assessment (USEPA, 2005) acknowledge that a nonlinear dose response may be operable even when there are positive genetic toxicity data.

5 RISK ASSESSMENT AND DOSE-RESPONSE RELATIONSHIPS

5.1 NOAEL/LOAEL

Approaches for characterizing threshold dose-response relationships include identification of "no observed adverse effect levels" (NOAELs) or "lowest observed adverse effect levels" (LOAELs).

In Figure 14, the threshold represents the dose below which no additional increase in response is observed. The NOAEL is identified as the highest nonstatistically significant dose tested. The LOAEL is the lowest dose tested with statistically significant effect.

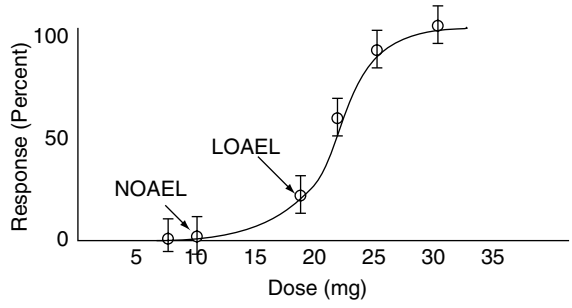

FIGURE 14 Dose-response curve.

5.2 Benchmark Dose

The benchmark approach is gaining attention as an alternative to the NOAEL approach. A benchmark dose (BMD) is defined as the statistical lower confidence limit on the dose producing a predetermined level of change in adverse response compared with the response in untreated animals (the benchmark response, or BMR) (Crump, 1995). It is determined by modeling a dose-response curve in the region of the dose-response relationship where biologically observable data are available. The BMR is generally set near the lower limit of responses that can be measured directly in animal experiments of typical size. There are multiple advantages to using BMD in place of NOAEL. The BMD method does not extrapolate to doses far below the experimental range. BMD is not constrained to using only one experimental dose. BMD accounts for variability in the data and incorporates response data from groups other than the experimental group determining the NOAEL. A BMD can be defined even when all experimentally observed responses would be considered effect levels (i.e., there is no NOAEL), and thus can avoid application of additional uncertainty factors. However, current guidelines for the design of toxicity tests are based on assessing a NOAEL. It has been suggested that the current study design may not be optimal for assessing a BMD.

To further investigate three simulation studies in which a large number of designs were compared focusing on continuous endpoints were performed (Crump, 1995). Four fictitious endpoints were considered, their underlying dose-response curves having a linear, sublinear, supralinear, or sigmoidal shape. In each simulation run, the BMD was derived from a model fitted to the generated data, where the selection of the model was based on that particular data set (according to a formal likelihood ratio test procedure). Thus, the model used for fitting the BMD in a single generated data set may not be the same as the one used for generating the data. In this way, model uncertainty is taken into account as well. The results show that the performance

of a design is first of all determined by the total number of animals used. Distributing them over more dose groups does not result in a poorer performance of the study, despite the smaller number of animals per dose group. Dose placement is another crucial factor, and to minimize the risk of inadequate dose placement, the use of multiple dose studies is favorable. As a concomitant advantage, the use of multiple doses mitigates the disturbing effect of potential systematic errors in single dose groups. However, for endpoints with large residual variation (CV≥18%), there is a substantial probability of not detecting the overall dose response, and this probability increases in designs with increasing number of dose groups. In such situations, six dose groups may be used as a compromise. Designs with high dose levels (i.e., associated with relatively high effects) are helpful in estimating doses with smaller effects (such as the BMD), and it seems bad practice to omit higher dose groups to improve the fit at lower doses. The typical 28-days study design of four dose groups with five animals (per sex) may not be adequate to assess endpoints with large residual variation (CV ≥ 18%), both in assessing a benchmark dose and in assessing a NOAEL (Bokkers *et al.*, 2005).

5.3 Data Types and Benchmark Dose

Although software for several continuous models is available (USEPA, 2005), there may be occasions when it is advantageous to convert continuous data to quantal data, for example, before applying a BMD analysis (an alternative to the NOAEL approach for assessing noncancer risks associated with hazardous compounds). In some cases, converting continuous data into quantal data may more directly address a specific definition of adverse response. For example, body weight measurements for individual animals could be converted to the incidence of animals that have more than 10% lowering of body weight, if this is the criterion for a significant response. Rendering data quantal may also facilitate comparisons among data sets if the majorities are in quantal form. A major disadvantage of continuous to quantal conversion is the loss of information about the magnitude of response.

For quantal data, an excess risk of 10% is the default BMR, because the 10% response is at or near the limit of sensitivity in most cancer bioassays and in some noncancer bioassays as well. If a study has greater than usual sensitivity, a lower BMR can be used, although the ED10 (the dose corresponding to a 10% increase in an adverse effect, relative to the control response) and LED10 (the 95% lower confidence limit of the dose of a chemical needed to produce an adverse effect in 10% of those exposed to the chemical, relative to control) should always be presented for comparison purposes. For example, reproductive and developmental studies having generational nested study designs often have greater sensitivity, and for such studies a BMR of 5% has typically been used; similarly, epidemiology studies often have greater sensitivities, and a BMR of 1% has typically been used for quantal human data.

For continuous data with a minimal level of change in the endpoint that is generally considered to be biologically significant (for example, a change in average adult body weight of 10%, or the doubling of average level for some liver enzyme), that amount of change can be used to define the BMR. The BMD and the benchmark dose level [BMDL] corresponding to a change in the mean response equal to one control standard deviation from the control mean should also be presented for comparison purposes; if individual data are available and a decision can be made about which individual levels can be reasonably considered adverse (perhaps on the basis of a quantile of the control distribution, for example), the data can be "dichotomized" on the basis of that cutoff value, and the BMR can be set as previously for quantal data.

In the absence of any other idea of what level of response to consider adverse, a change in the mean equal to one control standard deviation from the control mean can be used. The control standard deviation can be computed including historical control data, but the control mean must be from data concurrent with the treatments being considered (Crump, 1995). Crump (1995) found that this gives an excess risk of approximately 10% for the proportion of individuals below the 2nd percentile or above the 98th percentile of controls for normally distributed effects.

6.0 DOSE-RESPONSE IN AN ERA OF -OMICS

Dose response and dose effect are fundamental descriptors of events occurring after an exposure. Although the field of toxicology continues to explode with the development of new techniques, models, and "-omics" areas of study, these underlying concepts remain invaluable and are the common threads that link these areas together. Newer terms including "biological monitoring" and "biomarkers," which have become more common in recent years, incorporate the responses to exposures and include information on dose response and effect. An in-depth discussion of these terms is found in Chapter 4 (Fowler, 2005).

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CHAPTER

7

Interactions in Metal Toxicology

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ABSTRACT

Human exposures to metals and metalloids such as arsenic frequently occur as mixtures, and hence it is important to consider interactions among these elements in terms of both mechanisms of action and for risk assessment purposes. Interactions among these elements may produce additive, synergistic/potentiative, or antagonistic effects that may be manifested as direct cellular toxicity (necrosis or apoptosis) or carcinogenicity. Dose-response relationships may further be influenced by constitutive factors such as age, sex, and the expression of specific proteins. The roles of molecular factors regulated by specific genes (so called gene–environment interactions) for the expression of metal toxicity are known only to a limited extent for most metals. However, for chronic beryllium disease causing fibrosis of the lung, it has been shown that beryllium sensitization, a prerequisite for developing the disease, depends on an antigen-specific immune response occurring predominantly among persons with a specific HLA–DBP1 genotype. Some gene–environment interactions in terms of genetic polymorphisms have been demonstrated such as those involving ALAD and arsenic methyl transferases, but the importance of these observations for development of human diseases has not been fully explored. Mechanisms of importance for interactions and the development of toxicity are the expression of metal-binding proteins (metallothioneins or lead-binding proteins). In many cases, direct primary data on interactions among toxic or essential elements are lacking, and so innovative derivative methods such as the binary weight of evidence (BINWOE) method have been used to predict potential interactions among groups of metals and metalloids. At present, there is much to be learned about interactions among both toxic and essential elements, but this is clearly a critical area of research.

1 INTRODUCTION

Toxic effects of metallic compounds may be modified in a number of ways by various factors, changing the character of effects or dose-effect and dose-response relationships. Nutritional and physiological factors may affect susceptibility. Constitutional factors like age, sex, and other genetically determined factors, often called host factors, may also be of importance. Toxicokinetics of metals can also be modified by simultaneous or previous exposure to certain environmental agents. Direct interaction with the metal in question at the molecular level explains some interactions, whereas in other cases, an indirect metabolic change is induced by the interfering environmental factor that gives rise to the change in dose-response relationship. Such indirect interactions are sometimes of considerable interest even though the mechanism may not be known. Important interactions may also occur in the general environment and exposure media (i.e., air, water, and food), and they are dealt with in Chapter 13. These interactions and environmental transformations, to a large extent, determine the type of exposure for human beings. Such interactions are not discussed in this chapter.

For several decades, chemical and physical interactions of metals have been studied in experiments both *in vitro* and *in vivo*. There is an abundance of data on

such interactions, and no attempt will be made in this chapter to review all these data. Many results from such studies have found practical application in the field of animal nutrition, but their significance for imbalance of trace elements and for toxic effects of some metals in the human being has been difficult to evaluate. The experimental work has usually been performed in healthy animals of the same strain in carefully controlled environments. Often, the doses or exposure routes have been of little relevance to the human situation. Nevertheless, such studies may sometimes be indications of what might happen in man and may form a basis for further studies with more relevance to human beings.

At meetings in Tokyo in 1974 (Nordberg, 1976) and in Stockholm in 1977 of the Task Group on Metal Interactions (Nordberg, 1978), results from such animal studies were discussed, and several interactions of possible importance for humans were recognized. Further considerations of interactions important for carcinogenicity were discussed during a meeting in Atlanta in 1980 (Belman and Nordberg, 1981; Nordberg and Andersen, 1981) and in Geneva in 1983 (Nordberg and Pershagen, 1984). Considerations discussed during these working group meetings are summarized in this chapter and complemented by more recent information from a meeting in Atlanta in 2004 (Fowler, 2005). This latest meeting in Atlanta focused on reports of original scientific contributions, and joint consensus conclusions were not developed. There is a need for an in-depth discussion of public health implications of exposure to mixtures of metals and the role of metal interactions in risk assessment of such exposures with an aim to reach consensus conclusions. The lack of such a document makes it difficult for the present authors to present a review that fills this gap, and this chapter can only give the views of the present authors. There is hence a pressing need for an international meeting to discuss interactions among metals and to reach a consensus, if possible.

Host factors such as age, sex, race, and other genetic factors are well recognized as modifying factors for the occurrence of adverse effects in humans. The following text gives a brief general discussion of such well-recognized factors. Emerging knowledge is reviewed about the influence of variation (i.e., genetic polymorphism) in specific identified genes for the occurrence of disease and other adverse health effects of metal exposures; a section on risk assessment of mixtures is included. As an introduction to these sections, some fundamental concepts will be introduced.

At the Tokyo meeting, the term "interaction" in the present context was considered to be a process by which metals in their various forms, or other factors, change the critical concentration or a critical effect of a metal under consideration. At the Stockholm meeting, this definition was accepted, and it was emphasized that the term "interaction" should be used to also describe the influence of these other substances, host factors, and external factors such climate. In the section of this chapter dealing with gene–environment interactions, the term interaction is used in a similar way, meaning how variation in specific genes may modify the dose-response relationship (describing the relationship between exposure or dose of a metal and the occurrence of disease or other adverse effects).

Finney (1971) described the classical mathematical interpretation of joint action of chemicals.

An example of a joint action (i.e., not a true interaction) occurs when two metals exert their action at the same site and when their modes of action are similar, but they do not influence the action of each other. The result of such joint action is an *addition* of effects that can be estimated from the dose-effect and dose-response relationships of the constituents of a mixture. Such types of joint action seem to apply to arsenic and lead in relation to coproporphyrin excretion (Fowler and Mahaffey, 1978). There are other types of joint actions in which the dose-effect or dose-response curve of the combination cannot be assessed from those of the individual chemicals (e.g., synergism and antagonism). Models have been developed later to describe the interaction between different factors (Breslow and Day, 1980). Under the assumption of an additive model and linear dose-response relationships, synergism takes place when the response of a combined exposure is greater than additive. A multiplicative action represents a special form of synergism. Further considerations on statistical aspects are given by Greenland and Rothman (1998) and in Section 6 of this chapter.

2 AGE, SEX, DRUGS, AND SOME OTHER FACTORS

2.1 Influence of Drugs, Alcohol, and Tobacco on Metal Metabolism and Toxicity

2.1.1 Drugs

Oral contraceptives are among the drugs taken by a large number of healthy women. Intake of such synthetic hormones is known to cause changes in copper metabolism, influencing the synthesis of ceruloplasmin and bringing about higher serum concentrations of copper. Zinc metabolism and iron metabolism are also influenced by oral contraceptives. In studies on women exposed to metals such as cadmium and lead, which might interfere with the metabolism of essential

metals, the use of contraceptive pills should be taken into account. Some of the drugs for the treatment of hypertension, which essentially act as chelating agents, also affect metal metabolism. Prolonged treatment with some of these may increase zinc excretion, but not cadmium excretion. Thus, such treatment may, in the long run, cause changes in the cadmium/zinc ratios. The use of chelating agents such as DMSA and penicillamine in the treatment of metal poisoning is a way of using interactions for therapeutic purposes (for details, see Chapter 15). Attempts have also been made to change the kinetics of methylmercury by the use of resins that prevent the reabsorption of methylmercury excreted through the bile (Chapter 15).

2.1.2 Alcohol and Tobacco

Ethyl alcohol has been shown to cause an increase in the absorption of lead. This might be of importance when evaluating dose-effect relationships in people exposed to lead in illicitly distilled alcohol. In cases of lead poisoning caused by such consumption, the symptoms in the central nervous system (CNS) seem to be more severe than what would be expected from lead exposure alone. Thus, some synergistic action between alcohol and lead might exist.

Consumption of large amounts of alcohol can temporarily depress ALA-dehydratase activity, which is also a sign of lead toxicity. However, it has also been shown that ALA-dehydratase activity may increase in animals exposed to both lead and alcohol. The depression caused by either of the substances is partially reversed when the two substances are combined (Chapter 31).

Alcohol influences the metabolism of mercury after exposure to mercury vapor. This is thought to be due to the interference of alcohol with some of the enzymes taking part in the conversion of mercury vapor to mercury (II) (Nielsen-Kudsk, 1965; Chapter 33). Simultaneous exposure to cobalt and alcohol has caused so-called beer drinker cardiomyopathy, where the effect was greater than would be expected from the single action of the compounds (see Chapter 25).

Smoking may affect the uptake and effects of metals in several ways. A discussion in relation to carcinogenesis of arsenic compounds is given in Section 3. The content of carbon monoxide, various organic compounds, and a number of toxic metals (e.g., cadmium, nickel, mercury, and lead) are probably of importance in explaining the increased carcinogenicity of combined exposures to carcinogenic metals/metalloids and smoking, compared with single exposures, as show in several studies performed in the working environment (e.g., Lundstrom *et al.*, 2006).

2.2 Influence of Age and Sex on Metal Toxicity

2.2.1 Age

It has long been claimed that certain age groups are at increased risk (i.e., young children and the elderly). During the past decade, quantitative data have become available to document these claims. Several investigations have dealt with young animals and children, and data on elderly people are also forthcoming. The fetus is regarded as especially vulnerable, because many functions are not fully developed until after birth, and several possibilities exist for contact between the fetus and toxic metals. The toxic effects caused by metals on development of the fetus are discussed in Chapter 12. Methylmercury is known to cross the placental barrier and to accumulate in the fetus. It is well documented that fetal exposure to methylmercury can cause intrauterine methylmercury poisoning (Chapter 33). Lead crosses the placental barrier, and prenatal lead exposure may play a role for later development. The fetal brain does not tolerate lead to the same extent as the adult brain does (cf Chapters 16 and 31). Extensive animal studies have shown that newborn and suckling animals have higher absorption of certain toxic metals, like lead and cadmium, because of their dependence on a milk diet. The neonate also has higher whole-body retention, higher blood levels, and a much higher accumulation in the brain compared with the adult exposed to similar doses (cf Chapters 12 and 31). Exposure of animals to lead in the early neonatal stages can cause changes in behavior, learning deficits, and increased motor activity. There are also data demonstrating that children develop signs and symptoms at lower blood-lead concentrations and have a higher absorption of lead than adults. Because the calorie intake per person increases rapidly during the period 6–20 years of age, it follows that the daily intake of a metal (e.g., cadmium) also increases during this time (cf Chapter 3). The urinary excretion of cadmium has a peak in the age range 6–12 years, followed by a second peak at ages 50– 70. The first peak is explained by the increase in per person intake in the mentioned age range and the growth rate of the kidney, implying that a peak in kidney cadmium concentration occurs. The later higher peak at age 50 is explained by the gradual accumulation of cadmium by age because of the long biological half-life of this metal in the human kidney (Choudhury *et al.*, 2001).

2.2.2 Sex

When women are referred to as a high-risk group, it is often meant that the exposure of pregnant women results in the exposure of the fetus. Such aspects are discussed in Chapter 12. The influences to be dealt

with here concern only those directly related to the differences in sex. Data from animal experiments are to some extent conflicting. Lead acetate (Kostial *et al.*, 1974) and cadmium chloride (Engstrom and Nordberg, 1978) have been shown to have higher acute toxicity in male than in female animals. On the other hand, compared with male rats, female rats were shown to retain twice as much cadmium after a single oral dose (Kello *et al.*, 1979). Because castrated males absorbed as much as the females, it was then concluded that male sex hormones were responsible for the decreased retention of cadmium in males. Pregnancy may create a change in sensitivity to metal exposure. Parizek (1965) found that pregnant rats were more susceptible to the action of injected cadmium than nonpregnant rats. These and other aspects on pregnant animals are discussed in Chapter 12.

Adult women before menopause lose iron with menstrual blood, and there is a high prevalence of low iron stores in this population group. Because it is known (cf Chapter 23) that persons with low iron stores have an increased gastrointestinal uptake of cadmium, women often accumulate more cadmium than men. Higher blood cadmium values have been reported among women than men in many epidemiological studies.

2.3 Influence of Some Other Factors on Metal Metabolism and Toxicity

There are important interactions between metals and nutritional factors, but this chapter cannot review all these interactions. Nutritional factors in terms of intake of essential metals will be discussed in Section 4 of this chapter. Several other dietary components have been shown to have a considerable influence on metal toxicology. Phytate in certain diets causes a reduction in the absorption of zinc and renders zinc less available from vegetable diets than from diets based mainly on meat. The protein content of the diet has been shown to play a role in cadmium and lead toxicity (i.e., the lower the protein content, the higher the absorption and the more severe the signs of toxicity), but these and other factors will not be reviewed here. The reader is referred to general textbooks of trace elements and nutrition (Mertz, 1987).

Because sweat is an important route of excretion for some metals (e.g., zinc and nickel), metal losses through sweat might influence metabolism and toxicity. Workers exposed to nickel in a hot environment had considerably lower nickel levels in blood than expected (Szadkowski *et al.*, 1969). The clearance of inhaled particles from the lung depends on the proper function of the mucociliary transport system. Smoking cigarettes over a long period and bacterial and viral diseases can impair this transport system (see Chapter 3). A decreased clearance of inhaled particles might increase the absorption of certain metals or prolong their retention time in the lungs. Simultaneous exposure to irritant gases, like SO_2, and metals may affect the distribution and toxicity of these metals. Another part of the pulmonary defense system consists of the alveolar macrophage, the function of which might be depressed by exposure to nitrogen dioxide. This might result in prolonged retention time and thus a greater absorption of slowly soluble metallocompounds in the alveoli.

3 GENE–ENVIRONMENT INTERACTIONS FOR METALS

It is generally known that there is a large interindividual variation within species in sensitivity to metal exposure. Different types of studies have shown that part of this variation in susceptibility is due to genetic causal components (e.g., studies of human twins, families, or epidemiological associations), as well as animal studies on knockout mice or inbred rodent strains (examples are given in the following for the different metals). This type of interaction in which a genetic component is thought to modify the exposure-disease relationship is often referred to as *gene–environment interaction* and the genes involved as *susceptibility genes*.

For most metals it is still totally unknown to what extent genetic components predispose for susceptibility to toxicity and which specific genetic factors are responsible. This is mainly due to the shortage of studies addressing these questions, which in turn is the result of lack of knowledge about mechanisms of metal action. Another reason for limited data is previous technical limitations. However, the molecular genetic technology has lately developed greatly, and there are now many fast tools for detecting genetic variation and performing association studies.

3.1 Genes of Concern

The metal susceptibility genes differ in several aspects from the so-called high penetrance genes that have been identified for some rare metal-associated hereditary diseases (e.g., Wilson or Menkes diseases) characterized by impaired copper homeostasis. Typically, high penetrance gene mutations associated with disease are rare (i.e., they have a frequency lower than 1% in the population). For example, the gene frequency for Wilson disease varies between 0.3 and 0.7% worldwide (Gollan and Gollan, 1998) and is the result of mutations in the *ATP7B* gene (Bull *et al.*,

1993; Tanzi *et al.*, 1993). This type of mutation has high penetrance, meaning that it confers a high absolute risk, irrespective of environmental factors. Hereditary hemochromatosis is a more common (gene frequency of approximately 5% in the Caucasian population) genetic disease (Cartwright *et al.*, 1979; Lalouel *et al.*, 1985). It is caused by mutations in the *HFE* gene, which leads to increased iron uptake from the gastrointestinal tract (Feder *et al.*, 1996). However, a recent study suggests that *HFE* mutations have much lower penetrance than what have previously been estimated (Beutler *et al.*, 2002). These aforementioned genetic diseases will be covered elsewhere in this handbook (namely, Chapter 26 on copper and Wilson and Menkes diseases and Chapter 30 on iron (hemochromatosis) (apart from some aspects on hemochromatosis, which will be mentioned here in relation to lead and cadmium).

In contrast, genetic variants of susceptibility genes generally have a low penetrance but are more frequent. Genetic variants that are present in greater than 1% of the population are called polymorphisms. When reviewing the literature, risk effects of odd ratios of 2–3 or less for different diseases are found for most susceptibility genetic variants, and, thus, there is much greater scope for interaction with specific exposures. However, although the individual risk for carrying a susceptibility variant might be increased by a small amount, a common polymorphism in the population may result in a substantial proportion of toxic effects attributable to this type of genetic trait.

The genetic variants influencing susceptibility to toxic effects by metals could be of many different kinds: they might affect the metal biotransformation by absorption—as suggested for lead and *HFE* polymorphisms (Wright *et al.*, 2004), metabolism—as exemplified for arsenic and genetic variants of the methyltransferase gene *CYT19* (Meza *et al.*, 2005), or excretion. They could also alter the cellular import and export of metals. Genetic differences in the defense against oxidative stress or DNA repair capacity probably modify the toxic effects of different metals as well. Because many metals are provoking hypersensitivity reactions (e.g., gold, mercury, beryllium, and nickel) (Garner, 2004), major histocompatability complex genes involved in presenting metals to the immune system are presumably of relevance, as has been shown for beryllium and the human leukocyte antigen (HLA) variant *HLA-DBP1* (Richeldi *et al.*, 1993), as well as other parts of the immune system.

Another type of genetic factors that might be important for individual susceptibility to metal toxicity is mediated by so-called epigenetic elements that can change genome function under exogenous influence. Epigenetic factors regulate changes in gene expression that are not transmitted by the DNA sequence itself, but by different modifications of the DNA nucleotides or DNA-associated proteins like the histones. DNA methylation patterns of the cytosines are the most studied epigenetic markers. Arsenic, cadmium, and nickel are affecting the methylation pattern of DNA (Chen *et al.*, 2004; Costa *et al.*, 2001; Takiguchi *et al.*, 2003). However, the impact of epigenetic factors on predisposition for metal toxicity is at present completely unknown.

3.2 Design of Gene–Environment Interaction Studies

There are many aspects of how to design a gene–environment interaction study; a few are mentioned in the following. For a more comprehensive overview, Vineis and Malats (1999) is recommended. Today, many gene–environment studies are based on the genotype for information of the phenotype (i.e., characteristics of an individual). Genotyping has several advantages over phenotypic analyses: the genotype is stable over time and not influenced by environmental factors, very small amounts of DNA are needed, and the technique is not as cumbersome. However, the genotype–phenotype association is not established for most polymorphisms. Probably, many genetic variants result in no or very limited effect on the phenotype. Thus, when no phenotypic information is present, careful selection of polymorphisms should be conducted based on position within the gene sequence (e.g., whether or not it results in an amino acid exchange or might regulate the expression of the protein). It is also important to note that the geographical distribution of polymorphisms varies worldwide, and thus, gene–environment interactions can differ between ethnic groups. For example, the frequency of the F allele of the *Fok*1 polymorphism of *VDR* was much higher among the African-American children than the non-African-American children in a study on *VDR* polymorphisms (Haynes *et al.*, 2003). The interaction observed for this *VDR* genotype and floor dust lead was not significant for African-American children, but it was significant for non-African-American children. In general, genetic effects are more easily observed in homogenous rather than mixed populations, and ethnic origin should, when possible, be taken into consideration.

Genetic factors for metal toxicity are most often handled as effect modifiers of the exposure-disease relationship. The interpretation of genotype as an effect modifier implies that studies should be planned to have sufficient power for detecting interactions and that an interactive term should be modeled into the statistical analysis. (However, it is important to note that interaction with one genetic variant does not warrant

conclusions on a causal relationship with the biochemical mechanism, because the genetic variant may be linked with the causal gene.) Another fact that stresses the importance of sufficient statistical power is that, in general, genes act in concert to exert effects. Therefore, effects on one gene may induce compensatory changes in others. Moreover, many metabolic enzymes have overlapping substrate specificities. Thus, there are several grounds for analyzing interactions between different gene loci, so-called gene–gene interactions. However, only a limited number of studies have until now examined the combined effects of two or more genes in relation to metal toxicity. One example is beryllium in which the combined effect of specific variants of HLA DPB1 and DRB1 and tumor necrosis alpha (TNF) have been analyzed in relation to clinical severity (Maier *et al.*, 2001). Considering multiple genes is more biologically relevant, but for these studies, a large number of individuals are needed. Moreover, it is necessary to formulate a strategy for handling the problem of multiple comparisons that very quickly arise when analyzing the effects of a large number of polymorphisms in different exposure groups, in different subgroups, or for different markers of effect. Simple corrections for multiple testing (e.g., Bonferroni correction) are not very fruitful when analyzing several hundreds of polymorphisms, rather it has been suggested that the prior credibility of the hypothesis is important for interpreting a set of observations. New statistical methods for genetic association studies are needed, and a recent article illustrates how a Bayesian approach can be used to estimate the probability that a positive association is false (Wacholder *et al.*, 2004).

3.3 Interactions for Specific Metals

A review of what presently is known about gene–environment interactions for different metals follows. For most metals apart from a few exceptions (i.e. beryllium, lead, and to some extent arsenic) there are no or only a few reports on gene–environment interactions. However, the literature within this field is rapidly growing.

3.3.1 Arsenic

Gene–environment interaction studies for this element have mainly focused on genes involved in the metabolism of inorganic arsenic. Of the total amount of arsenic excreted through the urine, 10–30% is usually inorganic, 10–20% monomethylated arsenic (MMA), and 60–70% dimethylated arsenic (DMA). However, there is a marked variability in metabolism of inorganic arsenic within and between population groups (Vahter, 2000). In some areas of Taiwan, people excrete an unusually high percentage as MMA in the urine (20–30%), whereas certain populations in Argentina and Chile only excrete a few percent as MMA. Support for the idea that part of this variation is determined by genetic causal components comes from a correlation study for arsenic performed within families (Chung *et al.*, 2002), showing that siblings have a higher correlation of arsenic methylation capacity than their parents. Moreover, there are two studies addressing intraindividual variability in arsenic methylation pattern, showing that arsenic methylation pattern remains relatively stable over time, suggesting that the predominant factors are either genetically influenced or associated with long-term environmental factors, such as dietary habits (Concha *et al.*, 2002; Steinmaus *et al.*, 2005).

Evidence for genetic effect modification on arsenic metabolism comes from studies on the methyltransferase CYT19 (also labelled AS3MT) that is believed to catalyze the methylation of arsenic to MMA and further to DMA. This enzyme is best characterized in rat (cyt19) (Lin *et al.*, 2002; Thomas *et al.*, 2004) but is not well defined in humans (Hayakawa *et al.*, 2005). Meza and colleagues showed in a study from Mexico that *CYT19* displayed three variants that were strongly associated with the second methylation index (DMA/MMA) among children but not in adults (Meza *et al.*, 2005). This finding suggests a role for polymorphisms regulating developmental differences in arsenic biotransformation. However, there was a difference in ethnicity between the groups: the children were to a larger degree of Indigenous and the adults of European ancestry. Wood *et al.* (2006) estimated the functional impact on AS3MT enzyme activity of genetic variants occurring in exons. However, for intronic polymorphisms, like those reported in Meza *et al.* (2005), their function on AS3MT activity is still completely unknown.

It is generally thought that many of the toxic effects associated with arsenic exposure were mediated through arsenic-induced oxidative stress. Thus, Ahsan *et al.* analyzed whether polymorphisms in the oxidative stress–scavenging enzyme catalase and the phase I metabolic enzyme myeloperoxidase (MPO) are associated with increased risks of hyperkeratosis in a highly arsenic-exposed population in Bangladesh (Ahsan *et al.*, 2003). The results indicated that the low-activity variant of the catalase was overrepresented among the cases of hyperkeratosis. Similarly, the variant of MPO producing an increased amount of the reactive oxygen species hypochlorous acid was overrepresented among the cases. Ahsan and coworkers also published data suggesting that genes involved in DNA repair, removing oxidative stress–induced DNA damage, may influence the risk of arsenic-induced

premalignant hyperkeratotic skin lesions (Ahsan *et al.*, 2003). However, in both studies the results were based on very small study populations. Genes involved in oxidative stress have also been shown to modify the risk for arsenic-related cardiovascular effects (Hsueh *et al.*, 2005, Wang *et al.*, 2006).

3.3.2 Beryllium and Cobalt

Genetic susceptibility for beryllium-associated sensitization and subsequent chronic beryllium disease (CBD) has been described and is maybe the clearest example of a gene–environment interaction for a metal. The immunopathogenesis of CBD and beryllium sensitization depends on the development of an antigen-specific, cell-mediated immune response (Saltini *et al.*, 1989). Beryllium-specific $CD4^+$ T cells probably recognize a form of beryllium as an antigen, acting in combination with MHC class II molecules on antigen-presenting cells. In a key study, Richeldi *et al.* (1993) reported an increased prevalence of HLA-DPB1 with glutamic acid in position 69 (Glu^{69}) in cases of CBD (97%) compared with beryllium-exposed nondiseased controls (30%). These results were later confirmed by further studies, although the frequency of the Glu^{69} variant was somewhat lower (Maier *et al.*, 2003; Rossman *et al.*, 2002; Saltini *et al.*, 2001). The results from these studies show that specific Glu^{69}-containing alleles and their copy number (homozygous or heterozygous) confer the greatest susceptibility to CBD in exposed individuals. However, because 15–20% of CBD patients lack Glu^{69}, other class II markers are likely to be involved in the Be-specific response as well. Recently, Amicosante and coworkers identified the HLA-DRPhebeta47 marker to be associated with beryllium sensitivity in Glu^{69}-negative subjects (Amicosante *et al.*, 2005).

Beryllium antigen stimulates tumor necrosis factor alfa (TNFα) from bronchoalveolar lavage cells in CBD. Thus, functional gene polymorphisms of this gene are suspected to modify the course of CBD. Several studies suggest that the −308 allele in the promoter region of TNFα determines susceptibility for CBD (Dotti *et al.*, 2004; Gaede *et al.*, 2005; Maier *et al.*, 2001). Moreover, the antiinflammatory cytokine transforming growth factor beta (TGFβ) is able to inhibit production of alveolar macrophages and monocytes, and it has recently been demonstrated that the frequency of a polymorphism associated with a low TGFβ release (labeled $TGF\beta_1$, codon 25) was strongly associated with CBD (Gaede *et al.*, 2005). However, this phenomenon was only seen among individuals from the United States, suggesting that different genetic factors are important in different populations.

Hard metal disease is a pulmonary disorder associated with occupational exposure to inhalation of cobalt ions, sintered or not to other metals. A strong association with hard metal disease and HLA-DP sharing a Glu at position 69 has been reported (Potolicchio *et al.*, 1997, 1999). On the other hand, no influence of Glu^{69} was observed in relation to lung function changes among workers from a cobalt-producing plant (Verougstraete *et al.*, 2004).

3.3.3 Cadmium

Support for genetic influences comes from a large number of studies performed on different inbred strains of rodents that differ greatly in metal susceptibility. As an example, for cadmium-induced testicular toxicity, more than 30 mice strains are sensitive at very low cadmium levels, whereas approximately 10 are resistant, even at very high cadmium concentrations (Liu *et al.*, 2001). Differences in the cadmium-binding protein metallothionein have generally been thought to be responsible for the strain susceptibility to cadmium toxicity. Metallothionein null mice, indeed, are more sensitive to certain effects of cadmium (e.g., nephrotoxic or testicular injuries) (Liu *et al.*, 2000). However, a study on cadmium-induced testicular damage showed that the genetic mouse strain and not the metallothionein genotype determined susceptibility to testicular injury (Liu *et al.*, 2001). Therefore, the resistance to cadmium-induced testicular injury among certain strains has been sought in other factors, most probably including genetic differences in the recently reported transporter ZIP8, located at mouse chromosome 3 (Dalton *et al.*, 2000; 2005).

Although cadmium is one of the major toxic metals, so far strikingly little is known about what extent and which genetic factors influence its toxicity among humans. One twin study has been performed for quantifying the genetic influence of variation in blood concentrations of cadmium and lead (Björkman *et al.*, 2000). Heredity had a substantial impact on blood cadmium and lead levels, but a striking sex difference was observed. Among nonsmoking women, the hereditary impact was 65%, whereas for nonsmoking men it was only 13%.

3.3.4 Lead

3.3.4.1 ALAD

It is now 20 years since the first study by Ziemsen *et al.* describing differences in blood lead levels by genotype of the gene δ-amino levulinic acid dehydratase (ALAD) (Ziemsen *et al.*, 1986). Since then, there have been a large number of studies performed focusing on

interactions for *ALAD* genotype, lead metabolism, and toxicity (reviewed in Kelada *et al.*, 2001). ALAD is the major binding site for lead in red blood cells (Bergdahl *et al.*, 1997; 1998). This protein is polymorphic, and the main variant analyzed is the Lys/Asn substitution at amino acid position 59. The two alleles are traditionally labeled *ALAD*-1 for the Lys variant and *ALAD*-2 for the Asn variant, and the resulting genotypes are named *ALAD* 1-1 for Lys/Lys carriers, *ALAD* 1-2 for Lys/Asn, and *ALAD* 2-2 for Asn/Asn. The frequency of the ALAD-2 allele varies considerably worldwide: the highest frequency is found in Northern Europe (up to 20%), whereas this allele is rarer among Asian populations and almost absent among Africans (Packer *et al.*, 2006). The Lys59Asn amino acid position is not positioned near the Zn-binding sites, where lead probably binds (Jaffe *et al.*, 2000; 2001). However, because asparagine is a neutral amino acid whereas lysine is positively charged, this amino acid substitution results in a more electronegative enzyme. This fact has generated the hypothesis that the ALAD-2 protein binds positively charged lead more tightly than the ALAD-1 protein and that a protective effect of ALAD-2 exists because of this tight binding, maintaining lead in the intravascular space in a less bioavailable form (Wetmur, 1994; Wetmur *et al.*, 1991). It is important to stress that, so far, limited proof exists of molecular differences between the two isozymes. Jaffe *et al.* have constructed recombinant ALAD variants in *E. coli*. They found no functional dissimilarities, apart from a small difference in the half-time for recovery from lead inhibition between ALAD-1 and ALAD-2 *in vitro* (Jaffe *et al.*, 2000; 2001).

In early studies of effect modification of *ALAD* on lead, the individuals analyzed were from populations with relatively high levels of exposure from occupational or home environment. These studies displayed association of the *ALAD*-2 genotype with higher blood lead levels. Wetmur and colleagues demonstrated in a study based on more than 1000 children from New York City that *ALAD*-2 individuals had higher lead levels in blood (Wetmur 1994; Wetmur *et al.*, 1991). However, conclusions of this large study are hampered by the selection of study subjects: they all had high protoporphyrin levels in an initial screening. This means that the conclusion may either be that *ALAD*-2 carriers demonstrate higher lead levels, which in turn result in higher protoporphyrin levels, or alternately, that *ALAD*-2 carries more lead relative to *ALAD*-1 but is protected from the negative effects of lead. Several other studies have shown an effect of *ALAD* genotype on blood lead levels among individuals with a high lead exposure (Alexander *et al.*, 1998; Fleming *et al.*, 1998), whereas others have not (Bergdahl *et al.*, 1997; Sakai *et al.*, 2000; Schwartz *et al.*, 1995; 1997; Sithisarankul *et al.*, 1997; Süzen *et al.*, 2003). Epidemiological studies of blood lead levels at background exposure levels show no clear association of effect of *ALAD* genotype (Bergdahl *et al.*, 1997; Hu *et al.*, 2001; Smith *et al.*, 1995), although Hsieh *et al.* found nonsignificant higher levels among *ALAD*-2 individuals in a Taiwanese population (Hsieh *et al.*, 2000).

Schwartz and colleagues found that the 1–2 genotype (Lys/Asn carriers) was associated with occupational exposures of more than 6 years (Schwartz *et al.*, 1995). This genotype distribution could be the result of genotype selection, and the authors suggested that *ALAD*-2 subjects were protected from effects of lead and could tolerate longer exposures to lead than *ALAD* 1-1. This is in line with the alternative conclusion of the New York study mentioned previously.

Kim *et al.* analyzed whether Korean lead workers with the *ALAD* 1-1 genotype were more susceptible to the hematological effects of lead exposure (Kim *et al.*, 2004). They found that *ALAD* 1-2/2-2 was associated with lower log zinc protoporphyrin (ZPP) and higher hemoglobin levels. Moreover, among individuals with normal iron status, those with the *ALAD* 1-1 genotype were more likely to be anemic. Alexander *et al.* (1998) found significantly lower levels of ZPP at high blood lead levels among *ALAD*-2 genotypes as well, and lower but nonsignificant ZPP levels among carriers of the *ALAD*-2 allele have been reported elsewhere (Sakai *et al.*, 2000; Schwartz *et al.*, 1997; Sithisarankul *et al.*, 1997). Reduced levels of plasma aminolevulinic acid among *ALAD*-2 carriers have also been seen in the studies on the Korean battery workers (Sakai *et al.*, 2000; Schwartz *et al.*, 1997; Sithisarankul *et al.*, 1997), as well as among Japanese workers (Sakai *et al.*, 2000). Accumulation of plasma aminolevulinic acid has been suggested to confer a greater risk for neurotoxic effects of lead. Bioavailable lead has also been analyzed by measuring the amount of dimercaptosuccinic acid (DMSA)–chelatable lead in urine. There is some evidence that *ALAD*-2 individuals display lower levels of DMSA-chelatable lead levels (Schwartz *et al.*, 1997; 2000).

ALAD genotype has been analyzed in relation to kinetics of bone lead as well. However, the results are difficult to interpret; some studies suggest an impact of *ALAD* on bone lead (Bellinger *et al.*, 1994; Hu *et al.*, 2001; Kamel *et al.*, 2003), whereas others do not find any effect modification of the polymorphism (Bergdahl *et al.*, 1997; Fleming *et al.*, 1998; Schwartz *et al.*, 2000). Moreover, a few studies have analyzed effect modification of *ALAD* on lead's effects on kidney parameters (Bergdahl *et al.*, 1997; Smith *et al.*, 1995; Weaver *et al.*, 2003). Also the results of these studies are contradictive.

The inconsistent findings of the impact of this ALAD polymorphism on lead levels and toxicity between different studies could be the result of different ways of selecting study subjects (e.g., from highly exposed environments or the general population, from lead levels in blood, or biomarkers of effect). Another problem regarding study design is the use of different statistical methods for analyzing interaction, which makes it difficult to compare the different studies. Thus, these data should still be interpreted with caution. Another reason for contrasting findings could be that *ALAD* may have impact, depending on the genetic background, and incoherent observations are made when different populations are studied. To explain the differences between populations, more genes related to lead metabolism and effects have to be analyzed in association with lead markers of toxicity.

In summary, at high levels of exposure and in comparison with *ALAD*-1 subjects, heterozygous or homozygous *ALAD*-2 carriers seem to have increased blood levels, lower levels of ZPP and plasma aminolevulinic acid, and perhaps lower amounts of chelatable lead. Theses associations are consistent with those reported by Scinicariello and colleagues (2006), who used meta-analyses to summarize the current state of knowledge. Still, much is unknown of genotypic effect modifications of lead on the bone, kidney, and the neurological system, and whether or not there are similar effects of *ALAD* genotype in the general low-exposed population as seen for high levels of exposure.

3.3.4.2 VDR

Lead uptake increases when calcium resources are limited (Mahaffey *et al.*, 1986). Thus, genetic differences in calcium absorption might, in turn, modify lead levels. Lead binds to calcium proteins, which are regulated by the vitamin D endocrine system and the vitamin D receptor (VDR). VDR displays polymorphisms, and Ames *et al.* (1999) demonstrated that the *VDR Fok1* genotype FF increases bone mineral density and calcium absorption by 30–40% among healthy children 7–12 years of age. Haynes and coworkers (2003) have shown that children from New York with the Ff genotype displayed lower blood lead than the FF genotype after exposure to floor dust lead, suggesting that this genotype is an effect modifier of the relationship of floor dust lead and blood lead concentrations.

Another polymorphism of *VDR*, labeled *Bsm*I, is affecting the bone mineral density: decreased density has been reported in those with the BB compared with the bb genotype (Cooper and Umbach, 1996). In Korean battery workers, individuals with the BB and Bb genotypes displayed higher blood and tibia lead levels (Schwartz *et al.*, 2000), as well as diastolic blood pressure, compared with participants with the bb genotype (Lee *et al.*, 2001). Also Theppeang and coworkers reported higher patella lead levels among VDR BB carriers compared with bb carriers (Theppeang *et al.*, 2004). In a study on renal function in Korean lead workers, the *Bsm*I polymorphism of *VDR*, as well as endothelial nitric oxide synthase (*NOS3*), involved in nitric oxide production were analyzed for possible effect modification (Weaver *et al.*, 2003). However, no obvious association was present for *VDR* and either lead levels or markers for renal function.

Studies analyzing gene–gene interactions for *ALAD* and *VDR* or different polymorphisms within *VDR* are very few in the literature. However, in the aforementioned study on Korean battery workers (Schwartz *et al.*, 2000), the authors analyzed the frequency of combined genotypes of *ALAD* and *VDR* among exposed subjects and controls and found that there was a tendency among exposed subjects homozygous for *ALAD*-1 to lack the *VDR Bsm*I bb genotype. Among the controls, the opposite genotype distribution was seen. This finding could indicate a genotype selection among the exposed workers.

3.3.4.3 HFE

Lead is also linked to the uptake of iron in the gastrointestinal tract: when the sources of iron are limited, lead absorption is increased (Cheng *et al.*, 1998). As previously mentioned, the autosomal genetic disease hemochromatosis is caused by mutations in the *HFE* gene. The *HFE* gene is mutated at amino acid position 282, which leads to a cysteine-tyrosine exchange, among 85% of the affected. Another variant of this gene, His63Asp, is also associated with hemochromatosis, but with a lower penetrance (Waheed *et al.*, 1997). Because mutations in the *HFE* gene affect iron uptake, variants of this gene or others affecting the iron status might have a modifying effect on the levels of lead as well. Barton *et al.* (1994) showed that subjects who were homozygous for the Cys282Tyr mutation had increased blood lead and that there was an allele-dosage effect. On the other hand, in a Swedish study, the blood lead levels were lower among hemochromatosis-affected subjects than among the controls (Åkesson *et al.*, 2000). Similarly, Wright *et al.* (2004) showed among elderly men that carriers of at least one variant *HFE* allele from Cys282Tyr or His63Asp were associated with lower levels of blood or bone lead. Adjusted analysis showed that one *HFE* variant was independent factor for significantly lower levels of lead in patella.

3.3.5 Mercury

There is substantial evidence for strain differences among mice in susceptibility to immune toxic effects of inorganic, methylmercury, as well as ethylmercury (Häggqvist *et al.*, 2005; Havarinasab *et al.*, 2005; Warfvinge *et al.*, 1995). The differences in predisposition to mercury-associated autoimmune diseases have been linked to genes in the major histocompatability complex (H-2), but genes outside this region also seem to be important (Hultman *et al.*, 1992; 1993). Kono and coworkers found evidence for a locus on mouse chromosome 1 determining susceptibility toward inorganic mercury-associated autoimmunity (Kono *et al.*, 2001). However, the candidate gene(s) has not yet been identified.

Glutathione (GSH) plays a central role in mercury metabolism, and there are also indications that the activity of glutathione S-transferases (GST) is involved in the conjugation of Hg^{2+} (Brambila *et al.*, 2002; McGuire *et al.*, 1997). A possibility for variation in toxic effect of mercury is genetic differences in mercury metabolism, because of polymorphism in genes that conjugate GSH to mercury (i.e., GSTs) or synthesize GSH (e.g., glutamyl-cysteine ligase encoded by *GCLC* and *GCLM*).

Westphal *et al.* showed that among individuals exposed to ethylmercury, those carrying the low-activity null genotypes of *GSTM1* and *GSTT1* were overrepresented among patients who had contact dermatitis develop (Westphal *et al.*, 2000). The mechanism for this observation could be that ethylmercury-sensitized individuals have a slower elimination of ethylmercury by GSH conjugation or even lack this route of elimination, and thus have a greater tendency for ethylmercury accumulation and sensitization. For methylmercury, a recent study tested the hypothesis that polymorphisms in GCL and GST genes could modify methylmercury elimination (Custodio *et al.*, 2004). Erythrocyte mercury concentration and plasma polyunsaturated fatty acids (a proxy for fish intake) were measured. The presence of one rare variant allele for either of the polymorphisms *GCLC*-129 or *GSTP1*-114 was associated with both higher EryHg levels and a steeper regression slope for the association between Ery-Hg and PPUFA. This is consistent with slower methylmercury elimination in these genotypes.

Also for inorganic mercury, the genotypes of GSH-related genes seem to modify the metabolism. Data from a study on gold miners and gold buyers in Ecuador, highly exposed to elemental mercury vapor, shows that the presence of the less GSH-producing *GCLM*-588T allele was associated with increased blood, plasma, and urine mercury levels and that it modifies the effect of exposure (Custodio *et al.*, in 2005). These results indicate that genotypes with decreased GSH availability for mercury conjugation affect the metabolism of inorganic mercury.

Inorganic mercury gives a porphyrinogenic response, which can be detected by increases of different coproporphyrins in urine, and this response has been suggested as a biomarker of mercury exposure and toxicity. A polymorphism in the coproporphyrinogen oxidase gene (*CPOX4*) was found to be overrepresented among subjects showing highly elevated levels of coproporphyrins in urine in response to mercury (Woods *et al.*, 2005). The *CPOX4* polymorphism and mercury exposure seem to exert negative effects in an additive manner on neurobehavioral tests among dentists and dental assistants (Echeverria *et al.*, 2006). However, no CPOX4-mercury interaction was investigated.

3.3.6 Nickel

Nickel allergy has been suggested to have a genetic causal component, partly from earlier twin and familial studies (Fleming *et al.*, 1999; Menne and Holm 1983), but also from the past finding of an association of an HLA-DQA polymorphism and nickel allergy (Olerup and Emtestam, 1988). However, associations with this or other HLA-types have not been confirmed (Emtestam *et al.*, 1993; Ikaheimo *et al.*, 1993). Recent data suggest that nickel mainly interacts with HLA-DR, but then with a conserved amino acid in the beta chain (Gamerdinger *et al.*, 2003). Still, other genetic factors not related to the HLA-system might have importance for the risk of developing nickel allergy. On the other hand, a more recent study based on female twins indicates that nickel contact dermatitis is mainly determined by environmental and only to a lesser degree to genetic factors (Bryld *et al.*, 2004).

3.3.7 Platinum

Interestingly, for platinum-containing substances like cisplatin and oxaliplatin used in high doses in chemotherapy, gene-exposure interactions have been reported that have an impact on treatment outcome and survival (Kweekel *et al.*, 2005). Variants in genes involved in cellular defense mechanisms that prevent platinum-DNA adduct formation (e.g., glutathione S-transferase *GSTP1*) or remove DNA adducts (base or nucleotide excision repair genes *XPD* and *ERCC1*) have been shown to affect the response of chemotherapy. These variants might as well have an impact on susceptibility toward platinum-related health effects from low-level occupational or environmental exposures.

3.4 Conclusions

Gene–environment interactions are unknown for most metals. So far, presence of *HLA-DPB1* and toxic effects of exposure to beryllium seem to be the strongest example of a genetic effect modification. *ALAD* genotype and lead exposure is the most studied interaction, but its impact on health effects of lead is still unclear. The heterogeneous results of studies of *ALAD*-lead stresses problems with gene–environment interaction studies, such as uncharacterized biological mechanisms, use of different biomarkers or markers of effect, small studies with low power, as well as the lack of a "gold standard" for statistical analysis for this study type. However, it is important to try to solve these problems for the identification of subpopulations that are more vulnerable to metal-induced toxic effects. Moreover, genetic variants may be significant in relation to low-level exposures, influencing the risk assessment and of setting tolerable limits of exposure.

4 METAL–METAL INTERACTIONS (NONCARCINOGENIC EFFECTS)

4.1 Arsenic and Other Metals

Lead, cadmium, and arsenic are among the most commonly encountered toxic trace elements to which humans may be exposed, but toxicity studies of interactions among these elements are relatively limited. Early *in vivo* studies concerning interactions between toxic trace elements (Fowler and Mahaffey, 1978; Mahaffey and Fowler, 1977; Mahaffey *et al.*, 1981) used "stressor dose levels'" of these elements in semipurified diets fed to rats for 10 weeks in a factorial design study, demonstrated additivity as the most common type of interaction among these elements for a variety of clinical and elemental concentration parameters. The organelle and biochemical aspects of interactions among these elements, as well as mercury, were reviewed and future research needs identified for understanding the molecular bases among these elements. Subsequent *in vivo* mechanistic studies evaluated interactions between gallium and arsenic (Goering *et al.*, 1988) and indium and arsenic (Conner *et al.*, 1993; 1995; Fowler *et al.*, 2005) semiconductors. More recent *in vitro* studies (Madden and Fowler, 2000; Madden *et al.*, 2002) compared cadmium and arsenic interactions in both rat and human cell lines and observed more than additive effects and documented differences in species sensitivity. These studies explained a number of the mechanistic molecular factors (e.g., stress proteins) that contribute to cellular resistance to toxicity until the cellular capacity is exceeded by either dose level or combined interactive toxic effects. Complementary *in vivo* studies (Fowler *et al.*, 2004) in rats exposed to lead, cadmium, or arsenic at LOEL dose levels in drinking water and fed semipurified diets for 30, 90, or 180 days also demonstrated additive, or more than additive, effects as a function of duration of exposure. As with the *in vitro* studies, biomarkers of cell injury showed clear responses when the capacities of cellular protective mechanisms were exceeded as a function of duration of exposure. Analogous studies on human populations exposed to Cd and As in China (Nordberg *et al.*, 2005) also showed positive interactive effects using biomarker endpoints with regard to increased renal toxicity among groups with combined exposures to both cadmium and arsenic relative to groups with increased exposures to only cadmium or arsenic.

Interactions between arsenic and selenium have been documented by a number of investigators. Previous studies (Kraus and Ganther, 1989) reported synergistic toxicity between arsenic and methylated selenium compounds. Studies by others (Csanky and Gregus, 2003) demonstrated alterations in the disposition of arsenate or arsenite in rats treated with selenite and demonstrated decreased tissue concentrations of MMA(III) and MMA(V) and altered biliary excretion of MMA(III) and DMA metabolites. The investigators also noted a pronounced dose effect with regard to these observations. Mechanistic studies by others (Styblo and Thomas, 2001; Walton *et al.*, 2003) using *in vitro* hepatocyte culture systems showed that selenite rather than its methylated metabolites was responsible for the observed inhibition of arsenical methylation and may stimulate the observed increases in cellular toxicity of inorganic arsenic. Studies by others (Zakharyan *et al.*, 2005) showed that three Se atoms and three GSH molecules were covalently bound to the monomeric form of human glutathione-S-transferase (hGST01-01), which also acts a the arsenical methyl transferase. The investigators conclude that the mechanism of Se inhibition of arsenical methylation involves complexation of Se with glutathionylated cysteines at the active site of the enzyme. Se–As interactions have also been reported (Davis *et al.*, 2001) to produce alterations in the methylated pathway and to alter DNA methylation in Caco-2 cells *in vitro*.

There is also a pressing need to consider the impact of factors such as age and gender on interactions among toxic trace elements. Such intrinsic biological factors are clearly important for assessing risks to sensitive subpopulations or groups of workers exposed to mixtures of these elements such as those found in the semiconductor industry (Fowler and Sexton, 2002;

Fowler and Silbergeld; 1989), because they may influence target molecular and cellular responses to mixtures of gallium and arsenic or indium and arsenic (Conner *et al.*, 1994).

4.2 Interactions Between Cadmium and Other Metals

Many studies show that zinc can counteract toxic effects of cadmium. Cadmium and zinc belong to the same group of the Periodic Table. It has been speculated that cadmium can displace or replace zinc in some essential systems in the organism, thus causing functional changes. Metallothionein, a low-molecular-weight cysteine-rich protein, can bind cadmium, copper, and zinc; and this protein will bind most of the cadmium stored and kidneys, being inert in this form and preventing cadmium ions from interfering with the essential metals. Some parts of the reproductive system have particularly high concentrations of zinc, which is of great functional importance. Lethal toxicity from injection of Cd compounds can be protected by pretreatment by Zn or Cd (Leber and Miya 1977; Nordberg, 1972). The induction of MT by the pretreatment, shown to take place in the liver, most probably is the explanation for such protective effects. Studies using MT transgenic and MT null mice have demonstrated lower lethality and lower liver toxicity from Cd in mice expressing increased levels of MT (Liu *et al.*, 1995) and increased such toxicity in MT-null mice (Liu *et al.*, 1998). Cadmium in single doses may cause testicular destruction, but this damage may be prevented by injection of large amounts of zinc (Parizek, 1957), selenium, or even pretreatment with smaller doses of cadmium. The latter phenomenon is likely to be due to the induction of a small cadmium-binding protein (Nordberg, 1971) protecting against the deleterious effect of the large dose. Although it has been speculated that this protein might be metallothionein, the fact that various strains of mice with variable susceptibility to this effect are not related to MT, makes it likely that the protective protein is different from metallothionein (see Section 3.2 of this chapter). Gunnarsson *et al.* (2004) showed that pretreatment with zinc protected against Cd-induced testicular prostaglandin increase, a probable mechanism by which Cd inhibits testosterone synthesis. In many studies on the relationships between zinc and cadmium, animals exposed to cadmium were given diets with extremely high concentrations of zinc, which might have prevented some of the expected effects of cadmium. Of greater interest are the studies in which animals have been exposed to cadmium, but the dietary concentrations of zinc have been just adequate or suboptimal. In such experiments, it has been shown that exposure to small amounts of cadmium may cause a redistribution of zinc in the organism (Petering *et al.*, 1971). Zinc concentrations increase in liver and kidney and might decrease in other organs where cadmium does not accumulate to the same extent (e.g., in the testes). It has also been shown that if pregnant animals are exposed to cadmium, the fetal concentrations of zinc and of copper may become lower (Pond and Walker, 1975). Cadmium, a potent inhibitor of iron uptake in mice fed a low iron diet, impaired zinc uptake under these conditions (Hamilton *et al.*, 1978). Such interactions influencing the fetal levels of essential elements may be important components for the explanation of the effects of low-level Cd exposures on the fetal brain (cf Chapter 23).

In rats, cadmium (Cd2+) and chromium (Cr3+) inhibited zinc (Zn2+) uptake, but chromium (Cr6+) did not show any affinity for the transport process (Lyall *et al.*, 1979). In animals exposed to cadmium over a long period of time, there is a marked accumulation of cadmium in the liver and kidneys; in addition, there is an increase in zinc and a decrease in iron in these organs, whereas the concentration of copper is usually unchanged (Sugawara, 1984). It could be expected that cadmium would cause changes in the activities of some enzymes that require zinc. It has been shown that the renal activity of leucine aminopeptidase, which is a zinc-requiring enzyme, is reduced in animals exposed to cadmium (Cousins *et al.*, 1973).

It is known from animal experiments that the dietary intake of iron, zinc, and calcium is of importance for the gastrointestinal uptake of Cd (see reviews by Andersen *et al.* [2004] and Chaney *et al.* [2004]).

There are indications that contributing factors for Itai-Itai disease were low intakes of calcium and other minerals, vitamin D, and protein. The poor nutritional conditions in many areas of Japan during World War II were discussed and a comparison between diets in the endemic region of Itai- , Toyama prefecture, Japan as a whole and Sweden was presented by Kjellstrom in 1986. Lower dietary intakes calcium and of fat-soluble vitamins compared with Sweden and the practice by women in the endemic area of wearing clothes covering their whole body, including hands and face, may have provided only marginally adequate calcium intake and marginal or less than marginal vitamin D activity. Such conditions most probably made this population more sensitive to the development of bone effects of Cd than populations in other countries (Kjellstrom, 1986; Nordberg, 1974). Although it is clear that the high cadmium intake in the endemic area was the main causal factor for development of the disease (cf Chapter 23), these other factors may have contributed to the development of severe osteomalacia and osteoporosis with many

fractures. Severe cases of bone disease with osteomalacia have not been observed in other countries (China) where exposures started in the 1960s, nutritional factors are similar to Japan in the 1960s–1990s (but better than in Japan during WWII), and where cadmium intakes have been almost as high as in the endemic area for Itai–Itai disease in Japan (Nordberg *et al.*, 2002).

The important interaction between iron and cadmium in the intestinal mucosa has also been shown in humans. Iron deficiency or low iron stores (indicated by low serum ferritin values) increases Cd uptake in humans (Flanagan *et al.*, 1978). Higher blood and urine values of Cd were found among Swedish women with iron deficiency compared with women without such deficiency (Akesson *et al.*, 2002). The probable mechanism of this interaction is the competition between Fe and Cd for the common transporter DMT-1 (divalent metal transporter 1) in the intestinal mucosa. Findings in human enterocytes of a close correlation between the expression of DMT-1 and Cd absorption (Tallkvist *et al.*, 2001) supports such a mechanism.

In a study in metal-contaminated areas in China, interactions between cadmium and inorganic arsenic exposure were demonstrated (Hong *et al.*, 2004, Nordberg *et al.*, 2005). Although there was a lack of persons with low arsenic exposure and concomitant high cadmium exposure, a multiplicative interaction is still indicated with regard to the prevalence of renal tubular dysfunction in subgroups exposed to various combinations of the two metals (Figure 1).

4.3 Interactions Between Lead and Other Metals

Zinc, a nutritionally essential metal, may affect both the absorption and toxicity of lead. Lead exposure in both humans and animals may lead to a decrease in the activity of the zinc-requiring enzyme ALA-dehydratase. Simultaneous administration of zinc can counteract this decrease. Soil and dust measurements of environmental lead were positively associated with the blood lead concentrations, regardless of the corresponding zinc concentrations in these samples. The strength of association was 20–46% lower in areas with high environmental concentrations of zinc. The findings suggest that zinc influences the relationship between soil and dust lead and the corresponding blood lead levels (Noonan *et al.*, 2003). Lead has been shown to interfere with the DNA-binding properties of Sp1 and Egr-1, both *in vivo* and *in vitro*, and also with the zinc finger protein transcription factor IIIA. Thus, by targeting zinc finger proteins (ZFP), lead may cause multiple responses through its action on a common site that is present in enzymes, channels, and receptors (Zawia *et al.*, 2000). Lead may also replace zinc on heme enzymes (Goyer, 1997).

Experiments in laboratory animals have shown that nutritional iron deficiency may increase the gastrointestinal lead absorption, thereby promoting lead toxicity (Mahaffey 1981), particularly in pregnant women and young children. Similar findings have been shown in epidemiological studies among children, suggesting that

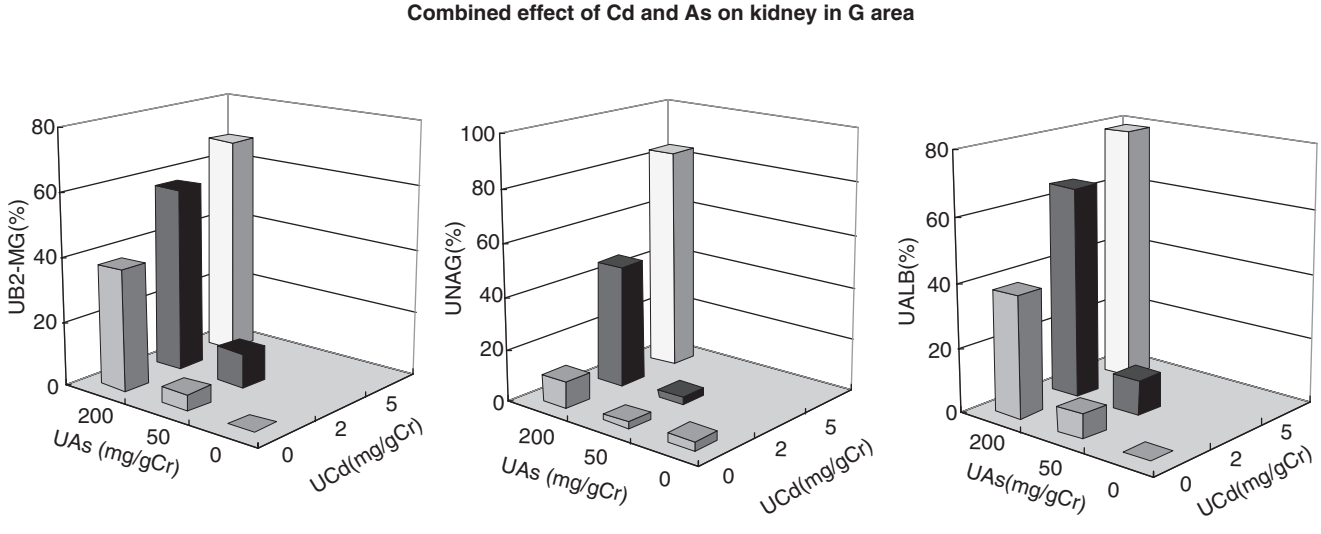

FIGURE 1 Prevalence (%) of elevated excretion for three biomarker proteins in a metal-contaminated area of China in relation to combined exposure to Cd and As as indicated by urinary As and Cd excretion. UB2MG, urinary beta2-microglobulin; UNAG, urinary N-acetylglucosaminidase; UALB, urinary albumin. Reproduced from Nordberg *et al.* (2005).

iron deficiency may increase the susceptibility to lead poisoning. This risk can be counteracted by high iron intake and sufficient iron stores (Kwong *et al.*, 2004).

In a study of 157 children from Katowice, and industrial area in Poland, blood lead concentrations showed a negative relationship with selenium levels in whole blood and serum. The relationship was mainly appearing at low blood lead levels, which was interpreted as an influence of selenium on the kinetics of lead, rather than an impact of lead on the selenium status. In the study, children with low serum ferritin concentrations showed a tendency to higher blood lead levels. Thus, the kinetics of lead may be influenced by both the iron and selenium status (Osman *et al.*, 1998).

Lead may interact with calcium in the nervous system, which can impair the cognitive development (Goyer, 1997). Lead competes with calcium, inhibiting the release of neurotransmitters, and interferes with the regulation of cell metabolism by binding to second-messenger calcium receptors, blocking calcium transport by calcium channels and calcium-sodium ATP pumps, and by competing for calcium-binding protein sites and uptake by mitochondria (Goyer, 1995). The capacity of lead to promote oxidative stress, affect cell signals, and induce cell death by apoptosis has been mostly attributed to its effect on different calcium-mediated cellular events (Oteiza *et al.*, 2004). Low concentrations of inorganic lead ions (Pb^{2+}) can disrupt transmitter release by causing aberrant augmentation of spontaneous and suppression of evoked release. These effects are probably caused by high-affinity interactions of Pb^{2+} with the voltage-gated calcium channels, as well as with Ca^{2+} binding proteins involved in the regulation of synaptic vesicle mobilization, docking, and exocytosis processes. In contrast to intracellularly mediated actions of Pb^{2+} causing an augmentation of spontaneous release, the inhibition of evoked transmitter release by Pb^{2+} is largely attributable to extracellular block of the voltage-gated calcium channels (Suszkiw, 2004).

In experiments on rats, the results indicated an interaction between arsenic and lead on lipid peroxidation of the erythrocyte membrane. The results showed that in the arsenic-lead–treated rats, the levels of lipid peroxidation did not change significantly. However, the concentration of blood glutathione and superoxide dismutase (SOD) activity decreased significantly. A synergistic effect of arsenic-lead on SOD activity was observed (An *et al.*, 1998).

4.4 Hg and Other Metals

The influence of metals and metalloids on the toxicity of inorganic mercury has been studied in animal experiments (Magos and Webb, 1980; Nordberg, 1978) and is also reviewed in Chapter 33. Selenite and other compounds that are metabolized to selenite have been shown to reduce the lethal effect of inorganic mercury in rats, preventing the development of renal tubular and intestinal necrosis (Parizek and Ostadalov, 1967). Dietary selenate prevents the depression of growth in rats receiving mercuric chloride by mouth (Potter and Matrone, 1974). In long-term experiments, dietary selenate reduces the chronic renal tubular damage produced by oral mercuric chloride in rats (Groth *et al.*, 1973; 1976). Parizek *et al.* (1969; 1974) demonstrated that administration of selenite to rats gives rise to a marked increase in mercury in blood and a reduced excretion of mercury. Similar distribution changes have been observed in rabbits (Imura and Naganuma, 1978) and in pigs (Hansen *et al.*, 1981). Both mercury and selenium are bound to a plasma protein with an atomic ratio of 1 (Burk *et al.*, 1974). The formation of a mercury-selenoprotein complex may explain these alterations in the kinetics and toxicity of mercury (Parizek, 1976; Parizek *et al.*, 1971; 1974), and it may also be related to the observation by Groth *et al.* (1976) and Carmichael and Fowler (1979) of intranuclear inclusion bodies containing mercury and selenium. A high-molecular-weight selenium–protein complex could be involved (Chen and Fang, 1974; Imura and Naganuma, 1978). The decreased passage of mercury and selenium across the placenta and into milk, when inorganic mercury and selenite are administered simultaneously, may also be explained by the formation of a mercury–selenium–protein complex (Parizek *et al.*, 1974). The formation of such a complex diminishes the binding of mercury to metallothionein in tissues (Mengel and Karlog, 1980).

More recent studies by a number of investigators have examined the role selenoproteins in mediating the binding and transport of mercury in blood and tissues. Yoneda and Suzuki (1997) first reported the detoxification of mercury by selenium via binding in an equimolar complex with a specific plasma protein in rats. They observed that both elements appeared to first from a complex and then bind to basic amino acids in the protein. This binding was inhibited by addition of polylysine. Subsequent studies using extended X-ray absorption fine structure (EXAFS) by Gailer *et al.* (2000) using plasma from rabbits demonstrated the formation of an Hg–Se–S chemical species.

More recent studies (Chen *et al.*, 2006a, b) have further examined the roles of selenoprotein P in serum samples from miners with occupational exposure to mercury and in porcine liver and kidney using pigs raised in an area of China with extensive mercury contamination. They found increased binding of Hg

to selenoprotein P and glutathione peroxidase in miners. In the pig tissues, they observed increased binding of mercury to metallothionein and both mercury and selenium binding in higher molecular weight protein fractions in a ratio of 1:1.

An indication that processes similar to those demonstrated in animals might occur in man is provided by the observations of elevated concentrations of mercury and selenium in organs of mercury miners many years after cessation of exposure (Rossi *et al.*, 1976).

The influence of other metals on the toxicity of inorganic mercury has not been adequately documented. One observation showed that pretreatment with cadmium protected (male) rats from the nephrotoxic effect of inorganic mercury (Magos *et al.*, 1974). This action is regarded to be mediated through induction of metallothionein. The Cd–Hg–Se interaction is limited by mercury, with respect to the distribution of both cadmium and selenium. Results from animals concomitantly exposed to inorganic salts of the three elements (Cd, Hg, Se) suggest that an observed increase in the whole-body retention of selenium is due to the mercury exposure. Mercury significantly increases the retention of selenium in the kidneys—all fractions—and liver—nuclear and mitochondrial fractions (Chmielnicka *et al.*, 1983). The toxic effect of methylmercury has been shown to be influenced by selenite in several animal species (Nordberg *et al.*, 1978; Skerfving, 1978). Thus, 0.5–8.0 mg/kg of selenite in the diet protected chicks, quail, and rats from the toxic effects of up to 40 mg/kg of methylmercury in the diet. Although it has been suggested that selenium present in marine fish might decrease the toxicity of dietary methylmercury (Ganther and Sunde, 1974; Ganther *et al.*, 1972; Ohi *et al.*, 1976), the evidence that selenium is the responsible factor is inconclusive; for instance, the difference in toxicity could be explained by differences in methylmercury intake or by the quantity and quality of protein in the diet (Nordberg *et al.*, 1978; Stillings *et al.*, 1974). However, selenium probably does have an influence. It has been shown (Kasuya, 1976; Naganuma and Imura, 1980) that bismethyl-Hg selenite ([CH3Hg]2Se) is formed in blood and that this compound more readily penetrates into the brain than methylmercury (Naganuma and Nobumasa, 1980). An increased mercury concentration in brain and even in fetal brain has been reported after combined exposure to methylmercury and selenium (Iijima *et al.*, 1978; Satoh and Suzuki, 1979). Bismethyl-Hg selenite seems to decompose readily in tissues. Selenium may also be involved in demethylation of methylmercury (Yamane *et al.*, 1977).

Dietary selenite and vitamin E diminished mortality in methylmercury-exposed quail (Welsh and Scares, 1976). Welsh (1976) showed that, in rats, vitamin E and other antioxidants had a protective effect. More recent neurodevelopmental studies in rats fed semipurified diets (Beyrouty and Chan, 2006; Newland *et al.*, 2006; Reed *et al.*, 2006) have reported alterations in the neurotoxic effects after methylmercury exposure that seem to be influenced by dose levels of both selenium and methylmercury.

In rats, the whole-body retention of mercury under the influence of zinc and cadmium was slightly lower than that observed in animals treated with mercury alone; however, copper decreased whole-body retention by more than 50% (Komsa Szumska and Chmielnicka, 1983). Urano *et al.* (1997) reported that selenite exposure reduced the biliary secretion of methylmercury in rats. More recent studies (Juresa *et al.*, 2005) reported that selenite treatment decreased the efficacy of DMSA and DMPS on facilitating mercury elimination in rats after concomitant treatment with mercuric chloride. Conversely, Gregus *et al.* (2001) reported that exposure of rats to methyl mercury, merbromin, mercuribenzene sulfonic acid, or mercuribenzoic acid alter selenium tissue concentrations in rats injected with selenite. Studies by Frisk *et al.* (2001a,b) reported that concomitant *in vitro* exposure of K-562 cells to selenium protected against the toxicity of mercuric chloride but had a lesser effect on methylmercury. Subsequent studies (Frisk *et al.*, 2003) showed that selenium protected against mercuric chloride–induced apoptosis in this cell line.

In a study on humans exposed to methylmercury, multiple regression analyses revealed an inhibition of ALA-dehydratase in red cells related to concentrations of both mercury and lead in blood (Schutz and Skerfving, 1975). The effects of equimolar concentrations of mercury and lead were similar. More recent studies (Farina *et al.*, 2003a,b; Perottoni *et al.*, 2004 a,b) demonstrated Se–Hg interactions with regard to increased inhibition of this enzyme with concomitant exposure to both these elements.

4.5 Molybdenum–Copper–Zinc Interactions

There is an interaction between copper and molybdenum studied for many years in animals and humans (cf Chapter 34). Thiomolybdates are formed by ruminants after oral intake of molybdenum causing copper depletion. This is the biochemical explanation of molybdenosis (Mason, 1986). Sheep and cows are more susceptible to imbalances between Cu and Mo than other animals like pigs and rats. Copper deficiency has been observed in ruminants grazing on pastures where the Mo content is high (Bremner, 1979). In human volunteers with an increased dietary intake

of Mo, increased Cu excretion was found (Deostahle and Gopalan, 1974). Copper toxicity in humans with Wilson's disease has been successfully treated with tetrathiomolybdate (Brewer and Yuzbasiyan-Gurkan, 1992).

Another efficient treatment of Wilson's disease is by oral doses of zinc salts (Brewer, 2000). This treatment is based on the findings that oral zinc intake induces the synthesis of metallothionein in the intestinal mucosa. Cu binds more readily to metallothionein than zinc, and thus Cu is sequestered in the mucosa and not taken up systemically but eliminated by the fecal route when the mucosal cells are shed. It has also been shown in groups of the general population that several manifestations of Cu deficiency can be induced by high oral Zn intakes in the form of supplements. When taking such supplements, it is important to make sure that there is a balance between intakes of Zn and Cu (Chapter 47).

4.6 Interactions Between Thallium and Potassium

Thallium and potassium seem to have some common receptor sites where they compete and thus undergo direct interaction. Potassium has been proposed for use in the treatment of thallium poisoning, because the administration of potassium increases excretion of thallium. For further data, see Chapter 41.

5 METAL–METAL INTERACTIONS IN CARCINOGENESIS

5.1 Arsenic

Arsenic is a known human carcinogen (see Chapter 10 for a detailed review) that seems to elicit the carcinogenic response via production of reactive oxygen species (ROS) with subsequent DNA damage secondary to oxidative stress, alteration of DNA methylation processes, gene expression patterns, and inhibition of DNA repair (NAS/NRC, 1999). Concomitant exposure to other metals that produce toxicity by oxidative stress such as cadmium (Hong *et al.*, 2004; Nordberg *et al.*, 2005) create a situation with additive or more than additive interactive effects. Such interactions are likely to exist for cancer but have so far only been demonstrated for noncancer effects among human populations exposed to both these elements in China (Figure 1).

5.2 Chromium

Crosslinking of DNA can be caused by chromium metabolites. The concentrations of chromium bound to DNA are related to the levels and stabilities of the chromium (V) species formed on reaction of chromium (VI) with various thiols. Thus, chromium–thiol interactions may play an important role in chromium (VI) genotoxicity (Borges *et al.*, 1991).

Exposure to lead chromate can induce chromosomal damage after extracellular dissolution, producing solubilized chromium and lead. Even if lead (II) is weakly clastogenic at high doses, hexavalent chromium seems to be the proximate clastogen in lead-chromate–induced clastogenesis. Pretreatment of cells with vitamin E can block clastogenesis induced by particulate chromates (Wise *et al.*, 1994).

Apoptosis in Chinese hamster ovary cells was observed 48 hours after treatment with 300 µmol/L chromium (Na_2CrO_4) for 2 hours. Cadmium alone at concentrations of 1, 5, or 10 µmol/L (as $CdCl_2$) did not induce apoptosis in these cells at times up to 72 hours after treatment. When these cells, however, were concurrently exposed to cadmium and chromium, chromium-induced apoptosis was markedly suppressed in a cadmium concentration–related fashion. The experimental findings indicate that cadmium can block chromium-induced apoptosis (Shimada *et al.*, 1998). The activation of the JNK, p38, and ERK mitogen-activated protein kinases by Cr (VI) is mediated through oxidative stress, but their activation does not affect cytotoxicity. In the experiments, the p38 activation by Cr(VI) showed a positive relationship to oxidative stress, whereas the JNK activity was enhanced by either a quencher (e.g., mannitol) or activator (H_2O_2) of redox reactions in Cr(VI)-exposed human non-small cell lung carcinoma CL3 cells (Chuang *et al.*, 2000).

At the genomic level, Cr genotoxicity manifests as gene mutations, several types of DNA lesions, and inhibition of macromolecular synthesis. At the cellular level, Cr exposure may lead to cell cycle arrest, apoptosis, premature terminal growth arrest, or neoplastic transformation. Cr-induced DNA–DNA interstrand crosslinks, the tumor suppressor gene p53, and oxidative processes are some of the major factors that may play a significant role in determining the cellular outcome in response to Cr exposure (Singh *et al.*, 1998). Chromium (VI) may induce cell growth arrest through hydrogen peroxide–mediated reactions in experiments on human lung epithelial A549 cells. The authors found that ROS were generated in Cr (VI)-stimulated A549 cells through reduction of molecular oxygen. Among the reactive oxygen species generated, H_2O_2 played a major role in causing G2/M phase arrest in the human lung epithelial cells (Zhang *et al.*, 2001).

N-acetylcysteine (NAC) and ascorbic acid (AsA) showed additive effects in reducing chromium (VI) and reverting its mutagenicity in experiments on mice

(D'Agostini *et al.*, 2000). NAC prevented the adverse effects of AsA on spontaneous mutagenicity. At the same time, this thiol and AsA showed additive effects, inhibiting the mutagenicity of Cr (VI) and the lung tumorigenicity of urethane in mice (D'Agostini *et al.*, 2000).

Experiments on Chinese hamster ovary cells using a host cell reactivation assay showed that the repair of BPDE-DNA adduct in a luciferase reporter gene was greatly inhibited after Cr(VI) exposure in NER-proficient cells but not in NER-deficient cells. Accordingly, Cr(VI) exposure can greatly enhance the mutagenicity and cytotoxicity of PAHs by inhibiting the cellular NER pathway, and this may constitute an important mechanism for Cr(VI)-induced human carcinogenesis (Hu *et al.*, 2004).

5.3 Iron

Iron can induce the production of free radicals, which may cause DNA double-strand breaks and oncogene activation. Iron can also maintain the growth of malignant cells, as well as the growth of pathogens. Breast cancer cells have been shown to display 5–15 times more transferrin receptors than normal breast tissue cells. On the other hand, iron chelators can counteract cell damage (Reizenstein, 1991). Animal experiments have shown that magnesium antagonizes nickel-induced carcinogenesis in the rat kidney, whereas iron tends to enhance it (Kasprzak *et al.*, 1994). Experiments on hamsters have shown that estrogen treatment increased the non-heme iron in liver of both high and low iron treatment groups and in kidney of the hamsters on the low-iron diet. Thus, the dietary iron enrichment may enhance the incidence and severity of estrogen-induced tumor induction (Wyllie and Liehr, 1998).

Iron may act as a promoter of dimethylnitrosamine-initiated hepatocytes in rats. Fibrogenesis was not an absolute requirement for this promotion. The authors suggest that iron may also act as a promoter of already initiated hepatocytes in the development of liver cancer in humans (Carthew *et al.*, 1997).

In experimental studies, 2-butoxyethanol increased liver tumors in B6C3F1mice after chronic exposure. Continued treatment with 2-butoxyethanol resulted in hemosiderin deposition in the liver, indicating that the liver tumors were mediated by oxidative iron catalyzed stress and Kupffer cell activation (Park *et al.*, 2002). Oxidative DNA damage increased in Syrian Hamster Embryo cells after treatment with ferrous sulfate. On the other hand, the DNA lesions decreased by cotreatment of ferrous sulfate with antioxidants. The authors suggested that iron, produced indirectly through hemolysis and not 2-butoxyethanol or its metabolites, might be responsible for the observed carcinogenicity (Park *et al.*, 2002).

An elevated dietary iron intake enhances the incidence of carcinogen-induced mammary tumors in rats and estrogen-induced kidney tumors in Syrian hamsters. Estrogen administration increases iron accumulation in hamsters and facilitates iron uptake by cells in culture. In humans, increased body stores of iron have been shown to increase the risk of several estrogen-induced cancers (e.g., breast cancer; Liehr and Jones [2001]).

In cell experiments, addition of catalase, which can increase Fe^{2+} concentrations, further increased the plasmid-induced DNA damage; on the other hand, diethylenetriaminepentaacetic acid (an iron chelator) significantly inhibited DNA damage. Thus, iron-dependent DNA damage has been suggested as one mechanism of action of human arsenic carcinogenesis (Ahmad *et al.*, 2002). The iron chelator phenanthroline suppressed the sodium chloride–enhanced gastric carcinogenesis induced by N-methyl-*N*′-nitro-*N*-nitrosoguanidine in experiments on Wistar rats (Tatsuta *et al.*, 2003). Intraperitoneal administration of ferric iron-ATP (FeATP) has been studied in an animal model. The morbidity and mortality rate were higher with FeATP, indicating two different interactions (intensity and duration) with the cell plasma membrane (Anghileri *et al.*, 2002).

Ex vivo and *in vitro* protective effects of kolaviron against oxygen-derived radical-induced DNA damage and oxidative stress have been found in human lymphocytes and rat liver cells. One mechanism behind the protective effects versus oxidative damage to molecular targets may be by means of scavenging of free radicals and iron binding (Farombi *et al.*, 2004).

5.4 Lead

Lead chromate can induce chromosomal damage. In cell experiments, high doses of lead glutamate were weakly clastogenic. It induced a different spectrum of chromosomal aberrations compared with lead chromate. Pretreatment of cells with vitamin E reduced the lead chromate–induced clastogenesis by 54–93% (Wise *et al.*, 1994).

Lead, as well as cadmium, may act as a tumor promoter in diploid human fibroblasts. In the experiments, ROS had a greater impact on cadmium than on lead-induced cytotoxicity and anchorage independence (Hwua and Yang, 1998).

It has been suggested that lead on a cellular and molecular level may permit or enhance carcinogenic events involved in DNA damage, DNA repair, and regulation of tumor suppressor and promoter genes

(Silbergeld, 2003). The formation of lead inclusion bodies in normal human lung cells exposed to lead chromate indicates that ionic lead is released from the particles and thereafter may contribute to the cell toxicity caused by lead chromate. Internalization and dissolution of lead chromate particles and the interaction of ionic chromium and lead with DNA may be components of the mechanism of lead chromate carcinogenesis. Lead chromate–induced apoptosis may be one mechanism to eliminate cells with chromium- and/or lead-damaged DNA (Singh *et al.*, 1999).

Lead in blood can, together with other parameters, influence the level of DNA single-strand breaks (DNA-SSB) in human mononuclear blood cells. Although lead alone did not increase the level of DNA-SSB, an increase of the lead air concentrations for the exposed workers from 1.5–50 $\mu g/m^3$ in the presence of constant exposures to cobalt ($8 \mu g/m^3$) and cadmium ($3.8 \mu g/m^3$) caused an almost fivefold increase in the odds ratio. The mechanism behind these interactions might be repair inhibition of oxidative DNA damage, because a decrease in repair capacity will increase susceptibility to ROS generated by cadmium or cobalt (Hengstler *et al.*, 2003).

5.5 Nickel

Nickel is a human and animal carcinogen that primarily affects the initiation process in the lung cancer development (Chiu *et al.*, 2004). Transition metals can damage DNA by free radicals generated by the Fenton reaction. Zinc finger proteins act as transcription factors binding specifically to short DNA sequences and controlling the transcription of a number of genes. Experiments have shown that metals like cobalt, cadmium, copper, nickel, and iron may substitute for zinc in zinc finger proteins. An important mechanism of the metal-mediated carcinogenesis is the enhancement of cellular redox processing by metals. A redox metal substituted for zinc in the zinc finger protein is expected to generate free radicals that may cause DNA damage (Sarkar, 1995). Another mechanism may include mediation of promutagenic oxidative DNA damage that depends on Ni (II) binding to chromatin (Bal *et al.*, 1995). Nickel ($NiCl_2$) may also inhibit specific DNA–protein interactions (Coogan *et al.*, 1989). In experiments on rat kidney, magnesium antagonizes nickel carcinogenesis, whereas iron tends to enhance it. The findings may be caused by the attenuating or enhancing effects of magnesium and iron on the inflammatory response to nickel compounds (Kasprzak *et al.*, 1994).

Nickel (II) and cadmium (II) can both inhibit the incision step of nucleotide excision repair. By applying a gel-mobility-shift assay and HeLa nuclear extracts, the effects of both metals on the damage recognition step of the repair process was studied. When applying nuclear extracts from HeLa cells treated with 50 µmol/L Ni (II) and higher, there was a dose-dependent decrease in protein binding. This effect, however, was largely reversible by addition of magnesium (II) to the binding reaction. Nickel compounds seem to disturb DNA–protein interactions essential for the initiation of the nucleotide excision repair, probably by the displacement of essential metal ions (Hartmann and Hartwig, 1998). A molecular mechanism of nickel carcinogenesis has also been suggested, involving oxidative damage processes catalyzed by weak Ni (II) complexes with cellular components (Bal *et al.*, 1996).

Nickel compounds can increase UV-induced cytotoxicity and mutagenicity and interfere with the repair of UV-induced DNA lesions by disrupting DNA–protein interactions involved in DNA damage recognition. Experiments suggest that Ni (II) at noncytotoxic concentrations inhibits nucleotide excision repair and possibly crosslink repair by interference with distinct steps of the respective repair pathways (Krueger *et al.*, 1999).

Oxidative stress induced by nickel sulfide caused loss of the tumor suppressor gene p16 and activation of the mitogen activated protein kinase (MAP kinase) signaling in experiments on mice. The findings indicate that there may be synergistic interactions between MAP kinase activation and p16 loss in carcinogenesis (Govindarajan *et al.*, 2002). The mechanism of DNA damage induced by carcinogenic Ni (II) in the presence of SH compounds has been studied for several compounds. The intensity of Ni (II)-mediated DNA damage that was induced by dithiothreitol (DTT) was stronger in comparison with other tested model endogenous SH compounds, 1,4-dithio-L-threitol and dithioerythritol. The finding that DNA damage induced by Ni (II) in combination with DTT was only evident when DNA was treated with piperidine suggests that Ni (II) and DTT caused only base damage. The results indicate that Ni (II) and DTT form reactive species that might be responsible for the observed guanine-specific DNA damage. Endogenous SH compounds, which have similar chemical structures as DTT, would participate in nickel carcinogenesis through causing oxidative DNA damage (Oikawa *et al.*, 2002).

Ni (II) genotoxicity may be enhanced through the generation of DNA-damaging ROS and the inhibition of DNA repair. Epigenetic effects of nickel include alterations in gene expression resulting from DNA hypermethylation and histone hypoacetylation, as well as activation or silencing of certain genes and transcription factors, particularly those that are involved in the cellular response to hypoxia (Kasprzak *et al.*, 2003a).

Several pathogenetic effects of nickel are due to the interference with the metabolism of essential metals such as Fe (II), Mn (II), Ca (II), Zn (II), or Mg (II). One way of treatment would be to find ways to inhibit or prevent nickel (II) interactions with critical target molecules and ions, Fe (II) in particular, and thus avert the respiratory tract cancer and other adverse health effects in nickel-exposed workers (Kasprzak *et al.*, 2003a). Ni (II) binding to some chromatin proteins in somatic as well as in sperm cells may cause oxidative and structural damage to the proteins and DNA. These effects may alter the fidelity of DNA replication and gene expression and thus facilitate carcinogenesis, including paternally mediated cancer in the progeny (Kasprzak *et al.*, 2003b).

5.6 Selenium

In an animal experiment, Ip (1986) studied the interaction between vitamin C and selenium supplementation in the modification of mammary carcinogenesis in rats. The protective effect of selenite in tumorigenesis was counteracted by vitamin C, whereas the chemopreventive action of seleno-DL-methionine was not affected. One hypothesis is that vitamin C reduces selenite to elemental selenium, which is not available for uptake by tissues. The results indicate that high concentrations of vitamin C can interfere with the accumulation of tissue selenium and that an increased level of selenium in cells is essential for hampering tumor development (Ip, 1986). In another experiment, the chemopreventive effect by a synthetic retinoid, selenium, and the combination of these elements during the postinitiation stages of carcinogenesis induced in rats by azaserine was investigated (Curphey *et al.*, 1988). Retinoid inhibited the progression of pancreatic carcinogenesis in a dose-related way, but selenium alone showed no effect. The combination of retinoid and selenium, however, was more effective than retinoid alone (Curphey *et al.*, 1988). Ip and Ganther (1988) compared the efficacy of trimethylselenenium versus selenite in cancer chemoprevention and its modulation by arsenite, using the dimethylbenz(a)anthracene-induced mammary tumor model in rats. Supplementation of selenite (3 ppm Se) alone caused approximately a 50% reduction in tumor yield. Arsenite (5 ppm As) reduced the response to selenite. On the other hand, arsenite greatly enhanced the protective effect of trimethylselenium (40 ppm Se). This combination proved to be nearly as effective as selenite. Thus, arsenite seems to be able to influence the anticarcinogenic properties of selenium. It can either potentiate or attenuate the protective effect, depending on the methylation state of the selenium compound (Ip and Ganther, 1988). It has been suggested that arsenite increases the production of a critical metabolite from methylated selenides (Ip and Ganther, 1992).

The effect of deficiencies of selenium and vitamin E alone or in combination on the induction of mammary carcinogenesis by 1-methyl-1-nitrosourea was studied in 21-day-old female Sprague–Dawley rats. Compared with rats fed the adequate diet, final cancer incidence and number were higher and cancer latency time shortened in rats consuming the diet deficient in both selenium and vitamin E. Single deficiencies of either nutrient failed to significantly alter the tumorigenic response (Thompson, 1991). Ip and Ganther (1991) investigated the dimethylbenz(a)anthracene (DMBA)-induced mammary tumor model in rats using three sets of combinations of blocking and suppressing agents (diallyl sulfide/Se-methylselenocysteine; ellagic acid/selenomethionine; diallyl sulfide/quercetin). In all three cases the combination regimen was much more effective than the single-agent treatment in tumor suppression (Ip and Ganther, 1991). Similar results have been reported by Rao *et al.* (1990) studying the chemopreventive effects of six agents (tamoxifen, 10 mg/kg; retinyl acetate, 50 mg/kg; tocopherol, 200 mg/kg; aminoglutethimide, 1 mg/kg; ergocryptine, 5 mg/kg; sodium selenite, 1 mg/kg) when given singly or in combinations on the initiation of mammary carcinogenesis induced by 20 mg of DMBA in virgin female rats. When modulators were given singly or in combinations of two, tumor incidences were not altered significantly. The range of tumor incidences was between 13 and 30% when the agents were given in combinations of three, four, or five. When all six modulators were given together, the tumor incidence decreased significantly to 8.3% (Rao *et al.*, 1990). High selenium intake significantly reduced the tumor incidence of pancreatic cancer, induced by *N*′-nitroso bis(2-oxopropyl)amine in female Syrian golden hamsters (Kise *et al.*, 1990). McGarrity and Peiffer (1993) studied the effects of difluoromethylornithine (DFMO), an inhibitor of ornithine decarboxylase (ODC), and selenium supplementation on tumor formation induced by the carcinogen 1,2-dimethylhydrazine (DMH) in Sprague–Dawley rats fed a fiber-free diet. In distal colon, 19 tumors developed in the DFMO group, 22 tumors in the high selenium group, and only 12 tumors in the combined high selenium/DFMO treatment group compared with 32 tumors in the regular diet group. Thus, selenium supplementation in combination with DFMO treatment selectively inhibited distal colon tumor formation in an additive way (McGarrity and Peiffer, 1993).

Inhibition of experimental benzo(a)pyrene-induced carcinogenesis by vitamin C alone and by vitamin C/vitamin E and selenium/glutathione was studied in

224 female Wistar rats (Charalabopoulos *et al.*, 2003). By calculating the carcinogenic potency of benzo(a)pyrene and the anticarcinogenic potency of substances used, as well as histological examination of developed tumors and survival time of treated animals, it was shown that vitamin C exerts a significant anticarcinogenic effect of 8.3 units. The combination of the two studied anticarcinogens produced a significant prolongation of the animals' survival time with anticarcinogenic potencies of 22.1 and 22.2 units, respectively (Charalabopoulos *et al.*, 2003). In another experiment, it was found that there is a synergy between sulforaphane and selenium in the induction of the enzyme thioredoxin reductase 1. The synergy can result from a combination of induction at the levels of transcription, as well as translation (Zhang *et al.*, 2003).

Oxidative stress may increase the risk of prostatic carcinogenesis. In a nested case-control study from the prospective Physician's Health Study, 567 cases and 764 controls were investigated, including baseline determinations of plasma antioxidant concentrations (selenium, lycopene, alpha-tocopherol) and beta-carotene. Among men with the AA genotype, high selenium levels (quartile 4 vs. 1) were associated with a relative risk (RR) of 0.3 (95% confidence interval [CI], 0.2–0.7) for total prostate cancer, and a RR of 0.2 (95% CI, 0.1–0.5) for clinically aggressive prostate cancer. In contrast, among men with the VV/VA genotype, the corresponding RRs were 0.6 (0.4–1.0) and 0.7 (0.4–1.2), respectively. Similar patterns were observed for lycopene and alpha-tocopherol. The protective effect was particularly strong when these antioxidants and selenium were combined. Men with the AA genotype had a 10-fold gradient in risk for aggressive prostate cancer across quartiles of antioxidant status (Li *et al.*, 2005).

5.7 Zinc

Zinc supplementation tends to inhibit carcinogenesis based on a systemic effect (Kasprzak and Waalkes, 1986). The binding of cadmium to DNA *in vitro* and the effect of calcium, magnesium, and zinc on the cadmium binding have been investigated by Waalkes and Poirier (1984). All three metals were competitive antagonists of the cadmium binding to high-affinity sites in double-stranded DNA. Zinc had the highest antagonizing capacity followed by magnesium and calcium. The relative capability of these metals to antagonize high-affinity Cd-DNA binding probably parallels their *in vivo* capacity to antagonize Cd-induced carcinogenesis (Waalkes and Poirier, 1984). Esophageal carcinogenesis in rats induced by methylbenzylnitrosamine (MBN) was enhanced by zinc deficiency. Zinc deficiency also caused esophageal tumors in rats exposed to the hepatocarcinogen dimethylnitrosamine (Newberne *et al.*, 1986). Zinc may prevent cadmium-induced testicular tumors of interstitial cell (IC) origin in rats. One mechanism may be zinc-induced synthesis of metallothionein (MT), a metal-binding protein with high affinity for cadmium. Thus, sequestration of cadmium may account for cadmium tolerance in most tissues (Waalkes and Perantoni, 1988). In experiments on isolated interstitial cells of the rat testes, the nonpassive component of the cadmium accumulation was markedly reduced by addition of zinc *in vitro*, indicating a competition for uptake at the cellular level (Waalkes and Perantoni, 1988). Further experiments by Wahba *et al.* (1994) showed that zinc pretreatment had little effect on metallothionein RNA in the testes and did not alter the testicular cadmium-binding protein capacity. In contrast, zinc-pretreated rats showed marked increases in MT RNA and MT protein in liver cells. Thus, the testicular MT gene did not seem to play a major role in the zinc-induced tolerance to cadmium toxicity and carcinogenesis (Wahba *et al.*, 1994).

Zinc finger proteins is a class of DNA-binding proteins, which act as transcription factors binding specifically to short DNA sequences and controlling the transcription of a number of genes. Several metals (e.g., cobalt, cadmium, copper, nickel, and iron) may substitute for zinc in zinc finger proteins. A redox metal substituted for zinc in the zinc finger protein is expected to generate free radicals to cause DNA damage (Sarkar, 1995). The disturbance of DNA damage recognition after ultraviolet (UV) irradiation by nickel (II) and cadmium (II) has been studied in mammalian cells (Hartmann and Hartwig, 1998). In the case of cadmium (II), a dose-dependent inhibition of DNA–protein interactions was detected at a level of 0.5 μmol/L and higher, which was almost completely reversible by the addition of zinc (II) (Hartmann and Hartwig, 1998).

The p53 protein is a zinc-binding protein containing several reactive cysteines. The protein seems to control the timely production of reactive oxygen intermediates, which may initiate apoptosis. The activity, however, is under the control of changes in metal levels and in cellular redox status. This redox sensitivity may be one of the biochemical mechanisms by which p53 acts as a sensor of multiple stress (Hainaut and Mann, 2001). It has been shown that alpha-difluoromethylornithine (DFMO), a highly effective chemopreventive agent in esophageal carcinogenesis, reverses and counteracts esophageal cell proliferation/cancer initiation in zinc-deficient rats by way of stimulating apoptosis (Fong *et al.*, 2001).

The dietary intake and micronucleus index survey in South Australia have been used to study whether supplementation with beta-carotene, vitamin C, and

vitamin E together with zinc (ACEZn) improved genome stability in a randomized trial for 6 months in 190 healthy subjects. Supplementation with zinc significantly reduced the micronuclei assay index by 18% ($P = 0.038$) (Fenech *et al.*, 2005).

6 RISK ASSESSMENT OF MIXTURES OF METALS

6.1 Introduction

Risk assessment of chemical(s) is the practice of evaluating the degree of danger (risk) associated with chemical exposure. Assessing the risks associated with exposure to chemicals involves acquisition and interpretation of appropriate data; drawing and integration of conclusions from such data; and formulation of overall recommendations for the management of potential risks (Mumtaz *et al.*, 1993). Thus, the risk assessment process determines the kind and degree of hazard posed by a chemical(s), thereby permitting an estimate of the present or potential health risk. Typically, it involves four steps: exposure assessment, hazard identification, dose-response assessment, and risk characterization. In itself this process is a real challenge, but when applied to chemicals and/or their mixtures, it becomes that much more formidable. This section focuses on the major principles underlying metal toxicity and provides some basic risk assessment guidance to account for metal-specific toxicity assessment. Metals have a number of characteristics that should be carefully considered throughout the risk assessment process. Some metals are essential because they serve as macronutrients or micronutrients. Also, in contrast to other pollutants, metals are naturally occurring constituents of the environment. These characteristics of metals present additional challenges to risk assessors than those posed by environmental pollutants or other xenobiotics.

6.2 Toxicity Assessment of Mixtures

Metals can affect multiple organs and hence there exists potential of more than one metal occurring at the same time in a given target organ (Table 1). Thus, there exists a need for consideration of joint toxicity assessment of metal mixtures. Three different approaches, riddled with varying uncertainties and assumptions, are described for the toxicity assessment of metal mixtures (ATSDR, 2001; U.S. EPA, 1986). Selection of an appropriate assessment method depends on known toxic effects of chemicals comprising a mixture, availability of toxicity data, and quality of available exposure data.

6.2.1 The Mixture of Concern

The first approach is the "mixture of concern" approach, in which risk is estimated using toxicity data from the same mixture. It is the most direct and simplest method and entails the fewest uncertainties. This approach, however, is the least frequently applied, because very few mixtures have been studied adequately for this type of assessment. Some such mixtures for which MRLs could be derived include fuel oils, jet fuels, mixtures of polychlorinated biphenyls (PCBs), and polybrominated biphenyls (PBBs) (ATSDR,

TABLE 1 Potential Health Effects Caused by Some Metals Found in Human Body Fluids

	Cancer	Reproductive	Immune function disorders	Renal dysfunction	Liver dysfunction	Neurotoxic disorders
Aluminum						•
Arsenic	•	•	•	•	•	•
Barium				•		
Beryllium			•			
Cadmium	•	•		•		
Chromium	•			•	•	
Cobalt			•			
Copper			•	•	•	
Lead		•	•	•		•
Manganese						•
Mercury		•	•	•		•
Nickel	•		•			
Silver			•			
Thallium				•	•	•
Thorium					•	
Uranium				•		

2006). Similarly, a few occupational exposure limits (OELs) for asphalt fumes (0.5 mg/m^3), cotton dust (0.2 mg/m^3), coal dust (0.4 mg/m^3), and Stoddard solvent (100 ppm) have been established (*http://www.acgih.org*; *http://www.cdc.gov/niosh/homepage.html*; NIOSH, 2006).

6.2.2 The Similar Mixture Approach

The second approach used is the "similar mixture" approach and can be applied on a case-by-case basis to a candidate mixture or groups of mixtures that could act similarly (Durkin *et al.*, 1995). It is used when adequate information is not available for the mixture of concern and is often applied to complex mixtures that have been extensively investigated, such as coke oven emissions, diesel exhaust, and wood stove emissions. However, a minimum of information should be available to consider a mixture sufficiently similar to the mixture of concern. For example, if a risk assessment is needed for gasoline contamination of groundwater and information is available on the chronic toxic effects of gasoline, it may be possible to use the available information to assess risks from the contaminated groundwater. However, there are no set criteria to help decide when a mixture is sufficiently similar. Hence, the health assessor is left to determine whether the two chemical mixtures are sufficiently similar and whether this similarity justifies use of surrogate risk or toxicity data. The first two approaches, the mixture of concern and the similar mixture approach, are used for those mixtures that have been experimentally tested as a whole to some extent.

6.2.3 The Hazard Index Approach

The third approach, the hazard index (HI) approach, is the method most often used. This approach integrates the exposure level and the related toxicity into a single value by use of potency-weighted dose or response addition. The goal of the HI approach is to approximate the toxicity index that would have been determined had the mixture itself been tested (Mumtaz *et al.*, 2002b). Initially, the potential health hazard from exposure to each chemical is estimated by calculating its individual hazard quotient (HQ). The HQ is derived by dividing a chemical's actual exposure level (E) through an environmental medium by its acceptable/allowable exposure level (AE) such as a minimal risk level (MRL) or a reference dose (RfD). The hazard index (HI) of the mixture is then calculated by adding together all the component hazard quotients, as illustrated below for three chemicals in a mixture:

$$\mathrm{HI} = \frac{\text{Chem. Exposure}_1}{\mathrm{AE}_1} + \frac{\text{Chem. Exposure}_2}{\mathrm{AE}_2} + \frac{\text{Chem. Exposure}_3}{\mathrm{AE}_3}$$

In a manner analogous to the hazard index approach for noncarcinogens, a hazard index for carcinogens can be estimated by dividing chemical exposure levels by doses (DR) associated with a set level of cancer risk (U.S. EPA, 1986; 2000a):

$$\mathrm{HI} = \frac{\text{Chem. Exposure}_1}{\mathrm{DR}_1} + \frac{\text{Chem. Exposure}_2}{\mathrm{DR}_2} + \frac{\text{Chem. Exposure}_3}{\mathrm{DR}_3}$$

In terms of estimating risk, the HI values obtained using the HI approach should be interpreted carefully. For example, if chemical mixture "X" yields an HI value of 4, it need not be interpreted as twice as toxic as mixture "Y" that yields a value of 2. However, it can be said that mixture "X" is more toxic than mixture "Y". Thus the HI approach can be used for priority setting of mixtures. As the value of the HI increases toward unity, the concern for the potential hazard of a mixture increases. The potential health effects of a mixture are further analyzed and investigated if the HI value is equal to or greater than 1. The HI approach assumes that all components have similar joint action, that is their uptake, pharmacokinetics, and dose-response curves have similar shape (Teuschler and Hertzberg; 1995; U.S EPA, 2000). For carcinogens, the preceding equation assumes that each carcinogen has a linear dose-response curve and that each carcinogen is acting independently (U.S. EPA, 1986).

6.2.4 The Target-Organ Toxicity Dose (TTD)

In terms of estimating risk, it is important that the estimates be realistic. The use of acceptable exposure levels (MRLs or RfDs) that are based on a critical effect to assess secondary effects could lead to overestimation of risk (U.S. EPA, 1989). To circumvent this problem, target-organ toxicity doses (TTDs) can be used (Mumtaz *et al.*, 1997). TTDs, in essence, are effect- or organ-specific MRLs and are calculated by use of the same method and process. Thus, for a given chemical, there could be an MRL for hepatotoxicity and a series of TTDs for nephrotoxicity, neurotoxicity, and reproductive toxicity (ATSDR, 2001a). The TTD method is a simple modification of the HI approach and yields a series of HIs for various toxic effects. The values of endpoint-specific hazard indices are treated the same as that of a HI of a mixture (Mumtaz *et al.*, 1997).

The hazard index approach is a simple approach to implement but somewhat limited in its scope and, as a result, may either underpredict or overpredict risk estimates of mixtures of industrial, occupational, and environmental chemicals if synergistic and antagonistic interactions occur (Feron and Groten, 2002; U.S. E.PA.,1986; 2000a). It is important that the risk

assessment values with the HI approach also express the available information on chemical interactions. But, there is little guidance on how the interactions should be evaluated or incorporated into the overall risk assessment (Feron and Groten, 2002; Mumtaz and Hertzberg, 1993). The National Academy of Sciences has proposed the use of additional safety factors if synergistic interactions are of concern (NAS, 1989).

6.3 A Weight-of-Evidence (WOE) Method

To provide further guidance on this issue, a weight-of-evidence (WOE) method was developed (De Rosa *et al.*, 2002; Mumtaz and Durkin, 1992). The WOE method yields a composite representation of all the toxicological interaction evidence from animal bioassay data and human studies data; relevance of route; duration and sequence; and the significance of interactions. The method consists of a classification scheme used to provide a qualitative and, if needed, a quantitative estimation of the effect of interactions on the aggregate toxicity of a mixture (Table 2). The first two components of the scheme are major ranking factors for the quality of the mechanistic information that supports the assessment and the toxicological significance of the information. The last three components of the WOE are modifiers that express how well the available data correspond to the conditions of the specific risk assessment in terms of the duration, sequence, routes of exposure, as well as the animal models. This method evaluates data relevant to joint action for each possible pair of components of a mixture, and, as such, requires mechanistic information and direct observation of toxicologically significant interactions. Initially, for each pair of component chemicals of a mixture, two binary weight-of-evidence (BINWOE) determinations are made to estimate the effect of the first chemical on the second chemical's toxicity and to estimate the effect of the second chemical on the toxicity of the first chemical. A BINWOE determination is a qualitative judgment based on empirical observations and mechanistic considerations, which categorizes the most plausible nature of any potential influence of one compound on the toxicity of another for a given exposure scenario (ATSDR, 2001a; Mumtaz and Durkin, 1992).

Once all of the qualitative WOE determinations have been made for each pair of compounds in the mixture, these are arrayed in a qualitative WOE matrix (Table 3). This matrix lists each potential binary classification along both axes. The diagonal line running from the upper lefthand corner to the lower righthand corner corresponds to chemical identities. These are, by definition, dose additive and are left blank in the interaction matrix. The column headings indicate the chemicals that are affected by

TABLE 2 Weight-of-Evidence Scheme for the Qualitative Assessment of Chemical Interactions

Determine whether the interaction of the mixture is additive (=), greater than additive (>), or less than additive(<).

Classification of Mechanistic Understanding

I. Direct and Unambiguous Mechanistic Data

The mechanism(s) by which the interactions could occur has been well characterized and leads to an unambiguous interpretation of the direction of the interaction.

II. Mechanistic Data on Related Compounds

The mechanism(s) by which the interactions could occur is not well characterized for the compounds of concern, but structure/activity relationships, either quantitative or informal, can be used to infer the likely mechanisms and the direction of the interaction.

III. Inadequate or Ambiguous Mechanistic Data

The mechanism(s) by which the interactions could occur have not been well characterized, or information on the mechanism(s) does not clearly indicate the direction that the interaction will have.

Classification of Toxicological Significance

A. The toxicological significance of the interaction has been directly demonstrated.
B. The toxicological significance of the interaction can be inferred or has been demonstrated in related compounds.
C. The toxicological significance of the interaction is unclear.

Modifiers

1. Anticipated exposure duration and sequence
2. A different exposure duration or sequence
   a. *In vivo* data
   b. *In vitro* data
      i. The anticipated route of exposure
      ii. A different route of exposure

Source: Modified from Mumtaz and Durkin (1992).

TABLE 3 Matrix of BINWOE Determinations for Intermediate or Chronic Simultaneous Oral Exposure to Chemicals of Concern

Effect of	On toxicity of			
	Lead	*Manganese*	*Zinc*	*Copper*
Lead		=IIICii n	=IIB h	=IIIC p
Manganese	>ICii n >IIB2ii h		? h	? p
Zinc	<IB n <IA h	? n		<IB p
Copper	<IC n <IB h	? n	<IIA h	

n, Neurological; h, hematological; p, hepatic. Direction of interaction: =, additive; >, greater than additive; <, less than additive; ?, indeterminate. Mechanistic understanding: I, clear; II, inferred; III, unclear. Toxicologic significance: A, clear; B, inferred; C, unclear. Modifying factors: 2, different exposure duration or sequence than anticipated; ii, different route of exposure than anticipated.

the compounds listed in the row headings. For example, the classification for the effect of zinc on the toxicity of lead is given in the first column (lead) of the third row (zinc). Similarly, the classification for the effect of copper on the toxicity of zinc is given in row four (copper) column three (Zn). Binary classifications starting with an "=" indicate an additivity; those starting with a ">" indicate a greater than additive interaction; and those starting with a "<" indicate a less than additive interaction.

The qualitative WOE matrix may then be used as a tool to quantify the risk assessment for a particular site. The quantitative BINWOE can have an absolute value of 0 to 1, with 1 indicating the highest degree of confidence in the assessment. Ultimately, this WOE method can be used so as to modify the HI and include the role of chemical interactions (ATSDR, 2001a, De Rosa *et al.*, 2002; Mumtaz and Durkin, 1992). Improvements in assessing human health risks from chemical mixtures can only come about by reducing inherent uncertainties in risk assessment methods. This will require an understanding of how chemicals behave in biological systems, as well as explaining their collective mechanisms of action.

When performing an exposure-based assessment of joint toxic action of the mixture, the BINWOEs developed for the endpoints of concern can be used qualitatively to predict the impact of interactions. An analysis to evaluate neurological, hematological, and hepatic effects of varying pairs of lead (Pb), manganese (Mn), zinc (Zn), and copper (Cu) yielded the following WOE matrix (Table 3). The BINWOE for the hematological effect of zinc on lead is <IA. The qualitative interpretation will be as follows:

6.3.1 Direction of Interaction

The direction of interaction is predicted to be less than additive based on a study in orally exposed children indicating a protective effect of zinc on the hematological effects of lead (Chisolm, 1981). This is supported by several intermediate-duration oral studies in rats that show supplemental zinc protects against a number of hematological effects of lead related to heme synthesis, particularly at higher lead doses (Cerklewski and Forbes, 1976; Flora *et al.*, 1982). The evidence for zinc inhibition of lead hematotoxicity is clear and toxicologically significant and is supported by clear mechanistic understanding that excess zinc protects and reactivates lead-inhibited ALAD (delta-aminolevulinic acid dehydratase), decreases the absorption and tissue distribution of lead, and may induce proteins that sequester lead and donate zinc to ALAD and for other tissue needs.

6.3.2 Mechanistic Understanding

A characteristic and sensitive effect of lead is the inhibition of ALAD, a zinc-containing enzyme in the heme synthesis pathway. Zinc protects the enzyme from inactivation by lead *in vivo* and *in vitro* (Abdulla *et al.*, 1979; Mauras and Allain, 1979), as assayed directly or by the appearance of greater amounts of ALA (delta-aminolevulinic acid) in the urine. Supplemental zinc decreased the gastrointestinal absorption of lead and decreased blood, bone, liver, kidney, and spleen concentrations of lead in rats in intermediate-duration studies (Cerklewski and Forbes, 1976; El-Gazzar *et al.*, 1978; Flora *et al.*, 1982; 1989). This effect was seen at higher, but not lower, doses and was not seen in humans at lower doses of both metals than those used in the rat studies. The evidence in general suggests that oral coexposure to zinc at levels significantly above essentiality decreases lead absorption and body burden at higher lead exposures. Zinc induces a metallothionein that has been shown to sequester lead *in vitro*, protecting against its cytotoxicity (Goering and Fowler, 1987; Liu *et al.*, 1991). A metallothionein-like protein in erythrocytes binds lead *in vitro* and was present in higher concentrations in lead-exposed workers than in controls. This protein was also present at exceptionally high levels in a worker with very high blood lead (180 μg/dL), but no signs of lead poisoning, compared with another worker with high blood lead (160 μg/dL) who was symptomatic (Church *et al.*, 1993a; 1993b). An acidic, soluble lead-binding protein in erythrocytes (and brain and kidney) that normally binds zinc is postulated to attenuate lead toxicity to ALAD through lead binding and zinc donation (Fowler, 1998). The preceding mechanistic data indicate that under conditions in which an interaction occurs, the effect of zinc on the hematological toxicity of lead will be less than additive.

6.3.3 Toxicological Significance

A study in occupationally exposed men given a low oral dose of zinc did not provide evidence of an effect on blood lead or urinary ALA. However, the inhalation route of exposure for lead circumvents potential interactions at the level of absorption, and the dose of zinc was very low in this study. In another study, an increase in urinary ALA above the normal range was significantly associated with a decrease in the chelatable zinc/lead ratio to 18.45 or less in children given chelation therapy for lead poisoning (Chisolm, 1981). Supplemental zinc protected against the inhibiting effects of lead on ALAD activity and against lead-induced increases in zinc protoporphyrin and urinary ALA excretion in rats given both metals orally for intermediate durations (Cerklewski and Forbes, 1976; El-Gazzar *et al.*, 1978; Flora *et al.*, 1982; 1989). These protective effects were seen at higher but not lower lead doses and when basal levels of zinc in the diet were adequate. The potential public health hazard of the joint toxic action of this mixture must also recommend inclusion of estimation of endpoint-specific hazard indexes (HIs) for neurological effects of lead and manganese and for hematological effects of lead and zinc. The HIs are estimated by use of exposure data and health guidance values:

$$\mathrm{HI}_{\mathrm{neuro}} = \frac{\mathrm{Exposure}_{\mathrm{Pb}}}{\mathrm{Guidance\ value}_{\mathrm{Pb}}} + \frac{\mathrm{Exposure}_{\mathrm{Mn}}}{\mathrm{Guidance\ value}_{\mathrm{Mn}}}$$

This HI approach should be used when the hazard quotients (HQ) of at least two of the components equal or exceed 0.1. If only one or if none of the mixture components has a hazard quotient that equals or exceeds 0.1, further assessment of the joint toxic action is not needed, because additivity and/or interactions are unlikely to result in significant health hazard (ATSDR, 2001a). With the information from the WOE matrix, the following summarizations can be made regarding the impact of interactions on some specific toxicities.

A deductive method such as the WOE should have built-in capabilities to integrate all observations on interactions, reflect the underlying principles and mechanisms that are operative, allow the users to express uncertainty comparable to existing methods, be able to be applied consistently in varying exposure scenarios, be used qualitatively and if possible quantitatively, and, most importantly, be able to be verified and validated through experimental studies.

6.4 Perspectives and Future Needs

The best measurement of exposure comes through biomonitoring, the measurement of chemicals in human tissues and fluids. This age-old practice has been used by the Centers for Disease Control and Prevention (CDC) to study the presence of natural and synthetic chemicals in human populations (CDC, 2005 the NHANES report). This has led to the development of programs to control emission of certain environmental pollutants such as lead, when the cause and effect has been established. The measurement of metals in biological fluids is the primary means of quantifying exposure for metals by occupational health organizations, such as the American Congress of Governmental Industrial Hygienists, and is the basic tool for population or molecular epidemiology studies of effects of exposure to humans of various metals.

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CHAPTER

8

Epidemiological Methods for Assessing Dose-Response and Dose-Effect Relationships

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By the term *epidemiology*, we mean the science of the occurrence of health and illness in *human* populations. The formal definition is "the study of the distribution and determinants of health-related states or events in specified populations, and the application of this study to control health problems" (Last *et al.*, 2000). Although the focal point in clinical practice is the *individual* and changes in the individual's health status, in epidemiology the interest is extended to *groups (populations)*. However, in modern evidence-based medicine, clinical decision making uses integrated epidemiological and clinical observations.

In this chapter, we will introduce basic concepts of epidemiology and discuss how they are applied in assessing dose-response or dose-effect relationships for metals. The chapter will not systematically explain all concepts and methods in epidemiology as such. The reader who is seeking more detailed information about epidemiology is referred to standard textbooks, such as *Basic Epidemiology* (Beaglehole *et al.*, 2000) (or one of the 20 translations), *Environmental Epidemiology* (Baker *et al.*, 1999), *Epidemiology Beyond the Basics* (Szklo and Nieto, 2004), or *Modern Epidemiology* (Rothman and Greenland, 1998). The terminology in this chapter is based on the *Dictionary of Epidemiology* (Last *et al.*, 2000), sponsored by the International Epidemiology Association. The structure of the chapter is inspired by Beaglehole *et al.* (2000), listing measurement concepts and terms in an early section. Systematic research on the dose-response and dose-effect relationships for metals developed in the 1970s, and we will make reference to the early studies, as well as recent studies, to highlight that useful evidence was accumulating long before we could do literature searches in *Google Scholar*.

1 EPIDEMIOLOGICAL MEASUREMENT OF OCCURRENCE OF HEALTH EFFECTS

Epidemiological studies are essential for assessing dose-response or dose-effect relationships for toxic metals (see Chapter 6), because humans and laboratory animals may differ in the metabolism and toxicity of metals. Results of animal experiments can, therefore, seldom be used directly to draw quantitative conclusions about human effects and responses. On the other hand, epidemiological studies can be used in this way, because they measure health effects of metals in groups of people with different exposure levels, different vulnerability, or other relevant characteristics. The measurement of health effects in these groups would focus on the *population at risk*. This population includes only the people who are actually able to suffer from the health effects under study. For instance, congenital malformations can only occur among newborn children who would thus be defined as the population at risk in a study of such malformations. If prostate cancer is being studied, only men are included in the population at risk.

The study population is the selected part of the total population that is actually studied. It should include people with a range of different metal exposure levels in the population at risk, but it may be restricted to selected age, gender, or occupation groups to reduce the number of variables that potentially influence the health effect and to draw more focused conclusions from the study.

The different approaches and terms used in expressing the results of epidemiological measurements will be presented here, because these terms will be referred to in the subsequent sections. Issues concerning the analysis of differences between groups with different levels of metal exposure will be discussed in Section 5.

The occurrence of disease or toxic effects is measured either as *incidence*, which is the number of newly developed cases during a defined period, or as *prevalence*, which is the number of existing cases at a defined point in time. Incidence and prevalence are usually calculated as rates in the population at risk (e.g., number of cases per 1000 persons at risk). Depending on the type of disease or effect that is measured, the time unit used for incidence rates may vary. The most common unit for chronic effects is years. For acute effects, days or even hours may be more appropriate. The time unit is included in the incidence rate. The rate may be expressed as cases per 1000 persons per year or cases per 1000 persons per day, depending on the type of disease or toxic effect studied. The size of the population at risk is often determined as the average population during a defined period. In epidemiological studies of groups with a rapid turnover of individuals entering and leaving the population at risk, the average population can vary greatly with time. For each individual, the *number of person-years of follow-up* can then be calculated, and the incidence would be expressed as the number of cases per 1000 person-years.

Incidence and prevalence rates are interdependent, because the number of existing cases depends on how many new cases occur and how many disappear because they die or are cured. The *duration* of the disease and the time unit for incidence are the factors on which this interdependence is based. A low incidence may give a high prevalence if the duration of the disease is longer than the time unit for incidence. If the time unit for incidence is very long, the calculation should be corrected for the increasing number of prevalent cases that cannot experience the effect again. The correction is made by calculating the *cumulative incidence rate* (Beaglehole *et al.*, 2000). This measure is particularly valuable in studies of chronic diseases such as cancer. In these studies, interest is often directed to the cumulative incidence over a 30- or 40-year period, and increasing incidence rates with age need to be taken into consideration. The cumulative incidence in the population at risk can be described as the *risk* (or probability) of an individual contracting the disease.

The occurrence measures mentioned previously are absolute measures. It is common to calculate relative measures when the occurrence of effects in the population at risk is compared with the occurrence in some defined *control population* (or *reference population)* without excessive exposure to the metal under study. If the population at risk for metal exposure is very small in comparison with the entire population of the country or the area, then this entire population can be used as a control population.

In mortality and cancer-morbidity studies, the *life-table method* for calculating relative risks has been used (e.g., studies on arsenic and heart disease mortality; Chen *et al.*, 1996). From death registers and cancer registers, the age-specific incidence rates for dying of a particular disease or of contracting cancer at a particular site are calculated for an entire country or province (the control population). For each individual in the population at risk, these rates are used to estimate the risk of dying or of contracting cancer within the period under study. The *expected number of cases* is calculated as the sum of the individual risks, and this number is compared with the *observed number of cases* to calculate the *risk ratio* (relative risk) and the *risk difference* (Beaglehole *et al.*, 2000). If the risk ratio is 2, this means that the effect is twice as common in the population at risk as in the control population. The risk difference is expressed in the same unit as the risk itself (e.g., cases per 1000 persons).

Two other important measures of the occurrence of effects are the *attributable risk among the exposed* and the *attributable risk in the population.* The former expresses the part of risk of the effect occurring among the exposed that is thought to be caused by the exposure. It can be computed as the risk difference divided by the total risk in the exposed group (Beaglehole *et al.*, 2000). The latter takes into consideration the fact that in any given population, only a fraction may be exposed. Thus, the attributable risk in the population is the part of the measured risk of the effect on the entire population that is thought to be caused by exposure to the metal.

Epidemiological studies for assessing dose-effect and dose-response relationships of metal toxicity have the advantage that they are usually dealing with only one specific exposure—the metal ion (or metal compound), which is not metabolized into any other substance. However, the speciation or oxidation state may change, which, in some cases, may be of toxicological importance. Another crucial factor is the measurement

of dose at the appropriate time or time span. It is often necessary to estimate long-term doses that take into account long half-times of the metal in the critical organ. This may involve the use of kinetic models (see Section 4.1), because direct measurements may not be possible.

Measurement of effects requires a clear case definition and attention to the timing of the effect (see Section 4.2). From the viewpoint of prevention, emphasis should be placed on early or subclinical effects that may occur in the most sensitive part of a population at risk. Measurements of relatively high response rates can be made in studies of limited size. Very low response rates (e.g., 1 case per 1000 persons) may, however, necessitate large-scale studies that are not always feasible because of cost or because the population at risk is not large enough to provide valid findings.

2 OBSERVATIONAL STUDIES AND MODELING STUDIES OF DOSE-RESPONSE RELATIONSHIPS

Dose-effect and dose-response relationships for humans can be ascertained in two different ways. *Observational studies* involve measurement of exposure and dose in groups within the study population and measurement of occurrence of effects and response in the same groups by use of clearly described methods. In the analysis, the relationship between dose and severity of effect and between dose and response are ascertained (Figure 1). *Modeling studies* do not assess the exposure at an individual level but involve the use of metabolic models, statistical distributions of the occurrence of the toxic metal in tissues or the daily intake, as well as estimates of critical concentrations in the critical organ to calculate the proportions of groups in the population at risk that are likely to have the effect (Figure 2). Another term for modeling studies would be health risk assessment or health impact assessment (Yassi *et al.*, 2001). In such cases in which a direct exposure at an individual level is not available, a variety of *proxy variables* (e.g., questionnaire data or survey results) may be applied.

Observational studies are more straightforward and avoid assumptions about the metabolism of the metal compound, which may increase the uncertainties of the dose-response evaluations. Even low incidence rates can be studied from routine records of deaths or of specific diseases. Less prominent effects, such as lead toxicity on hemoglobin synthesis (Roels *et al.*, 1975), have also been established through observational studies. In designing and evaluating these studies, the background occurrence of the effect in question must be taken into account. For instance, renal tubular damage of the type seen in chronic cadmium poisoning (Chapter 23) is rare in the general population, whereas paresthesias, an early symptom of methylmercury poisoning (Chapter 33), is not uncommon and mostly from other causes.

Modeling studies may be used as an extension of data from observational studies for parts of the dose-response relationship where no observations exist or as a complete substitute for observation studies. The metabolic model is the key (Figure 2). The models used range in complexity from the one-compartment model of methylmercury metabolism proposed by Berglund *et al.* (1971) to the eight-compartment model of cadmium metabolism (see Chapter 23) proposed by Kjellstrom and Nordberg (1978). The model is a means of linking together data on metal intake, metal concentrations in different tissues, and metal excretion. Data from groups with relatively low exposure levels

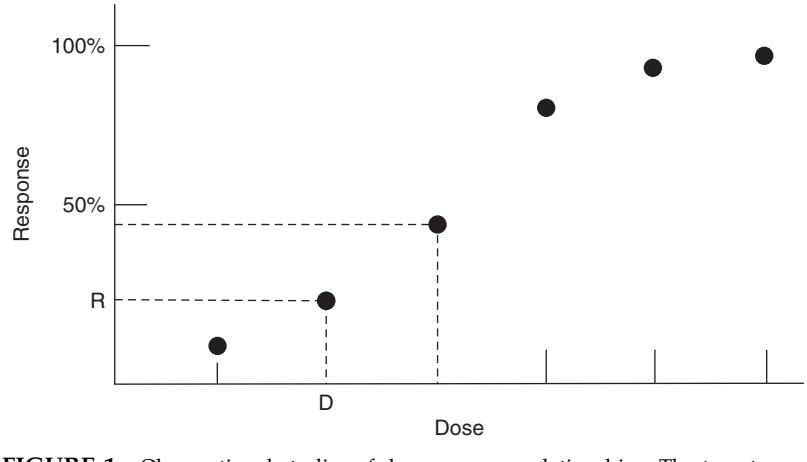

FIGURE 1 Observational studies of dose-response relationships. The target population is stratified into groups according to dose. The mean dose and the mean response are measured for each stratum, giving one combined point on the dose-response curve.

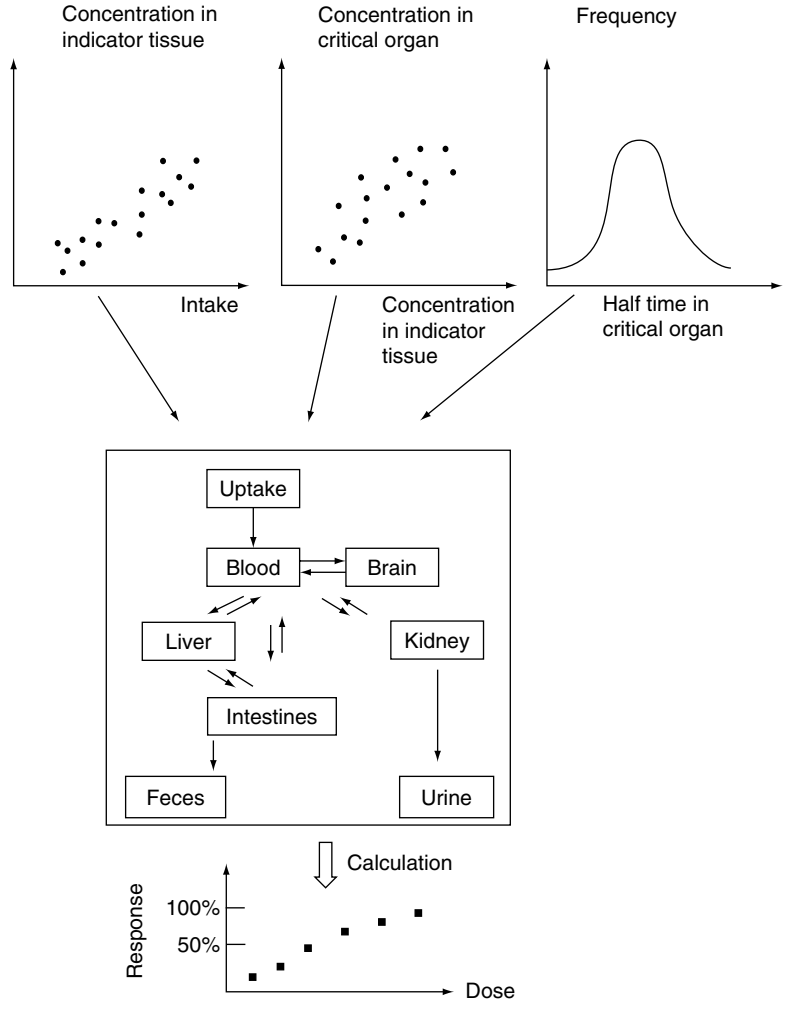

FIGURE 2 Modeling studies of dose-response relationships. Data on human metabolism of the metal and distribution of half-times, etc. are used to establish a metabolic model. The model calculates the proportion of a group with a particular dose that would have metal concentrations in critical organs above an estimated critical concentration. This is used as an estimate of response rate in each dose group.

can be used to quantify rates of transfer between compartments in the model. If it can be assumed that these rates are independent of the exposure levels (within the range of interest), critical organ concentrations under particular exposure conditions can be predicted. Individual variation in dose within the population at risk, as well as the individual variation in half-times of the metal compound in the main body compartments, can be expressed as statistical distributions. In the case of cadmium, for instance, it has been shown that both daily intake and renal cortex cadmium concentrations follow log-normal distributions, which coincide remarkably well with calculations that use a metabolic model (Kjellstrom, 1977).

The critical organ concentration is defined as a threshold level above which "adverse" changes occur in the cells of the critical organ (TGMT, 1976) in an individual. For most metals, it can be assumed that there is considerable individual variation in the sensitivity of the critical organ. This has been shown clearly by *in vivo* neutron activation studies of cadmium in renal cortex (Ellis *et al.*, 1984). Some controversy about critical concentrations for cadmium has developed, because of the application of the term to populations without consideration of individual variations. To overcome this problem, the term population critical concentration (PCC) has been proposed (Friberg and Kjellstrom, 1981; Kjellstrom *et al.*, 1984). The PCC-50 is the concentration in the critical organ at which 50% of the population has exceeded the individual critical concentrations; the PCC-10 applies to 10% of the population and so on. This approach to assess risk is similar to the benchmark dose concept (see later).

By use of metabolic models based on epidemiological data on metal intake, tissue distribution, and excretion, as well as statistical distributions and estimates of critical organ concentrations, the proportion of a population at risk that develops the critical effect (the response rate) can be calculated. Calculated dose-response relationships, such as those for methylmercury (Nordberg and Strangert, 1976) and for cadmium (Kjellstrom, 1977; 1985), can be of value to indicate what response rates can be expected at different doses. This information can be used to decide on sample size in direct studies and to decide on provisional hygienic standards for primary prevention.

Health risk assessments have provided new insights into the potential epidemiological impact of metal compounds. For instance, Trasande *et al.* (2005) analyzed the impact of mercury emissions from coal-powered electricity generation plants in the United States. The frequency distributions of different blood mercury levels were combined with estimates of dose-response functions (from other epidemiological studies) to calculate that between 300,000 and 600,000 children each year would be exposed to methylmercury levels in maternal blood high enough to cause loss of IQ.

3 STUDY DESIGN

Epidemiological study designs can be classified into three types (Beaglehole *et al.*, 2000) (Table 1). Descriptive studies provide data on the occurrence of exposures, dose, or effects with no specific comparison between dose and effects or dose and response. Studies on metal intake through food in a population or on the incidence of stomach cancer in different age and sex groups would be descriptive studies. Data from this type of study are commonly used for dose estimation in modeling studies of dose-response relationships (see Section 2). Analytical studies combine data on exposures or doses with data on effects or responses to estimate the causative role for a particular exposure. Intervention studies involve manipulation (or intervention) of metal exposure in a randomized group in the population and follow-up of the influence of intervention on the occurrence of effects. Intervention studies are, in a sense, equivalent to human experiments and can be used only for studying effects that are reversible and not injurious to the persons exposed. They are, therefore, primarily used to evaluate preventive interventions (e.g., Lanphear *et al.*, 1999; Markowitz *et al.*, 2004; Weihe *et al.*, 2005). Data from analytical studies are the basis of most observational studies of dose-response relationships. Epidemiological studies can also be classified with regard to data specificity as either macroepidemiological (ecological, population level) or microepidemiological (individual level) (Table 1).

The studies can also be classified with regard to the time relationship of observations as either longitudinal or cross-sectional (Table 1). In the longitudinal studies, data about exposures and effects are collected at more than one point in time, and antecedent–postcedent relationships can be analyzed. A cross-sectional study collects data on exposures and effects at only one point in time, which is often a disadvantage from the point of view of analysis. Longitudinal data can be collected either retrospectively or prospectively. Retrospective data collection involves the use of information about the past (usually from existing data), whereas prospective data collection makes it possible to design more precise and standardized data sets that fit the needs of the study. When the half-time in the critical organ is very long, as it is for cadmium (see Chapter 23), retrospective exposure estimates are essential for achieving reasonably rapid results.

If there is a good indicator of internal dose—for instance, blood lead for inorganic lead exposure (see Chapter 31) and hair mercury for methylmercury exposure (see Chapter 33)—data on dose and effects may be collected at the same time (cross-sectional study), because the indicator reflects exposures in the past. However, irregular exposure and other types of imprecision may render this measure unreliable. Thus, data often have to be collected longitudinally over time.

TABLE 1 Classification of Observational Epidemiological Studies

Type		
Descriptive	Describing the occurrence of exposures and effects	
Analytical	Testing associations between exposures and effects	
Intervention	"Experiments" in which exposures are assigned (includes randomized controlled trials)	
Direction		
Case-control	Start from cases with effect and probe into their exposures	
Cohort (follow-up)	Start from exposed people and see whether they get the effect	
Timing		
Cross-sectional	All data collected referring to one point in time	
Longitudinal	Retrospective	Data collected regarding the present and the past
	Prospective	Data collected regarding the present and the future

The study can either focus on cases with the effect as compared with a control group without the effect, or it can focus on persons with the metal exposure and compare them with people without exposure. The first type of study is called a case-control study (Table 1). The cases and non-cases are compared concerning their metal exposures in the past or the present based on analysis of indicator tissues. Higher doses in the group of cases may be interpreted as an association between the metal and the effect. *Absolute* occurrence and dose-response relationships cannot be measured using a case-control study alone, but a *relative* occurrence between groups with different dose levels can be calculated in the form of an *odds ratio*. If both the cases and the controls are stratified into groups according to metal dose, the odds ratio for each stratum can be calculated; this ratio can be expressed as a dose-response relationship. Case-control studies are often retrospective, because the analysis starts out from the people with effects, and their relevant metal exposure for these effects must have occurred in the past. The term retrospective study has, therefore, often been used in the past as a synonym (Beaglehole *et al.*, 2000). It should be pointed out that retrospective data collection might increase errors in exposure estimations, because of the inherent difficulties in "reconstructing the past."

To measure the *absolute* incidence or prevalence rates needed to establish complete dose-effect or dose-response relationships in different dose groups, a cohort study (follow-up study) would be the most suitable. In this type of study, an exposed group and a nonexposed group (controls) are selected. The occurrence of effects of the metal is measured in each group. The exposed group can be stratified into groups according to dose level. The measurement of incidence or prevalence of effects in each group forms the basis for dose-effect and dose-response relationships.

One of the most important steps in an epidemiological study is the selection of the groups under study. In both descriptive and analytical (follow-up) studies, it should be ascertained that the people in the exposed group are really exposed and belong to the population at risk. Ideally, the entire group that is and has been exposed should be studied, or if the group is too large, a randomly selected sample from this group should be studied. In an industrial environment, this means that all workers who were ever exposed in the factory under study should be included. An operational definition for "exposed" may be used such that an exposure duration limit is set (e.g., having worked in a metal-exposed area of a factory for 1 year or longer). If only workers actively working at the time of the study are included, those with the effect may be selectively excluded, because they had to stop working when they contracted the effect. In a dose-response study of cadmium-induced tubular proteinuria (Kjellstrom, 1977), it was shown that the possible underestimation of response because of selective exclusion might be considerable. A similar bias may occur in studies based on population screenings where only those healthy enough to come to the screening can participate. Selection bias may also be introduced in studies of industrial workers by the procedure of preemployment selection of workers. Mainly healthy young men would be selected for the work that involves metal exposure, and the results of such a study may not be applicable to the general population. This is called the *healthy worker effect* and is a problem in, for example, retrospective follow-up studies of mortality (Kitagawa and Hansen, 1973; McMichael, 1976).

The selection of the control group in analytical follow-up studies is as important as the selection of the exposed group. Selection bias caused by, for example, sick people who are not able to participate, should be avoided. If any sampling of the exposed group takes place, the same criteria for sampling should be used for the control group.

In case-control studies, similar problems of selection occur as in follow-up studies. A clear definition of "a case" is needed, and often this has to be based on an operational definition from symptoms, signs, and biochemical or physiological measurements. The selection of a "control" in a case-control study is sometimes a problem, because it is necessary to find cases and controls in the same source population, the study base (Rothman and Greenland, 1998) (which may be restricted by age, gender, etc.). For example, if cancer is the effect studied, ideally the wider population in which the cancer cases occur should be the source of the controls. In some studies of hospitalized cancer cases, other hospital patients without cancer have been selected as controls. These controls naturally have some other disease, because they are also hospital patients, and if this disease is also associated with the metal exposure, the epidemiological analysis may be invalid. In the choice between follow-up and case-control at the design stage of the study, it should also be pointed out that the size of the study group needed for a particular statistical power in the conclusions depends on whether the exposure or the effect is rare. If the exposure is rare, but the exposed people can be identified, a follow-up study needs a smaller group than a case-control study. If the effect is rare, the opposite is true. If both exposure and effect are relatively common (e.g., in more than 10% of groups studied), either type of study can effectively be used.

Confounding factors can interfere with epidemiological analyses. A confounding factor is something that is associated with both metal exposure and the effect; for example, heavy drinking is associated with

gout. If the exposed group in a study of molybdenum and gout had a greater proportion of heavy drinkers than the control group, the drinking habits would be a confounding factor. At the stage of selection of target groups for the study, confounding can be controlled by stratification of the target group to subgroups of the population at risk according to drinking habits (stratified samples). Confounding can also be controlled by restriction. If drinking is a confounding factor, the study could be restricted to nondrinkers only, but the results may then only apply to the selected group (nondrinkers). One way of stratifying (and restricting) the sample in cohort studies is matching. For each exposed person, one or more controls are selected that have the same characteristics (age, gender, occupation, etc.) as the exposed person. Confounding can also be controlled for at the analysis stage of the study.

By controlling the confounders, a more precise estimate of the metal effects will be expected. However, this may not be true when the exposure parameter is imprecise (Budtz-Jørgensen *et al.*, 2003). Also, even when information on several suspected confounders has been collected, some residual confounding may remain. An important question is, therefore, whether the estimated effect of the metal exposure should be considered reliable. Although smoking has often been invoked as a confounder that may explain occupational lung cancer risks, simulations have clearly shown that any residual confounding because of unmeasured differences in smoking patterns is unlikely to explain risks that are above a certain level (Axelson, 1989). A balance, therefore, needs to be achieved, without invoking unjustified "ghost" confounders.

A study design often used in studies of subjective symptoms, for example to study short-term symptoms of air pollution, is the panel method. Groups of people in an area with anticipated variations in exposure to air pollutants are asked to keep a diary of symptoms as they occur each day. During the same period, exposure levels are measured, and any association between exposure and effects on a day-to-day basis is analyzed. The people studied serve as their own controls during periods of low exposure. This approach is also the basis for time-series studies and case crossover studies (Szklo and Nieto, 2004).

4 DATA COLLECTION

4.1 Measurement of Dose

Ideally, the metal dose should be measured in the tissue where the metal elicits its earliest effect, the critical organ (TGMT, 1976). In many cases, this is not possible, and, instead, indirect measurements of varying degree of accuracy have to be made (Figure 3). The critical organ is often an internal organ that is not directly accessible for metal measurements. During autopsy, samples may be collected and analyzed, and in some cases biopsy specimens have been used (e.g., renal biopsies from cadmium workers—Axelson and Piscator, 1971). Autopsy data (or stored organ analysis data) may be compared with earlier data on effects, as was done in some early studies of Minamata disease (methylmercury poisoning) (Harada *et al.*, 1977) and Itai–Itai disease (cadmium poisoning) (Kjellstrom, 1985). For certain metals, the *in vivo* neutron activation technique (Ellis *et al.*, 1984; Harvey *et al.*, 1975; Morgan *et al.*, 1990) can be used to measure excessive doses. *In vivo* methods have been used primarily for cadmium and lead (see, Chapters 23 and 31).

Indicator tissues, such as blood (for lead), urine (for cadmium), or hair (for methylmercury), can be used to estimate the dose in the critical organ. To translate

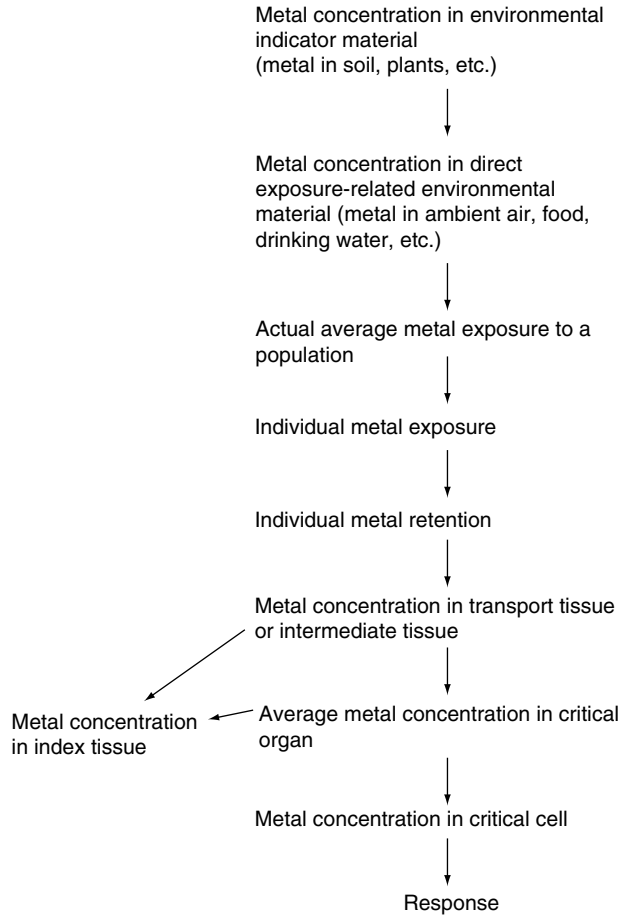

FIGURE 3 Sequential relationship between dose variables and a specific response. (From Kjellstrom, 1976).

indicator tissue concentrations into critical organ dose, metabolic models (see Section 2) are useful. The amount of a metal in feces is a good indicator of daily intake by ingestion when the gastrointestinal absorption is low. In Chapter 23, a comparison between cadmium body burden and daily fecal and urinary amounts of cadmium is given, which shows good agreement between body burden and urine cadmium on a group basis after long-term low-level exposure. Body burden and kidney burden are also well correlated (Kjellstrom, 1977). Hair mercury is a good indicator of body burden of methylmercury (Friberg and Vostal, 1972), and because of the relatively even distribution of methylmercury in the body (see Chapter 33), it is also assumed to be a good indicator of the amount of methylmercury in the brain. Hair has the advantage that it may be a source of retrospective dose data, because the metals stay in the hair as the hair grows. It was shown after the methylmercury poisoning outbreak in Iraq (Bakir *et al.*, 1973), sectional analysis of hair could pinpoint the time when mercury consumption started and the time when it reached its maximum.

The measurement of dose by analyzing indicator tissues or the critical organ itself involves analysis of the metal compound in the tissue. The concentrations are usually very low, in the order of μg/kg to mg/kg, which may make accurate analysis difficult. The problems of getting accurate chemical analysis results have been highlighted by interlaboratory comparisons (Kjellstrom, 1979; Nordberg *et al.*, 2000). Alternately, the dose may be estimated from exposure data if uptake kinetics and half-time in the critical organ are known. The product of exposure level and exposure duration, corrected for the ratio between duration and half-time and also for the absorption rate, can give an estimate of dose. The calculation of dose should be based on the exposure that is relevant for uptake. Measurements of exposure to metals, particularly through air, should ideally include an estimate of particle size distribution and the metal concentration in each size fraction. The absorption of metals in the intestines can be affected by other elements in the diet (see Chapter 3). Therefore, additional information on diet content would be necessary to calculate dose from metal levels in different foodstuffs.

Other ways of estimating the dose involve "proxy variables," which can reasonably be expected to be associated with actual exposures. One example is the use of the number of amalgam surfaces on teeth to estimate past exposures to inorganic mercury (Bates *et al.*, 2004). Because they are elements, most metals occur naturally in air, food, and drinking water. Whichever particular exposure conditions are being studied, the total exposure by adding all possible uptakes must normally be considered. Skin exposure may be significant in occupational environments.

Smoking can increase exposure both through the natural metal content of cigarettes and pipe tobacco and through external contamination of the smoking gear from hands, overalls, or the workroom air (Hassler, 1983; Piscator *et al.*, 1976). A smoker may be sensitive to airborne metal exposures in other ways. Smoking is associated with chronic bronchitis and emphysema (which can also be caused by cadmium inhalation), and it slows down lung clearance (Albert *et al.*, 1969; see also Chapter 3).

For acute effects of metals, the peak exposure levels will determine the risk of effect. We can assume that in some cases, for chronic effects also, the dose may not be a function of exposure level times the exposure duration, but rather a function of the frequency of peak exposures above a certain level (impulse exposures).

As indicated previously, all exposure measures involve some element of imprecision. None of the exposure biomarkers or other variables amply reflect the concentration profile occurring in the critical organ during the time period of relevance. In general, such imprecision is random, although it may sometimes be relatively greater at low concentrations. Random or nondifferential exposure measurement error results in underestimation of the risk. The larger the imprecision of the exposure parameter, the greater the underestimation. Unfortunately, a "gold standard" is rarely available, and the degree of misclassification is, therefore, seldom known for sure, and its magnitude must therefore be assumed (e.g., from comparisons between exposure indicators). Recent studies suggest that such comparisons result in an underestimate of the total imprecision (Grandjean *et al.*, 2004).

Although imprecision is generally expressed as the coefficient of variation (CV) obtained from laboratory quality assurance studies, other factors may play a role as well. For example, the degree to which an exposure parameter reflects the concentration at the sensitive target or compartment within the body may vary, and the laboratory accuracy and precision will not reflect the total degree of uncertainty associated with a particular exposure parameter. In studies on methylmercury toxicity, the laboratory CV was approximately 5% or less (i.e., of minimal significance for the outcome of the studies). However, an exposure indicator can be expressed as a function of the "true" exposure plus an intercept and an error function. This equation has three unknowns, but it can be modeled (e.g., by factor analysis) if at least three exposure variables are available from each subject. Using this approach, the

hair-mercury result obtained in a large population study was found to have a total CV of approximately 50% (Grandjean *et al.*, 2004). The laboratory variability alone was a small contributor. Irregular exposures, external contamination of the hair, leaching of mercury because of hair treatment, and differences in hair structure and hair growth rates may all have contributed to this large imprecision. These findings suggest that efforts to minimize laboratory imprecision need to be seen in regard to their contribution to total error levels. A greater impact may be achieved by collecting samples that better reflect the dose level (e.g., the cord-blood mercury concentration).

4.2 Measurement of Effect and Response

Whenever health effects are measured, biological variation and random errors in the measurement procedure will affect the results so that false-negative and false-positive results will occur. The ideal test of effects gives only true-positive and true-negative results, but such tests are difficult to find. A clinical sign or symptom, which is typical for a particular exposure, is called pathognomonic. Under certain circumstances, the skin granulomas seen in beryllium exposure, the cardiopathy and polycythemia associated with cobalt, and the characteristic breath after tellurium exposure may be considered pathognomonic (Grandjean *et al*, 1991). If the test yields very few false-negative results, it will have found almost all of those who had the effect (positives) and can then be called a *sensitive* test. Some of those who were classified as having the effect would be wrongly classified (i.e., the false-positive results). If the test, on the other hand, results in very few false-positive results, it is almost certain that a person classified as having the effect in fact has this effect. Such a test is called a *specific* test. Sensitivity and specificity are important qualities of health effect measurements in epidemiological studies (Beaglehole *et al.*, 2000). In studies of persons affected by a metal compound present in the environment, a sensitive measurement of the health effect is important to find all affected cases. However, in an epidemiological study aimed at quantifying a dose-response relationship, it may be more advantageous to have a specific measurement, so as not to "dilute" the analysis with false-positive results. The concepts of sensitivity and specificity can also be applied to exposure variables that are dichotomous (i.e., each person is classified as either exposed or unexposed).

Effects can be classified as subjective symptoms, psychological effects or learning deficiencies, social or behavioral changes, biochemical or physiological changes, clinical disease, and death. Subjective symptoms can be, for example, the shortness of breath associated with beryllium exposure (Chapter 21) or the weakness, fatigue, and anorexia associated with mercury-vapor exposure (Chapter 33). In this group of effects, we can also include subjective psychological changes, mood changes, and annoyance reactions to dust, skin irritation, and smell. Data may be collected through standardized interviews or self-completed questionnaires. When interviews are carried out, it is important to avoid observer bias caused by, for example, different interviewers in the exposed group and the reference group. The way questions asked may affect the answers. Ideally, the interviews should be blind (i.e., the interviewer should not know to which group a particular person belongs). In a double-blind study, neither the interviewer nor the person studied knows the exposure status of this person.

Psychological effects or learning deficiencies have been measured as effects of, for example, lead (Chapter 31). Many standardized "psychometric" tests are available, but problems of validity may arise when effects are measured in populations with a language or cultural background different from those of the population the test was designed for. Quality-assurance procedures incorporated into the testing program are, therefore, essential and should preferably be complemented with studies of case groups that are known from independent evidence to suffer the particular deficits that are sought in the exposed group. The problems of observer bias may be limited by standardized instructions for how the test should be carried out, but it is still desirable that the tests are carried out on a double-blind basis.

By social and behavioral effects we mean, for example, family strife caused by behavioral changes in the affected person, decreased work efficiency, lagging behind in school work, absenteeism, and workers compensation or other insurance claims. The latter two effects are usually based on diagnosed clinical disease, but this is not always the case, and absenteeism may be an unspecific "annoyance" effect of metal exposure in the workplace. Data collection can be carried out in a way similar to collection of subjective symptoms or by using existing records of absenteeism at school or work, school results, insurance claims, etc. Using this method, it was found that high arsenic exposure during early life affected children's school results 15 years later (Ohira and Aoyama, 1973). The effects mentioned here are quite nonspecific, and the risk of confounding may be greater than for other effects.

Biochemical and physiological changes are likely to be the critical and subcritical effects of metal compounds (see Chapter 6). Only a few have been established—for example, decreased erythrocyte ALA-dehydratase

activity after lead exposure (Chapter 31) and increased urinary beta$_2$-microglobulin excretion after cadmium exposure (Chapter 23). Histopathological changes may also be included in this type of effect. Measurement problems are related to finding the specific type of change caused by a metal compound. Animal studies can be of great value here, but species differences have been shown, for instance, for the type of proteins excreted in urine as an effect of cadmium poisoning. Another problem concerns the possibility of taking samples of the tissue where the effect occurs. The biochemical change we measure may be an indirect measure of the effect, as it is for cadmium-induced urinary β_2-microglobulin excretion, or a direct measure, as in lead-induced ALA-dehydratase inhibition.

When the effect is measured as changes in a normally occurring tissue compound or physiological variable, the dose-effect relationship can be evaluated from the quantitative increase in the variable with dose. For calculations of dose-response relationships it is necessary to have an operational definition of what level of the variable constitutes the effect. The distribution of the variable in the control group may be used to calculate a certain percentile as the cutoff level. When urinary concentrations of specific compounds are used for measuring effects, it is necessary to correct the individual concentrations for variations in urinary dilution. When compounds in whole blood are measured, it may be necessary to correct for percentage of packed cell volume (PCV).

Another problem with biochemical, physiological, and histopathological changes as measures of effects is whether the changes measured should be interpreted as an "adverse" toxic effect, adaptation to the metal, or just physiological variations influenced by the metal. There is no metal for which we have detailed knowledge about all the chemical steps in its toxicity from the first changes observed to severe clinical disease. Any change occurring in the critical organ that may be related to more severe effects in this organ should be considered at least as a subcritical effect (TGMT, 1976). In support of this notion, lead exposure is known to adversely affect the formation of hemoglobin, but the hematological parameters of most subjects will remain within normal ranges. However, significant differences in the rate of blood regeneration may occur when the subjects have been exposed to a blood loss (*in casu*, donation for a blood bank) (Grandjean *et al.*, 1989a). Decreases in reserve functions may not be apparent from most cross-sectional studies but are revealed during the additional stress of a blood loss.

Effects of metals can also be measured as clinically diagnosed disease. Medical practice records, hospital records, and data registers, such as a cancer register, may be used as information sources. Such records or registers may not be complete, however, which may result in an underestimation of response. There may be factors that cause a biased selection of the people in the records or the register, for example, economic barriers or social class-dependent attitudes to medical care. A complete clinical examination of the entire group under study may overcome such shortcomings of existing records. In industrialized countries where reporting systems are good and where autopsy rates are high, cancer registers or congenital malformation registers are becoming more and more complete and accurate. Cancers with a relatively short and characteristic course, such as lung cancer or breast cancer, would be more completely recorded than, for example, prostatic cancers, which occur at older ages and may be overshadowed by other diseases that cause death. When this type of effect is measured, it is necessary to collect data for the exposed group and the control group in an identical fashion. If a cancer register is used for calculating national reference rates for a "life-table study," all cancer cases in the exposed group should also be picked up through the cancer register. Whether the exposure itself makes it more likely for the cancers to be diagnosed and recorded in the cancer register must also be taken into account (e.g., metal-exposed factory workers may have an autopsy rate higher than normal, because of the suspicion of occupational cancer).

Finally, the type of effect that is most easily defined (death) has been used in some studies as an indicator of long-term prognosis after metal exposure (e.g., Cooper and Gaffey, 1975; McMichael and Johnson, 1982; Sorahan and Waterhouse, 1983). After very high exposures to metal compounds, death can be one of the main effects (Bakir *et al.*, 1973). Depending on the character of the recording system and autopsy rates, data on causes of death vary in quality. Mortality studies need long-term follow-up, which poses problems of tracing the place of death of the target group and problems of identification. Case-control studies of mortality involve finding data on exposures of people one cannot interview. One study has shown, however, that interviews with next of kin can produce valid data (Pershagen and Axelson, 1982). The major difficulty in mortality studies is linking up individual dose data with the effect data rather than measuring the effect itself.

When the target group is well defined and the effect has been measured accurately, the measurement of response is straightforward; the proportion of the group that contracts the effect in a specific period (incidence response), or the proportion that has the effect at a specific point in time (prevalence response). Selection bias (see Section 3) would be the most likely cause for errors in response measurement.

5 DATA ANALYSIS

Data analysis is the stage in an epidemiological study where the data are arranged and calculations made so that conclusions can be drawn. In descriptive studies, the conclusion would be in the form of a description of the occurrence of a variable in the population group studied. In an analytical or intervention study it would be in the form of rejecting or accepting a hypothesis about an association between an exposure and an effect. If the study was designed for ascertaining a dose-effect or dose-response relationship, the conclusion may be that this has or has not been found, and the result is often expressed as a mathematical relationship with confidence intervals. In drawing these conclusions, a number of statistical techniques are used, which will not be discussed in detail here. The reader is referred to more detailed texts, such as Jewell (2004).

We can express the dose as population dose, which is the sum of all individual doses in a population. This is the approach often used in radiation protection, and basically it assumes a linear dose-response relationship down to very low doses. A certain population dose is assumed to be associated with a certain number of affected cases. If the dose data are in the form of environmental measurements of emissions, it may also be relevant to estimate the dose commitment. This is the estimated total future population dose that will arise from the emissions. Quantitative effect data can also be expressed as distributions.

When the effects are measured as the number of cases of a particular disease (e.g., primary liver cancer) and incidence data based on a total population are available, the life-table method (see Section 1) can be used to estimate incidence rate ratios between the exposed group and the total population. The rate ratios for groups with different dose levels are compared with the average dose in the groups to yield a dose-response relationship.

If the data show an apparent increase in the response with increasing dose, a statistical test is used to determine whether it is likely that the data have emerged this way just by chance. A confidence interval for the dose-response relationship can also be calculated. These calculations are simplified if any of the three mathematical transformations mentioned in the following are used. The shape of the dose-response curve is often sigmoidal as a cumulative normal distribution. Such a curve can be transformed to a straight line by probit transformation. Another method of mathematical transformation that produces a sigmoidal dose-response curve for binary data is the logit transformation (Ashton, 1972; Cox, 1970). This has been used to assess the prevalence of prenatally induced CNS effects of methylmercury (Figure 4). It can be adjusted for a background prevalence. However, the dose range may represent only part of this curve, which would then be either concave or convex. For some outcome variables, there may be a "ceiling," so that a result cannot exceed a certain level. Vice versa, other outcomes may exhibit a "floor" effect, where the result cannot be recorded below a certain level. These properties may affect the shape of the dose-effect curve. The assumption of a"floor" and a straight line dose-response is the basis for the "hockey stick" method (Figure 4).

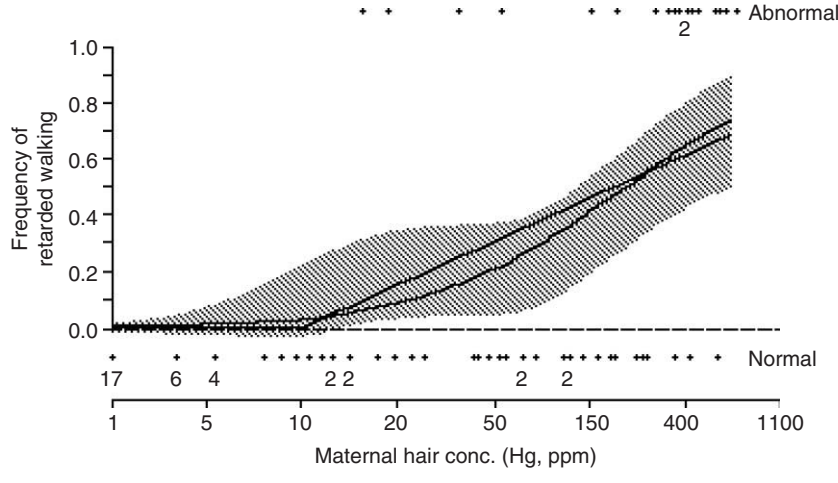

FIGURE 4 The frequency of retarded walking in prenatally exposed children versus the estimated maximum body burden in the mother during pregnancy, as expressed in terms of the hair-mercury concentration: (Straight line) "Hockey stick" method. (Curve) Logit analysis. The data are taken from Marsh *et al.* (1979) and clarkson *et al.* (1981). +, Motor retardation is defined as failure to walk by the age of 18 months.

When the shape of the dose-response curve is unknown, as is often the case in studies of, for example, metal-induced cancer, the margin of exposure (MoE) concept may be used. This approach is based on the calculation of a predefined dose, which is associated with a defined acceptable risk (e.g., a 0.001% cancer risk) or a benchmark dose (see later). The MoE is then calculated as the ratio between this parameter and the average exposure of the population at risk. The MoE is meant as a tool for risk assessors and needs to be interpreted with care, because the risk associated with a particular MoE is unlikely to be the same for different carcinogenic metals.

Of particular interest in regard to risk assessment is the existence of a threshold. However, this concept rests on limited theoretical grounds, and empirical studies have often found lower and lower "thresholds" using more sensitive measures and larger populations. For practical purposes, dose measures that represent the level at which certain changes begin to occur would be useful as a guide for preventive efforts. One simple approach is the so-called hockey-stick method of expressing a dose-response relationship (Figure 4). It is based on the assumption that there is a background prevalence and at a certain threshold dose, the response rate starts increasing linearly. The dose-response function is fitted to the data with a maximum likelihood method (Yanagimoto and Yamamoto, 1979).

Regulatory agencies have more recently started to apply the so-called benchmark dose (BMD), as originally proposed by Crump (1984). This parameter is statistically well defined and has several advantages to approaches that aim at identifying a possible threshold. The BMD is the dose that increases the risk of an abnormal response by a benchmark response (BMR) (i.e., from P_0 (most frequently 5%) for an unexposed subject to P_0+BMR for a subject exposed at the BMD). A BMR of 5% is often used, so that an exposure at exposure at the BMD doubles the risk of an abnormal response. To take the statistical uncertainty into account, a lower 95% confidence limit (BMDL) for the BMD is then determined. The BMDL is, therefore, not a threshold, but the statistical definition has the advantage of taking into account the variability of the dose-response relationship. It has sometimes been compared with LOAELs (Lowest Observed Adversed Effect Level) or NOAELs (No Observed Adversed Effect Level) no obtained in experimental toxicology studies. The benchmark approach has more recently been extended so that it can be used with continuous data, also those obtained in epidemiological studies, where confounder adjustment is needed (Budtz-Jorgensen *et al.*, 2001; Crump, 1995).

Measurement imprecision in the dose scale will, of course, also affect the BMD. At greater imprecision levels, the BMD is overestimated. However, if the imprecision level is known, an adjustment can be made (Budtz-Joergensen *et al.*, 2004). Interestingly, a comparison of different approaches showed that the hockey-stick method is less vulnerable to imprecision (Keiding and Budtz-Jorgensen, 2004).

For metals, exposures generally take place over time, and the effects may vary over time. Time-series analyses may be used to explore such relationships. In ecological or aggregate-level studies, the population exposures are drawn from centrally located pollution monitors, whereas the individual-level studies obtain exposure estimates for individual participants from monitors, perhaps in combination with personal records of environments where participants spend time. Geographical information systems (GIS) may be a helpful tool for such studies (Scoggins *et al.*, 2004). Measurement error is of particular importance to consider (Zeger *et al.*, 2000).

As a new approach to data analysis recently introduced, structural equation models provide several advantages (Bollen, 1989), although these methods require substantial statistical expertise and software experience. This multivariate approach is potentially more powerful than traditional statistical methods, because it may incorporate all data available; it includes adjustment for imprecision in the exposure variable, and it will eliminate the need for adjustment for multiple comparisons. The principle is that all exposure variables—and similarly the outcome variables—are joined into a latent composite variable. After confounder adjustment, the effect of the latent exposure on the latent outcome variable(s) is then computed. Figure 5 shows how the model is constructed. If more than one exposure parameter is used (e.g., prenatal and postnatal), the relative contributions by these exposures to the effects can be estimated. This technique has now been applied in studies of methylmercury toxicity (Budtz-Joergensen *et al.*, 2002; 2004).

To facilitate different approaches in the evaluation of dose-response data in metal toxicity studies, it is of value that the original detailed data are published rather than that data are just aggregated from groups. This will clearly show the individual variations, which are often great. Even the most sophisticated statistical methods can seldom prove or disprove that a particular mathematical dose-response function is the "correct" one.

It should also be pointed out that metal exposures may cause effects in combination with other exposures, and various factors can modify the dose-response relationship. For instance, the lung effects of cadmium exposure through air can be exacerbated by tobacco smoking, and the bone effects are influenced by dietary vitamin D or calcium intake. These interactions can be

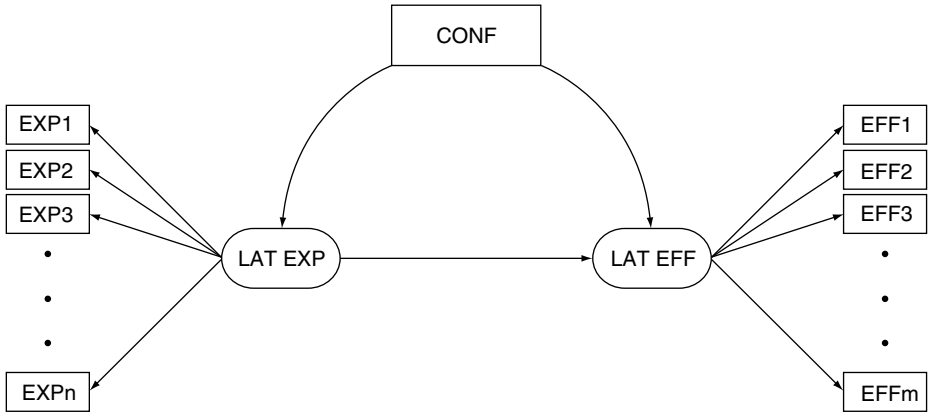

FIGURE 5 Structural equation model that includes a latent exposure variable modeled on the basis of several exposure parameters, and a latent effect variable based on a series of related parameters that reflect the same outcome. Confounder adjustment is included. This technique allows incorporation of incomplete cases.

studied at the analysis stage of a study, if the required data about other exposures are collected. The methods for analysis of interactions can be found in most textbooks of epidemiology or biostatistics (e.g., Rothman and Greenland, 1998).

6 INFERENCE

Epidemiological evidence often presents associations between frequency or severity of disease on the one hand and levels of metal exposures on the other. In human experimental studies, the exposure may be tightly controlled, but in most population studies, the exposure is not a matter of design, and it must instead be judged from various measurements, each of which is imprecise to some degree. Because all circumstances cannot be taken into account in the data analysis, each study will by necessity underdetermine the causal association.

One recent controversy relates to the possible effects of low-level lead exposure on blood pressure, where findings seem to differ between population subgroups (den Hond *et al.*, 2003). However, some variability in findings is only to be expected. First, most studies have used assessments of blood pressure and blood-lead concentration only at one point in time, and temporal changes have been ignored. Second, several factors, such as the blood-hemoglobin concentration, tobacco smoking, and alcohol drinking (Grandjean *et al.*, 1989b), can affect both blood pressure and the lead concentration in blood, thereby complicating scrutiny of the cause-effect relationships. Particularly when the causative exposure has been measured with some imprecision (and perhaps not at the right point in time), problems with confounding and possible adjustments may become insurmountable.

Scientific inference in regard to disease causation is a hotly debated issue in epidemiology, also in philosophical terms (Rothman and Greenland, 2005). Although most philosophers agree that a cause cannot be proven, certain criteria may be applied for logical analysis of the strengths and weaknesses of a hypothesis in light of the results available. The most frequently cited criteria are those developed by Bradford Hill. However, this list should not be applied too stringently, because very few environmental exposures seem to satisfy every single criterion. Because metal-associated adverse health effects are affected by dose level, timing, and vulnerability factors, each study of this association should not be interpreted as a test whether or not the association is true, but rather as a relative contribution to the understanding of this possible relationship.

Given that data quality may be far from ideal, that too few subjects have been followed for too short a time, and other limitations, epidemiology can only provide insight into causal associations to the extent allowed by the underlying information. In addition to critical scrutiny of an epidemiological study, the full perspective also needs to be appreciated: What is it possible to know, given the types of data that would be accessible for epidemiological study of this particular topic? For example, blood pressure is thought to be affected by prenatal programming and, therefore, also by developmental lead exposure. Studies of current blood-lead concentrations may show only weak associations with blood pressure, but this evidence has to be interpreted in light of the fact that the true underlying cause may be the unknown lead exposure some time in the past, of which the current blood-lead concentration is only a mere reflection.

In addition, a public-health angle is frequently relevant and deserves separate consideration (Grandjean, 1999). Thus, the scientist may conclude that the

difference in blood pressure between exposed and unexposed subjects may be less than the normal imprecision for repeated blood pressure readings. However, from a population viewpoint, even a small average increase may push a substantial number of subjects into the hypertension range with its associated increases in risk of disease and mortality. For a full interpretation of the findings, one, therefore, also needs to consider the possible effects at the extremes of the distributions. Furthermore, adjustments for imprecision of the data should explore how large the true difference between the two groups may be (e.g., the upper 95% confidence limit). Such calculations are particularly relevant for studies that report a high *P* value (>0.05) normally interpreted as denoting a lack of statistical significance. Although such studies are often called "negative," the lack of significance needs to be supplemented with a statement that some difference between the two groups would be in agreement with the results. Thus, the statistical power of the study, along with its other characteristics, needs to be taken into account when exploring whether or not the study adds support to a causal hypothesis.

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CHAPTER

9

Essential Metals: Assessing Risks from Deficiency and Toxicity

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ABSTRACT

Recommendations aimed at protecting the public from toxicity of essential elements have usually been developed separately from those recommendations aimed at protection from deficiency. Because of the uncertainties involved in the evaluations, these recommendations have sometimes been in conflict, emphasizing the need for a new approach, including a balanced consideration of nutritional and toxicological data.

In developing these new principles of evaluation, some basic concepts based on interindividual variability in sensitivity to deficiency and toxicity must be considered. Such variation translates into one interval of (low) daily intakes, at which there is risk of developing deficiency, and another interval of (high) dietary intakes at which toxicity may occur. In most instances, there is a third set of intakes in between, which represents the acceptable range of oral intakes (AROI) in which no adverse effects occur. It must be noted, however, that such a range cannot be found that protects all persons from adverse effects. Those persons with genetically determined sensitivity may require higher intakes to avoid deficiency or lower intakes to avoid toxicity than those defined by the AROI. AROI is defined as protecting 95% of an unselected human population from minimal adverse effects of deficiency or toxicity.

1 INTRODUCTION

Risk assessment of essential metals (EMs) in human health involves methods for analyzing the limits of deficient and excess intakes or exposures of EMs. Risk assessment of EMs needs a multidisciplinary scientific approach. Only a combination of data on required nutritive intakes, deficiency, and excess exposure can give appropriate intake recommendations from both the nutritional and toxicological point of view. Principles and methods for developing such recommendations will be described in this chapter. Assessing risks includes evaluation of the essentiality of metals, nutritional requirements and related adverse effects of deficiencies, homeostasis, maximum allowable concentration, and the critical adverse effects of intakes above this concentration.

The traditional criteria for essentiality for human health is that the absence or deficiency of the element from the diet produces either functional or structural abnormalities and that the abnormalities are related to, or a consequence of, specific biochemical changes that can be reversed by the presence of the essential metal (WHO, 1996). At present, the following trace elements are regarded as essential for human health: iron, zinc, copper, chromium, cobalt, molybdenum, and selenium (WHO, 1996). Details of their essentiality and toxicity are reviewed in the respective chapters in this Handbook. As explained in Chapter 32, a basic intake of this metal through the diet is required by humans to maintain health, and based on this information manganese should be added to the list of essential metals.

In this chapter the principles for risk assessment of EMs are outlined and discussed. For the assessment of risks from toxic effects, methods are similar to those used for metals in general (cf Chapter 14) with the addition that the essential nature of the metal has

to be balanced against its toxic effects. In the past, risk assessment of the toxic effects of EMs has sometimes been developed separately from those for deficiency, and conflicting recommendations have occurred. To avoid such a situation, explicit methods that use a combined approach have been developed and discussed by Mertz (1994), Nordberg and Skerfving (1993), WHO (1996), (Oskarsson, 1995), Mertz (1998), Olin (1998), Sandstrom (1998), Nordberg *et al.* (2000), IOM (1998, 2001), and WHO/IPCS (2002). Some aspects from those documents will be summarized and discussed in this chapter. Considerable weight is given to the principles described by WHO/IPCS in the Environmental Health Criteria Document on Principles and Methods for Assessment of Risk From Essential Trace Elements (WHO/IPCS, 2002).

The acceptable range of oral intake (AROI) is designed to limit deficient and excess intakes in healthy populations and is set for different age-sex groups and physiological states such as pregnancy and lactation. To facilitate comparisons, AROIs are discussed in relation to other risk assessment approaches. The principles and methods used to develop an AROI for an EM are not necessarily applicable to toxic nonessential metals or other chemicals.

A number of abbreviations and acronyms used in this chapter are given in Table 1. IUPAC official recommendations (Nordberg *et al.*, 2004) for defining some of these key terms are given below.

TABLE 1 List of Abbreviations and Acronyms

ADI	acceptable daily intake
AROI	acceptable range of oral intake
BMD	benchmark dose
CV	coefficient of variation
DRI	dietary reference intakes
EM	essential metal
FAO	Food and Agriculture Organization, United Nations
IUPAC	International Union of Pure and Applied Chemistry
JECFA	Joint Expert Committee on Food Additives, United Nations
LOAEL	lowest- observed-adverse-effect level
PRI	population reference intake
PTDI	provisional maximum tolerable daily intake
PTWI	provisional tolerable weekly intake
RDA	recommended dietary allowance
RfD	reference dose (oral)
SCF	EU Scientific Committee on Food
SD	standard deviation
SF	safety factor
SRPMI	safe range of population mean intake
TDI	tolerable daily intake
TI	tolerable intake
TWI	tolerable weekly intake
UL	tolerable upper intake level

Acceptable daily intake, ADI

Estimate by JECFA of the amount of a food additive, expressed on a body weight basis, that can be ingested daily over a lifetime without appreciable health risk.

Note 1: For calculation of ADI, a standard body mass of 60 kg is used.

Note 2: Tolerable daily intake (TDI) is the analogous term used for contaminants.

Tolerable daily intake, TDI

Estimate of the amount of a potentially harmful substance (e.g., contaminant) in food or drinking water that can be ingested daily over a lifetime without appreciable health risk.

Note: Acceptable daily intake (ADI) is normally used for substances not known to be harmful, such as food additives.

Tolerable weekly intake, TWI

Estimate of the amount of a potentially harmful substance (e.g., contaminant) in food or drinking water that can be ingested weekly over a lifetime without appreciable health risk.

Toxicity

1. **Capacity to cause injury to a living organism defined with reference to the quantity of substance administered or absorbed, the way in which the substance is administered and distributed in time (single or repeated doses), the type and severity of injury, the time needed to produce the injury, the nature of the organism(s) affected, and other relevant conditions.**
2. **Adverse effects of a substance on a living organism defined as in 1.**
3. **Measure of incompatibility of a substance with life: this quantity may be expressed as the reciprocal of the absolute value of median lethal dose ($1/LD_{50}$) or concentration ($1/LC_{50}$).**

2 BASIC CONCEPTS

2.1 Definition of an AROI (Acceptable Range of Oral Intake)

Considerable interindividual variation exists in the human population. For an essential metal, this applies to the expression of toxic effects from higher doses and the expression of adverse effects of deficiency from too low intakes. In Figure 1, this interindividual distribution of sensitivity is shown

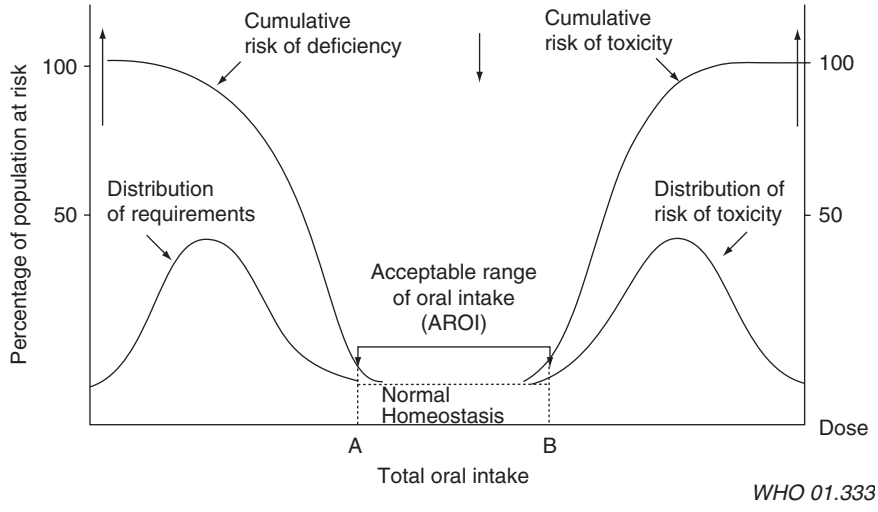

FIGURE 1 Percentage of population at risk of deficiency and toxicity effects according to oral Intake. As ETE intakes drop below A (lower limit of AROI where 2.5% of the population under consideration will be at risk of deficiency), an increasing proportion will be at risk of deficiency. At extremely low intakes all subjects will manifest deficiency. As ETE intakes exceed B (where 2.5% of the population under consideration will be at risk of toxicity), a progressively larger proportion of the population will be at risk of toxicity. From WHO/IPCS (2002) with permission.

for nutritional requirements and for expression of toxicity. For a specific adverse effect of deficiency, there are individuals in a population who display average sensitivity to developing symptoms. There are, as well, more sensitive individuals, that is, those who develop deficiency symptoms at somewhat higher intakes and, those with less sensitivity, that is, people who develop deficiency symptoms first when intakes are lower than the average. The same applies for a specific toxic effect. Some individuals develop symptoms from doses lower than those that cause symptoms in people of average sensitivity. In addition, some people have less than average sensitivity and require higher doses before they develop symptoms. In summary, neither the upper or lower boundaries of the AROI are absolute values below or above which adverse effects will occur. This situation can be shown as two bell-shaped curves describing the distribution of sensitivity to deficiency and toxicity (Figure 1 lower curves). In most cases, an interval between these two curves describes the acceptable range of oral intake (AROI) in which no adverse effects occur. If these conditions are, instead, shown with curves in cumulative forms, a U-shaped curve occurs, and the AROI appears at the bottom of the U, between points A and B in Figure 1.

2.1.1 Groups with Special Sensitivity/Resistance

2.1.1.1 Genetically Determined

The low limit of the AROI covers the requirement of most of the population, and the high limit should protect most people from toxic effects. Special population subgroups, such as those with Wilson's disease, may exhibit toxicity at relatively low intakes of copper, lower than AROI for normal persons. In contrast, some population subgroups may have requirements higher than the upper limit of acceptable range (e.g., zinc intake in subjects with acrodermatitis enteropathica). Further aspects on genetically determined variation in sensitivity are given by WHO (WHO, 1996; WHO/IPCS, 2002).

2.1.1.2 Nongenetically Determined

Many diseases are genetically determined, but this is not considered here if the disease is not known to involve a specific metabolic defect related to essential elements. In celiac disease, there is a deficient uptake of several nutrients including essential elements such as iron (Kushner, 1988) and zinc (Solomons *et al.*, 1976). In addition, gastrointestinal losses of trace elements can be increased because of diarrhea. If the disease is not well controlled by exclusion of gluten from the diet and/or the decreased uptake is not compensated by

an increased intake of these elements, iron and/or zinc deficiency may develop.

Increased urinary losses of zinc are observed in patients with alcoholic cirrhosis (Vallee *et al.*, 1957) and diabetes (Cunningham *et al.*, 1994).

It has been shown that iron deficiency gives rise to an increased uptake of manganese (Mena *et al.*, 1969). It can, therefore, be assumed that there would be an increased risk of manganese toxicity if persons with iron deficiency were exposed to high oral intakes of manganese.

Sensitivity to manifestations of zinc deficiency is known to be dependent on certain metabolic situations. When discussing effects of deficiency or toxicity of an essential element, it is, therefore, of fundamental importance to specify the background conditions of the group under consideration. Such conditions can be all determining for dose-response relationships. For example, at a certain low zinc intake (e.g., under conditions of total parenteral nutrition), clinical symptoms of zinc deficiency may not develop in a group of individuals who are in metabolic balance but may be clinically manifest in persons who undergo growth or who are in an anabolic phase (Kay *et al.*, 1976). Dose-response relationships for zinc deficiency thus can be quite different depending on metabolic state.

2.2 Other Concepts Used in Risk Assessment of Essential Metals

Terms and definitions used in this chapter are taken from IPCS Environmental Health Criteria 170, 210, and 228 (WHO/IPCS, 1994; 1999; 2002), "Trace Elements in Human Nutrition and Health" (WHO, 1996), (IOM, 1998), and (SCF 1993, 2000a,b,c). To avoid ambiguity and ensure scientific accuracy, these principles and definitions are often presented here as written in references with appropriate attribution

2.2.1 Toxicological Terms

IUPAC recommendations defining ADI (acceptable daily intake), TDI (tolerable daily intake), TWI (tolerable weekly intake), and toxicity were given in the Introduction. ADI is used for food additives and TDI for contaminants. Other terms of interest in relation to risk assessment of EMs are "reference dose" and "upper level of tolerable intake (UL). The US Environmental Protection Agency has replaced ADI and TDI with the single term, reference dose (RfD), which is defined as: "an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure for the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime." The term RfD is intended to avoid any implication that exposure to the chemical is completely "safe" or "acceptable," as may be implied by use of the term ADI (Barnes and Dourson, 1988). A basic assumption when using the terms ADI, TDI, and RfD in risk assessment is that zero exposure to the element of concern poses no health risk. For EMs, this is not an appropriate default position. For the upper intake level of the AROI (toxicity), the United States Food and Nutrition Board/Institute of Medicine (IOM, 1998) and the European Scientific Committee on Food (SCF, 2000a,b,c) have replaced the term TDI with "tolerable upper intake level (UL)."

2.2.2 Nutritional Terms: Definitions and Approaches Used to Assess Individual and Population Requirements for EMs

In developing AROIs, the term "population" does not refer to geographically or culturally defined groups or to groups with genetic abnormalities in the metabolism of EMs. Rather, the term population refers to a group that is homogeneous in terms of age, sex, and other characteristics that might affect nutrient requirements or alter toxicity (WHO/IPCS, 2002; WHO, 1996).

2.2.2.1 Factorial Estimation of Nutrient Requirement

The factorial approach calculates nutrient requirements by summarizing the minimum amounts of a nutrient needed to maintain growth and for maintenance of body stores at low intakes. Such intakes are usually sufficient for the prevention of clinical deficiencies in adults. These maintenance requirements are derived from the estimation of unavoidable losses in urine, sweat, hair, and so on. There are complex interrelationships between such losses, dietary intakes, nitrogen metabolism, and other interdependent nutrients. A lack of understanding of these interrelationships can lead to much uncertainty in the results of factorial methods. In general, they are used only as a first approximation for the assessment of individual requirements (WHO, 1996)

2.2.2.2 Requirements for the Individual

Requirement for the individual, as stated in WHO (1996), is the lowest continuing level of nutrient intake that, at a specified efficiency of utilization, will maintain the defined level of nutriture in the individual. The defined level of nutriture may be given as a basal requirement or a normative requirement or both. The level of intake needed to prevent pathologically relevant and clinically detectable signs of impaired function attributable to inadequacy of the nutrient is termed the basal requirement, whereas the normative

requirement refers to the level of intake that serves to maintain a level of tissue storage or other reserves that are judged to be desirable. Because intakes meeting the normative requirement provide for systemic stores, homeostatic adaptation resulting in mobilization of such stores when intakes are below the normative requirement would be considered evidence of "deficiency." Below the basal level, homeostatic adaptation is more likely to be inadequate, and metabolic and other disturbances would be expected.

2.2.2.3 Dietary Reference Intakes (DRI), Population Reference Intakes (PRI), Recommended Dietary Allowance (RDA), and Safe Range of Population Mean Intake (SRPMI)

Many different terms, definitions, and values are used by various countries and organizations in developing recommendations for dietary intakes of EMs. The recommended dietary allowances (RDA) developed by US National Research Council are set to maintain normal physiological functions from a nutritional point of view, and they have been widely used and discussed (IOM, 2001; Mertz *et al.*, 1994; SCF, 1993). Where adequate data are available, the dietary reference intakes (DRI) (IOM, 2006) will include, for a defined population, one or more of the following intake estimates: estimated average requirement (EAR); recommended dietary allowance (RDA); adequate intake (AI), and tolerable upper intake level (UL). The RDA and UL represent the boundaries of an AROI (see Section 2.1). The population reference intake (PRI) estimated in the European Union (SCF, 1993) is conceptually similar to the RDA, that is, the average requirement for a defined population plus 2 SD. The tolerable upper intake levels (UL) estimated by the IOM (1998) and the SCF (2000a,b,c) are conceptually the same and are based on the use of a risk assessment method similar to that discussed in Chapter 14. Although the terms DRI, AROI, PRI, and SRPMI used in North America, Europe, and within WHO have similarities, there are often subtle differences in their meaning and application. There is an urgent need to harmonize universally the terms and definitions used to describe recommended dietary intakes, including the upper level of intake based on toxicological data, and the methods used in nutrition and toxicological risk assessment.

Before 1996, most recommendations for the intake of EMs provided estimates that applied to individuals with the assumption that the average intake of a defined population plus 2 SDs would protect the majority (97.5%) of the individuals in the group. However, in estimating the "safe range of population mean intake" (SRPMI), WHO (1996) is concerned with population (group) mean intakes rather than intakes of individuals. The lower limit of the SRPMI is set so that very few (less than 3% of individuals in the defined population) would be expected to have intakes below their requirement. The upper limit of the SRPMI is set to protect most persons in the population from (minimal) adverse effects. The concept of SRPMI and the problems involved in its derivation are discussed fully in WHO (1996).

3 EFFECTS OF DEFICIENCY AND TOXICITY

3.1 Factors Affecting Dose-Response Relationships

The early literature on factors affecting dose response relationships of metals was reviewed by Nordberg (1976). A review by WHO (1996) highlights factors affecting nutrient dose-response relationships. Such alterations in dose-response will markedly affect the intake level of an EM at which signs of toxicity and deficiency will be observed.

3.1.1 Homeostatic Mechanisms

A defining characteristic of EMs is that there are homeostatic mechanisms that maintain optimum tissue levels over a wide range of intakes. Homeostatic mechanisms involve regulation of absorption, tissue retention, and excretion. There are specific homeostatic mechanisms for each EM that may vary in efficiency within individuals of defined populations (WHO, 1996; WHO/IPCS, 2002). For EMs handled as cations (zinc, iron, copper, manganese, and chromium) homeostatic mechanisms operate largely via gastrointestinal absorption and the liver, whereas the more soluble anionic EMs (molybdenum and selenium) are absorbed rather efficiently and total body burden regulated by alterations in oxidative state, level of methylation, and renal excretion. A third category of homeostatic mechanism is illustrated by cobalt. It is a highly reactive element with several oxidative states and can effectively compete with other cationic EMs. Such interactions are avoided, because the physiologically active form is the highly regulated bio-inorganic complex cobalamin. There is no evidence that humans require inorganic cobalt.

3.1.2 Bioavailability, Speciation, and Interactions

One important aspect determining both the likelihoods of deficiency symptoms and toxic manifestations is the bioavailability of the essential element in question. Variation in uptake and use of EMs varies, depending on chemical form, salt or ionic form, food source or dietary matrices, age, gender, interactions with other

nutrients, and nutritional state. Of these factors, nutritional status is most critical. It influences adaptive processes or biokinetics in response to deficiency or excess and needs to be taken into account when developing an AROI (WHO/IPCS, 2002). These aspects are discussed in detail elsewhere (Mertz *et al.*, 1994; Nordberg and Skerfving, 1993; WHO, 1996; WHO/IPCS, 2002).

Another aspect of fundamental importance is the chemical species of the EM ingested. Variations in solubility between chemical species of an EM may markedly alter its solubility and thus its bioavailability, in many cases altering the risks from deficient or excess intakes. Examples of EMs where speciation plays an important role in determining bioavailability and use are iron (ferrous/ferric), copper, and selenium (WHO, 1996; WHO/IPCS, 1998; 2002).

In addition to bioavailability and speciation, interactions between EMs and interactions between EMs and nonessential metals need to be considered during the development of an AROI. These interactions have been summarized by WHO (1996), and only a few examples are given here. There is a competitive interaction for absorption between copper and zinc (WHO/IPCS, 1998; 2001a). Also, copper can interact with a number of other nutrients (e.g., molybdenum, manganese, selenium, and iron), leading to a decreased copper availability. With regard to zinc toxicity, groups with a high dietary copper intake may not develop adverse effects of high zinc intakes to the same extent as persons with low dietary copper intake. The critical effects of zinc toxicity are considered to be related to induced copper deficiency (WHO/IPCS, 2001a).

3.2 Basic Principles for Classifying Effect

Evaluation principles concerning adverse effects from metal toxicity have been previously developed by the ICOH Scientific Committee on the Toxicology of Metals—Task Group on Metal Toxicity 1976 (Nordberg, 1976; 1992; Nordberg *et al.*, 2000) and Chapter 14 in this Handbook). Adverse effects can occur from too low or too high an intake of an EM. As mentioned (see Section 2), it may not be possible to arrive at recommendations that protect *all* individuals in a population from such adverse effects, because some individuals with genetically determined metabolic disorders may require intakes that are higher or lower than those that represent AROI for normal persons in a defined population (cf Section 4 and Chapter 7). AROI can, therefore, only be applicable for persons without such disorders. It is further considered that the use of sensitive indicators of adverse effects with limited functional significance as markers of critical effect will provide further safety from more severe clinical disease caused by deficiency or toxicity. Based on present knowledge, it seems possible in most cases to define an AROI for the essential elements that will protect 95–98% of the population from adverse effects (i.e., the protective level at each end of the U-shaped curve would be 97.5–99%) (cf Nordberg *et al.*, 2000, WHO/IPCS, 2002).

These considerations are based on the assumption that adverse effects from deficiency or toxicity follow an S-shaped (or Z-shaped for deficiency) dose-response curve. As mentioned, such curves represent the cumulative conversion of a Gaussian distribution (or other defined distribution) of individual thresholds of effect in a population. Such effects, which occur when a threshold concentration is exceeded in a specific tissue of an individual, are termed *deterministic effects*. Many adverse effects, which result from for example enzyme inhibition and similar biochemical mechanisms, are considered to be of this nature.

In Figure 2 (right hand side), theoretical examples of dose-response curves for a metallic compound are illustrated. Effects of increasing severity occur when the daily oral intake increases above a certain level (curves 5–8). Slight changes in biochemical markers that have been shown in epidemiological studies to be without functional or clinical significance (curve 5) are of limited importance in relation to public health. Subclinical markers of effects that are related to functional deficits or that signal the development of clinical disease are more relevant to public health, and such markers are often crucial for preventive action. These effects and their dose-response curves can be regarded as critical pieces of information when discussing preventive measures such as exposure limits and recommendations of safe intakes. Such effects are termed *critical effects* (for further discussion of the "critical effect" concept see Chapter 14). Sometimes subclinical biomarkers of effects have not been identified, and the clinical effect that occurs at the lowest dose must be used as the critical effect.

These evaluation principles focusing on the critical effect (i.e., the adverse effect that occurs at relatively low exposures and which implies some kind of clinical or functional impairment) have been adopted by several international bodies and has been used by WHO/IPCS (1994) and WHO/IPCS (2001b).

In toxicological cancer research and in radiation biology, considerable research efforts have been devoted to characterizing another type of effects, so called *stochastic effects*, which are considered to occur as a result of a random process of interaction between the agent and DNA in the cell nucleus. Cancer induction is considered to be such a stochastic effect. For such effects it has been assumed that the dose-response curve has

a linear component in the low-dose range, and for safety reasons it has been the policy in some situations to set very low acceptable exposures, because only extremely low risks have been considered as acceptable for cancer from environmental pollution with chemical substances. Because carcinogenic effects of this stochastic nature are unlikely to appear as a result of exposure to essential metals, except in persons with specific genetically determined susceptibility (cf Chapter 30), stochastic effects of essential EMs will not be discussed in this chapter.

3.3 Examples of Effects of Varying Severity

In the following text, examples of effects of deficiency and of toxicity are given, explaining the various theoretical curves in Figure 2.

3.3.1 Lethal Deficiency

Severe deficiency sometimes with lethal outcome may result from situations in which individuals are completely devoid of the nutritional element in question. Severe iron deficiency when combined with otherwise benign conditions may lead to severe anemia with lethal outcome in rare cases (Solomons *et al.*, 1976). In China, Keshan disease, with myocardial abnormalities and sometimes death, may occur as a result of selenium deficiency, possibly in combination with viral disease (Chen *et al.*, 1980; Keshan Disease Group, 1979). Lethal outcomes in deficiency cases are often related to some interacting agent or intercurrent disease and can then be considered as deficiency in a sensitive subgroup of the population.

3.3.2 Deficiency—Clinical Disease

Iron deficiency with clinically observable anemia with pallor and fatigue is still not uncommon among women in child-bearing age (Frey, 1995). Psychomotor development has been shown to be impaired in children whose mothers were iron deficient during pregnancy (Walter, 1989).

Clinical deficiency as a result of copper deficiency is more rare, but anemia unresponsive to iron therapy sometimes can be ascribed to copper deficiency (Pettersson and Sandstrom, 1995; Sandstead, 1993). Growth failure, poor immunity, impaired wound healing, and impairment of special senses and cognition are clinical signs of zinc deficiency (Sandstead, 1993; WHO/IPCS, 2001a; Chapter 47). Selenium deficiency has been shown to be related to an endemic disease in China with cardiomyopathy and sometimes changes in the thyroid called Keshan disease, and also to Kashin Beck disease, a joint and muscle disease occurring in the same area (Alexander and Meltzer, 1995). Muscular symptoms have been reported in patients on total parenteral nutrition (WHO, 1996).

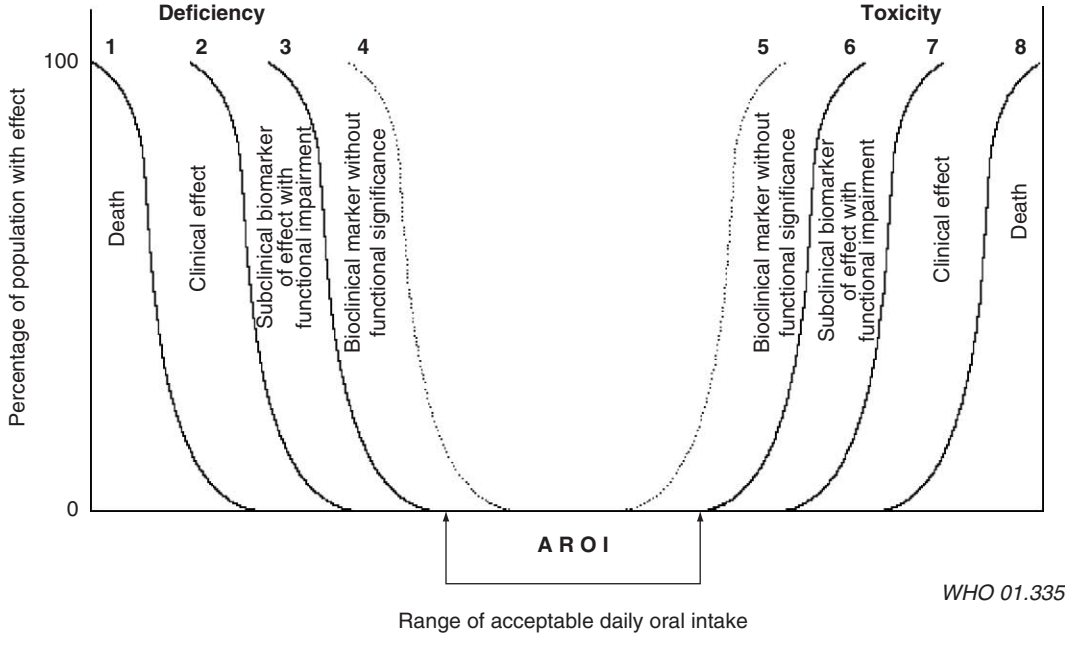

FIGURE 2 Theoretical dose-response curves for various effects occurring in a population at various levels of intake (doses) of an ETE. The lower end of the dose-response curve for such critical effects related to deficiency (curve 3) and toxicity (curve 6) defines the range of acceptable daily oral intakes. From WHO/IPCS (2002) with permission.

3.3.3 Subclinical Biomarkers of Deficiency with or without Clinical Significance

A number of markers related to decrease in enzyme levels have been shown to occur in persons with low intakes of some essential metals. In some cases, such an influence on enzyme levels is believed to be of functional significance and may even be a precursor of clinical disease. In other cases, a slight decrease in an enzyme level may be tolerated without any functional significance, namely, if there is a great surplus of this enzyme in the medium in which it is measured.

An example of such a subclinical marker of deficiency is the saturation of glutathione peroxidase (GSHPx) in plasma, erythrocytes, and platelets that is impaired when selenium intakes are low. Incomplete GSHPx saturation in plasma does not occur until Se intakes fall below approximately 40 μg/day (Yang *et al.*, 1989a,b). For saturation of GSHPx in erythrocytes higher intakes are required, and for saturation in platelets more than 100 μg/day is required (Whanger *et al.*, 1988). The clinical or health importance of such incomplete GSHPx saturation is debated, particularly in the most sensitive compartment (i.e., platelets).

For some essential metals (e.g., zinc) specific and sensitive biochemical indicators of deficiency are lacking. In absence of such indicators, the estimates of requirements have to be evaluated by a factorial technique (i.e., by adding together the requirements for tissue growth, metabolism, and endogenous losses). It is notable that the activity of zinc-dependent processes and plasma and tissue zinc concentrations can be maintained over long periods of time at low intakes by substantial reductions in endogenous losses of zinc. This adaptive ability was taken into account by WHO (1996) for estimates of the physiological requirement of absorbed zinc using data from long-term balance studies at very low intakes (Baer and King, 1984; Hess *et al.*, 1977). At the basal requirement level, the ability to increase the efficiency of zinc retention has been fully exploited. Observations made during the early phase of the same studies of zinc depletion were used to estimate the normative physiological requirement. For adult males, an uptake of 1.4 mg/day was judged to maintain zinc equilibrium without the need of adaptive changes in endogenous losses. With 30% fractional uptake from the diet, this corresponds to a daily normative dietary requirement of 4.7 mg/day. Lacking corresponding long-term studies in other age groups, endogenous losses in relation to basal metabolic rates were used as the basis for extrapolation.

A reduction of maximal oxygen consumption has been demonstrated in physical performance tests in young women with low serum-ferritin concentrations without anemia, suggesting effects on tissue level unrelated to oxygen-transport capacity (Zhu and Haas, 1997).

Another example is a decrease in ceruloplasmin levels in low copper intakes and decreased erythrocyte superoxide dismutase (ESOD) when copper intakes are low (WHO/IPCS, 1998). Although these changes are considered useful indicators of decreased biological copper activity, their clinical significance has not been well defined.

When iron intakes are low, serum ferritin levels decrease, and transferring saturation and increased erythrocyte protoporphyrin are decreased. Although these indicators are excellent indicators of low body iron stores, slight decreases in iron stores are of debatable clinical significance.

3.3.4 Lethal Toxic Effects

Excessive doses of soluble salts of iron or copper by the oral route that may be ingested by accident or with suicidal intent give rise to extensive and severe gastrointestinal manifestations, systemic toxicity, shock, and death (Borch-Johnsen and Petersson-Grawé, 1995; WHO, 1998).

3.3.5 Toxic Effects with Clinical Significance

Clinical disease (without fatal outcome) may occur as a result of ingestion of high doses of soluble selenium salts. Such poisoning cases display nausea, vomiting, and subsequently hair and nail changes and skin lesions (Alexander, 1993; Chapter 38). Persons ingesting large doses of copper may develop hematuria and jaundice in addition to vomiting, nausea, and diarrhea (WHO/IPCS, 1998; Chapter 26). Also ingestion of soluble iron salts may give rise to gastrointestinal manifestations with vomiting and diarrhea often with bloody stools, and later cirrhosis may occur (Borch-Johnsen and Peterson-Grawé, 1995; Chapter 30).

3.3.6 Subclinical Toxic Effects with or without Functional Significance—Biomarkers of Critical Effect

Like the situation with biochemical markers of deficiency, subclinical markers of toxicity have been identified in the form of changes in enzyme levels. To use such a biomarker as a critical effect, it is of great importance to be able to determine the extent to which increased levels of such biomarkers in media easily accessible for analysis (blood, urine, hair) signals a subclinical stage of a disease or abnormality in organ function. Unfortunately, for several potentially useful markers, there is a lack of such information, and further definition of the clinical and functional

significance of biomarkers is needed. ESOD levels may occur as a result of excessive zinc intakes. Most probably, this is a result of interference by zinc with uptake of copper, and the balance between zinc and copper is of fundamental importance for the expression of this effect (WHO/IPCS, 1998). For excessive iron intakes, excessive transferrin saturation and excessive levels of serum ferritin can be used as markers of iron overload (Borch-Johnsen and Peterson-Grawé, 1995; Chapter 30). The clinical significance of marginally increased iron overload is still under discussion. Large excesses like those occurring in persons with hemochromatosis are clearly of clinical importance. Homozygotes for hemochromatosis are at several hundred fold increased risk of liver cancer and an increased risk of coronary heart disease (Bradbear *et al.*, 1985; Niederau *et al.*, 1985; Chapter 30). Moderate levels of iron overload may also be related to increased risks for carcinogenicity in several organs, including the liver, but such effects are not well documented. A relatively large proportion of the population is heterozygotic for the hemochromatosis gene and potentially at risk for iron overload.

High selenium intakes may give rise to prolonged plasma prothrombin time and increased ALAT (see also Chapter 38).

TABLE 2 Principles Underlying Use of the Homeostatic Model in Human Health Risk Assessment of EMs (From WHO/IPCS 2002 with Permission.)

- Homeostatic mechanisms should be identified for the selected ETE.
- Variations of the population's homeostatic adaptation must be considered.
- There is a "zone of safe and adequate exposure for each defined age and gender groups" for all EMs – a zone compatible with good health. This is the acceptable range of oral intake (AROI).
- All appropriate scientific disciplines must be involved in developing an AROI.
- Data on toxicity and deficiency should receive equal critical evaluation.
- Bioavailability should be considered in assessing the effects of deficiency and toxicity.
- Nutrient interactions should be considered when known.
- Chemical species and the route and duration of exposure should be fully described.
- Biological end-points used to define the lower (RDA) and upper (toxic) boundaries of the AROI should ideally have similar degrees of functional significance. This is particularly relevant where there is a potentially narrow AROI as a result of one end-point being of negligible clinical significance.
- All appropriate data should be used to determine the dose–response curve for establishing the boundaries of the AROI.

4 SUMMARY OF PRINCIPLES FOR HUMAN RISK ASSESSMENT OF EXPOSURES TO EMs

WHO (WHO/IPCS, 2002) developed a set of principles for the use of the "homeostatic model" in the assessment of human health risks from exposure to essential trace nutrients. These are presented, with permission, in Table 2. Many of these principles were examined at a workshop in 1992 (Mertz, 1993; Mertz *et al.*, 1994) and have been discussed briefly elsewhere in this chapter or in the references provided.

It is beyond the scope of this chapter to discuss in detail the known physiological adaptation to changing levels of intake of EMs and the variables affecting this adaptation. However, as noted by WHO (WHO, 1996; WHO/IPCS, 2002), this will play an important role in the risk assessment of essential trace nutrients, including EMs.

4.1 Application of Principles for Determination of AROI

A proposed scheme for the application of the principles in Table 2 for the determination of an AROI has been presented by WHO (WHO/IPCS, 2002). This scheme is reproduced with permission in Figure 3.

The scheme in Figure 3 should not be considered a new paradigm for assessing human health risks from exposure to EMs. In fact, the steps shown are essentially the same as those shown for the risk assessment of metals in general (see Chapter 14). One major difference is the need to apply each step to data related to essentiality and toxicity for the EM. This requires a multidisciplinary team of scientists, applying the principles in Table 2, and using a weight of evidence approach, applying sound scientific judgement at each step. The entire scheme is an iterative process.

In step 6, risk characterization, data on exposure and the AROI are integrated. This process takes into consideration the variability in exposure and dose-response for multiple subpopulations, evaluating the strengths and weaknesses of the evaluations at each step (WHO/IPCS, 1999). Transparency during each step is important but particularly so during the risk characterization. It is essential to ensure scientific conclusions are identified separately from policy judgments. Also, the use of default values or methods during the risk assessment must be clearly identified and discussed (WHO/IPCS, 2002). Based on the wide-ranging expertise of groups carrying out risk assessments of EMs, an important role of risk characterization is the identification of research

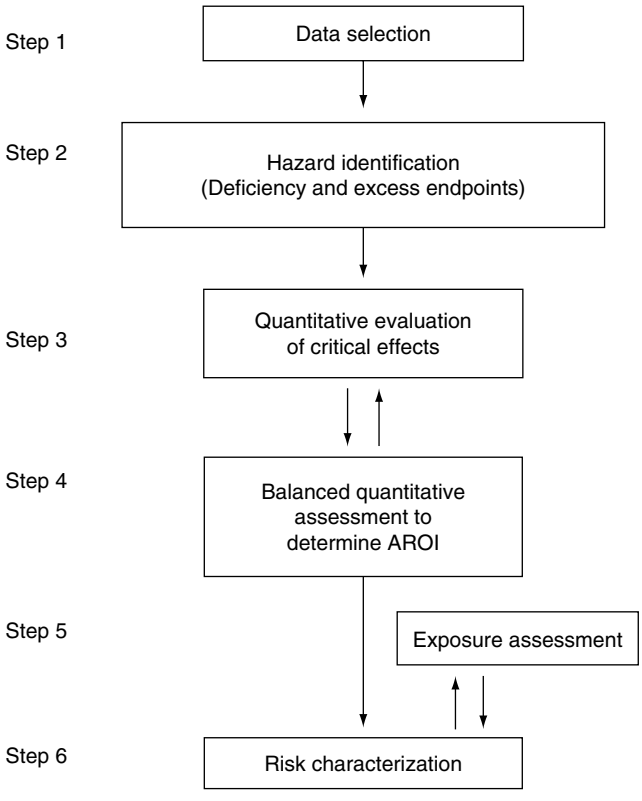

FIGURE 3 Application of the principles for the assessment of risk from essential trace elements. From WHO/IPCS (2002) with permission

needs that could lessen the uncertainty of any recommendation for an AROI.

With appropriate consideration of physiological adaptation during the application of the scientific principles in Table 2, and following the steps outlined in Figure 3, it should be possible to develop a range of intakes that meet nutritional requirements and identify intakes associated with toxicity in specific age-gender groups that do not overlap. However, as with any recommendation based on the evaluation of scientific data, the AROI should not be considered a static number over time. As new data on exposure, mechanism of action, and toxic effects of an EM become available, there needs to be national and, hopefully, international mechanisms developed to review the new data in a timely manner and, if necessary, to update the AROI.

5 ESTIMATION OF AROI

The overall goal in developing an AROI for an EM, or a SRPMI, is to make recommendations on a range of intakes necessary to maintain adequate nutrition in healthy members of a defined population while preventing signs of toxicity and avoiding conflicts between these two intake values. Such conflicts have occurred previously (e.g., zinc), largely the result of scientists evaluating the toxic risks from EMs in isolation from those developing the recommended nutritive requirement without consideration of the human requirements and, as discussed in Section 3 of this chapter, using biological markers of toxicity with markedly different biological significance than those used for the assessment of deficiency in humans (Mertz *et al.*, 1994).

When developed by a multidisciplinary group of experts using the principles and methods summarized in Section 4 of this chapter, an AROI or SRPMI affords an equal level of protection against deficiency and toxicity. Generally, an AROI and a SRPMI afford protection of 97–98% to the members of a defined population, that is, 2 SD from the mean of the distributions for nutritional requirements and risks of toxicity (IOM, 2001; WHO, 1996; WHO/IPCS, 2002). Although the AROI and SRPMI are based on different concepts, the scientific method used to evaluate the data on nutrition and toxicity is very similar, if not identical (WHO, 1996; WHO/IPCS, 2002). Also, the method used by the European Commission SCF to derive PRIs and UL (equivalent to an AROI) is that suggested in this chapter (SCF, 1993; 2000a). All groups have used a weight of evidence approach, whereby the full data base on nutritional requirements and toxicity of any EM is evaluated to identify possible hazards to human health. A critical step in this method is the balancing of endpoints for deficiency and toxicity to ensure the chosen biomarkers have comparable health significance (Section 3 of this chapter). Choosing as a critical effect the inhibition by an EM of an enzyme without known clinical significance with respect to deficiency and/or toxicity should be avoided.

In developing the UL for EMs, both the European Union Scientific Committee on Food (SCF, 2000a) and the US Institute of Medicine/Food & Nutrition Board (IOM, 1998; 2001) followed the principles and method described in this chapter and in WHO/IPCS (2002), that is, the generic risk assessment model having a long history of use for the assessment of chemicals in food. The model assumes nutrients, including EMs, work through threshold mechanisms, where no adverse health effects are assumed to occur below the threshold dose (intake). In deriving an UL, a NOAEL (considered an approximation for the threshold dose) or LOAEL is identified and modified by use of UFs to take into account the uncertainty within the data. Although the BMD method uses the complete dose-response curve, rather than the point estimate of the NOAEL, and considers the variation in response within the studied population, its use in deriving ULs for nutrients is very limited. As discussed in the report from the FAO/WHO Consultation (FAO/WHO, 2006), the data on the adverse effects of high intakes of

nutrients are usually not suitable for the derivation of a BMD. The mathematical modeling used in the derivation of a BMD requires high-quality studies with multiple intakes showing graded responses at different levels of intake. Such studies on EMs in humans are usually not available. Even animal studies that might be useful in deriving the UL provide limited dose-response data. Therefore, the UL for the AROI of EMs is presently based on the use of NOAEL or LOAELs and the application of appropriate UFs based on expert scientific judgement (FAO/WHO, 2006; IOM, 1998; SCF, 2000a; WHO/IPCS, 2002). This risk assessment approach is necessitated by the quality of data now available. However, it is strongly recommended that research efforts be intensified to generate data on the toxicity of EMs that would permit the application of the BMD model. As pointed out by WHO/IPCS (2002), this approach provides a more precise estimation of the UL, because it analyzes the complete dose-response curve, including the confidence interval around the central estimate. Although the NOAEL/UF approach is a very useful risk assessment tool, where adequate dose-response data are available, the BMD model can decrease the level of uncertainty in risk evaluations and is thus a preferable approach for dose-response evaluation (WHO/IPCS, 2002). The BMD approach for risk assessment of metals is discussed in Chapter 14.

Although neither the USIOM/FNB nor the EU/SCF used the term AROI, the recommendations for RDAs, AI, and PRIs, when combined with the recommended UL, is equivalent to the AROI described in Section 2 of this chapter. The method described by each of these organizations follows the principles and steps described in Section 4 of this chapter.

The derivation of a SRPMI (WHO, 1996) used many of the steps described in this chapter, particularly the use of a weight of evidence approach. It can be considered similar to an AROI but based on observations of the distributions of population mean intakes and their distribution. The difference in percentage of a particular population at risk from the choice of the SRPMI compared with an AROI will be affected by the slope (shape) of the relevant intake distributions and the slope of the risk of toxicity or deficiency curves. Theoretically, if the same criteria for safety are used for both the SRPMI and the AROI, the SRPMI will cover a narrower range of intakes; however, this difference may be very small in practice as indicated in Table 3.

A summary of recommended intake ranges (considered here as AROIs) by the EU/SCF, USIOM/FNB, and WHO for five EMs in adult men is shown in Table 3. In comparing these ranges it must be remembered that the recommended intake for nutrition (RDA, AI, PRI) represents the amount from dietary sources only, whereas the UL and PMTDI represent the oral intake from all sources (food, water, and supplements) (IOM, 2001; SCF, 1993). The SRPMI represents dietary intakes only. However, when developing specific SRPMIs, it was realized water and supplements might provide added exposure that might result in toxicity. In those cases, the upper boundary of the SRPMI was adjusted to reflect such exposures (WHO, 1996). Although values are given for adult men only, all three organizations developed recommendations for intakes by other defined populations.

An examination of the intake ranges (considered as AROIs) for any one of the five EMs in Table 3 indicates they are very similar, with a few significant

TABLE 3 Comparison of Intake Recommendations for Five Essential Metals in Adult Males from Europe[a], North America[b] and WHO[c]

Essential Metal	SCF		IOM		WHO
	PRI	UL	RDA	UL	SRPMI
Copper (mg/day)	1.1	5	0.9	10	1.3–12
Iron (mg/day)	9	ND	8	45	ND (see note[d])
Manganese (mg/day)	1–10 (a safe & acceptable range)	ND	2.3 (an AI)	11	ND
Selenium (ug/day)	55	300	55	400	40–400
Zinc (mg/day) (moderately available)	9.5	25	11	40	9.4–45

[a] European Union Scientific Committee on Food [PRIs (SCF, 1993); manganese (SCF 2000b); selenium (SFC 2000c); copper (SCF 2003a); zinc (SCF 2003b)].
[b] United States Institute of Medicine/Food & Nutrition Board [selenium (IOM, 2000); copper, manganese & zinc (IOM 2001); DRI summaries (IOM 2006)]
[c] World Health Organization (WHO 1996)
[d] FAO/WHO recommended a requirement of 9 mg Fe/day for an adult male (WHO/FAO 2002) and WHO (1983) calculated a PMTDI of 48 mg Fe/day (0.8 mg/kg bw). The range 9 to 48 can be considered as an AROI for iron in adult males.
AI = Adequate Intake
ND = Not determined

differences that need to be examined. It is hoped this analysis will lead to further refinement of the method for EMs discussed in this chapter. The fact that a few significant differences in recommendations occurred is not surprising given the fact they resulted from the deliberations of five different groups of experts meeting over a span of 20 years.

For copper, the SRPMI (WHO, 1996), PRI (SCF, 1993), and RDA (IOM, 2001) were not markedly different. The variation in recommendations arises from the fact that the SRPMI and the PRI were based on normative and average requirement of 0.8 mg/day and a CV in intakes of 20%. The RDA of 0.9 mg/day was derived from an EAR of 0.7 mg/day assuming a CV of 15%. This evaluation was made on the basis of additional well-controlled deletion/repletion studies not available to WHO and the SCF experts. The most significant difference in the AROIs for copper is the UL recommended. The upper boundary of the SRPMI of 12 mg copper/day was derived from an analysis of the database on adverse effects of copper in food, where no effects were seen at intakes of around 10 mg/day. The SCF (2003a) and the IOM/FNB (IOM, 2001) both considered potential liver damage as the critical effect and evaluated the same small double-blind study in humans given 10 mg copper/day as a soluble supplement. No adverse effects on liver function were reported. In the professional judgement of the SCF, an UF of 2 was required to take into account potential variability of response within the normal population. The IOM/FNB concluded an UF of 1 was adequate, given the large international database indicating a lack of adverse effects in humans consuming 10–12 mg/day of copper in foods and the rarity of observed liver damage from copper exposures in human populations with normal copper homeostasis.

Iron is an example of an EM in which the intakes within the general population are skewed by the requirements for growth in children and adolescents, as well as the losses in females through menstruation. Also, the bioavailability of the iron consumed will markedly affect the determination of the AROI boundaries. Taking these facts into account, very similar lower boundaries of the AROI were recommended by SCF (SCF, 1993), WHO (FAO/WHO, 2002), and the IOM/FNB (IOM, 2001). All determined the basal losses and modeled the components of iron requirements to determine an average requirement. Based on a CV in losses of 15% and assuming 15% as the upper limit of iron absorption, both WHO and the SCF recommended 9 mg iron/day as the lower boundary of the AROI. The value of 8 mg/day, derived by the IOM/FNB results from the assumption of 18% as the upper limit of iron absorption. The UL of 48 mg/day recommended by WHO in 1983 was derived by the examination of data on the gastrointestinal effects from consumption of soluble iron supplements by humans, whereas the UL of 45 mg iron/day recommended by the IOM/FNB in 2001 was based on the determination of a LOAEL for gastrointestinal distress of 70 mg iron/day (60 mg as iron fumarate) in a controlled double-blind study and applying an UF of 1.5 to account for extrapolation from a LOAEL to a NOAEL.

Manganese is an example of an EM in which there are limited data of sufficient quality to support the development of an AROI. As shown in Table 3, WHO concluded that data on manganese requirements and toxicity were not sufficient to set a SRPMI, and the SCF (1993) concluded it was preferable to give a safe and acceptable range of intakes rather than a definitive AROI. Given the limitations of the human data and the fact that NOAELs are lacking for critical toxic endpoints in animal studies (particularly neurotoxicity after oral intake), the SCF (2000b) concluded it was not possible to set a UL of intake for manganese. The IOM/FNB concluded that balance studies for manganese were inadequate to set an EAR and thus a RDA. The mean intake of manganese in adult men within the United States is 2.1–2.3 mg/day (IOM, 2001). Because symptoms of manganese deficiency are not found in this population, the recommendation of an AI of 2.3 mg manganese/day was appropriate at this time. The UL of 11 mg/day was set on the basis of no adverse effects found in humans consuming Western-style diets containing up to 11 mg manganese/day and supported by a LOAEL of 15 mg/day reported from a study in humans where significant increases in serum manganese were found after 25 days of supplementation. A UF of 1 applied to the NOAEL was considered appropriate.

As shown in Table 3, the AROIs for selenium derived by the three organizations are very similar. All three groups considered the enzyme plasma glutathione peroxidase as an appropriate indicator of minimal selenium deficiency and for toxicity, the threshold where biochemical disturbances in selenium metabolism were seen in humans (850 µg/day). The differences shown can be explained by different scientific judgments made with regard to whether maximal peroxidase activity was the most appropriate indicator of nutrient need and the level of uncertainty in the epidemiological data that indicated a threshold of toxicity at intakes of 850 µg selenium/day. The lower boundary of the AROI recommended by WHO was 40 µg selenium/day based on the intake required to provide two thirds of the maximum glutathione peroxidase activity and a 16% interindividual variation in selenium intakes. However, experts advising SCF (2000c) and the IOM (2000) considered it more

appropriate to recommend intakes of selenium that resulted in maximum peroxidase activity, namely 55 μg/day. The differences noted in the UL were a result of applying an UF of 2 to the threshold for selenosis by WHO (1996) and the IOM/FNB (2000), whereas the SCF (2000c) thought an UF of 3 was more appropriate.

As for iron, the bioavailability of dietary and supplemental zinc must be considered in deriving an AROI. The values in Table 3 are the intakes for adult men consuming a diet in which zinc was moderately available (defined by WHO [1996] as 30–35%). All three groups used factorial modeling to determine the minimal quantity of absorbed zinc required to replace endogenous losses. The lower boundary of the SRPMI was based on a CV of intakes of 25%, and the IOM assumed a 10% CV in requirements to calculate the RDA from the EAR of 9.4 mg zinc/day. The upper boundary of the AROIs in Table 3 indicate a marked difference between the ULs derived by WHO and the IOM/FNB and that recommended by the SCF. The three ULs for zinc are based on the intake of zinc that affects relevant indicators of copper status as the critical effect. In all studies evaluated, humans were given extremely soluble (highly bioavailable) zinc supplements while consuming diets containing approximately 10 mg of zinc. The IOM/FNB used a study in which 50 mg of supplemental zinc was provided to humans for 10 weeks. Decreased activity of ESOD was noted at this total intake of 60 mg zinc/day. A UF of 1.5 was considered adequate to account for interindividual variability in response and the fact that a LOAEL was used. The SCF (2003b) evaluated four studies in humans in which several relevant indicators of copper status were measured after zinc supplementation. No adverse effects were observed at total zinc intakes between 40 and 53 mg/day for 90 days. An intake of 50 mg zinc/day was considered a NOAEL, and an UF of 2 was applied because of the small number of subjects in relatively short-term studies. The recommended UL of 25 mg total zinc/day raises an important point mentioned earlier, namely, recommending intakes without full consideration of nutritional needs, or as in this case, the fact that thousands of humans worldwide have daily intakes of zinc well above the recommended UL without any signs of adverse health effects. It was mentioned (SCF, 2003b) that the 97.5 percentile of total zinc intakes of all age groups are close to the UL. However, it should be noted that the 95th percentile intake of total zinc of adult men in the United States is 30.5 mg/day, pregnant women 39.7 mg/day, and lactating women 46.7 mg/day (IOM, 2001).

6 CONCLUSIONS

From this review, we recommend the following:

1. A set of principles and methods for the assessment of risks from essential metals.
2. The use of these principles and methods for the development of AROIs for EMs and other nutrients be expanded globally whenever such risk assessments are carried out.
3. Nutritional and toxicological information should be considered in a balanced approach on the basis of adequate human data.
4. Speciation, bioavailability, and homeostatic mechanisms should be taken into account.
5. Critical effects of deficiency and toxicity should have similar clinical significance.
6. During the risk assessment process for EMs, greater emphasis should be given to the risk characterization step to increase the transparency of the process and to provide guidance to those using the evaluations of the strengths and weaknesses of each step in the process.
7. An acceptable range of oral (individual) intakes (AROI) and a safe range of population mean intakes (SRPMI) can be defined if adequate data are available.

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CHAPTER

10

Carcinogenicity of Metal Compounds

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ABSTRACT

Epidemiological studies have demonstrated that nickel, chromium, arsenic, cadmium, and beryllium compounds are human carcinogens. Most of these epidemiological studies were retrospective in humans occupationally exposed to compounds of these metals. There are few environmental studies in humans exposed to any of these metal compounds, when possible speciation of the metal compound is considered in the epidemiological studies. Of compounds of the five metals, those of nickel, chromium, cadmium, and beryllium have been confirmed as carcinogens in experimental animals, although there was recent evidence in the case of arsenic. However, it should be noted that in many cases animal bioassays were conducted with exposure routes that were not relevant to human exposure such as intramuscular injection of insoluble metal compounds. The genotoxicity and mutagenic activity of metals is also discussed briefly; however, the reader is referred to more detail in chapters on individual metals and to the chapter on the mechanisms of metal toxicity and carcinogenicity. Cell transformation in tissue culture and other mechanistic information for each metal ion is included in summary style.

1 PRINCIPAL METALS SHOWING CARCINOGENIC EFFECTS

Exposure to compounds of five metals has been shown in epidemiological studies to correlate with increased incidences of cancer in humans. These metals are nickel, chromium, arsenic, cadmium, and beryllium. However, adequate epidemiological studies, and in particular prospective studies, have not been performed with other metals; at present, there is a great lack of human data. Most of the available epidemiological data have been retrospectively obtained on occupationally exposed groups. Of the five metals, compounds of nickel, chromium, cadmium, and beryllium have been confirmed as carcinogens in experimental animals; there was recent evidence in the case of the metalloid arsenic (Burns *et al.*, 2004; Rossman *et al.*, 2004; Uddin *et al.*, 2005). Some compounds of cadmium and beryllium have been shown to have an unequivocal carcinogenic effect in animals, although the exposure situations have not been comparable to humans. In addition to the preceding, certain compounds of cobalt, iron, lead, manganese, platinum, titanium, and zinc have produced tumors in experimental animals, but the doses used and modes of administration have been different from any known human exposure situation. In further consideration of these 12 metals, their speciation should be kept in mind. A number of compounds of nickel are carcinogenic in animal bioassay, but with manganese and titanium only one organic compound of each has been shown to have such a potential. Currently, there is inadequate evidence with regard to a metal itself as carcinogenic; only a limited number of compounds have been shown to give rise to tumors in an experimental situation. It should further be noted that in both the general and occupational environment, exposure situations are complex, giving opportunity for enhancement or inhibition of a carcinogenic effect by interactions among metals and between metals and organic compounds (Nordberg and Andersen, 1981). Those metals for which there is some epidemiological evidence for a

carcinogenic effect in humans are considered first, followed by those for which there is evidence of such an effect only in animal bioassay. Brief reference is made to their genotoxic effects only when this seems to be relevant to a consideration of carcinogenicity. Reference is also made to the property of these metals to transform cultured mammalian cells *in vitro* and to affect the fidelity of DNA synthesis in a cell-free system.

1.1 Nickel

1.1.1 Epidemiological Observations

A highly significant excess of both lung cancer and cancer of the nasal sinuses has been found in nickel-refinery workers in several countries (Doll, 1958; Mastromatteo, 1967; Morgan, 1958; Pedersen *et al.*, 1973). In the Mond Nickel Works in South Wales, where exposure to nickel carbonyl gas had occurred in the past, Doll *et al.* (1970) estimated the excess mortality from lung cancer at 10 times the incidence expected for men first employed before 1914, and at about five times that expected for those first employed between 1915 and 1924 (Doll *et al.*, 1970). For nasal cancer, those first employed between 1910 and 1914 experienced an almost 900-fold increase; at the same time as in those first employed between 1920 and 1924, the mortality was almost 100 times the national average (Doll *et al.*, 1970). Observations by Doll *et al.* (1977) showed that the risk to the refinery workers continued until 1930, after which time major changes occurred in the refinery processes (Doll *et al.*, 1977). More recently, the Landolph laboratory has shown that reduction, or elimination, of the sulfuric acid in the refinery process in 1923 reduced the amount of nickel arsenide (orcelite) present in the Clydach refinery, in Wales, operated by the International Nickel Company (INCO), which led to a reduction in the incidence of nasal and lung cancers. Furthermore, it has also been shown that the 1920 refinery dust sample (25% orcelite) did, but that the 1929 sample (2.5% orcelite) did not, induce morphological transformation in C3H/10T1/2 cells, consistent with the high nasal and respiratory cancer incidence from 1901–1923 and the reduced cancer incidence after 1925 (Clemens and Landolph, 2003). All the men studied were manual workers in the refinery, but only some of them were employed on the specific nickel carbonyl process at the same time; others were general laborers who changed jobs frequently. Thus, it was not possible to collect detailed information on exposure.

The theory that nickel carbonyl alone acted as a carcinogen had to be discarded when a similar risk was found in nickel-refinery workers in Ontario, where the electrolytic process was used. Mastromatteo (1967) and Sunderman (1973) collected data on 327 cases of lung cancer and 115 cases of cancer of the nasal sinuses in nickel-refinery workers with a variety of exposures to nickel in Norway, France, Germany, the United States, and Japan, as well as in Wales and Canada (Mastromatteo, 1967; Sunderman, 1973).

Apart from nickel-refinery workers, isolated cases of lung and nasal cancer have been reported in workers in nickel-plating, nickel-grinding, and nickel-polishing operations, but the significance of these cases cannot be assessed, because no definitive epidemiological study has yet been performed. However, from the extent of exposure in the old nickel-refining processes and from the decrease in cancer mortality when the exposure was reduced, it seems unlikely that the much lower levels of exposure encountered in nickel plating would constitute a risk.

Although Morgan (1958) gave a detailed account of the nickel-refining process in South Wales, no information was given on exposure levels encountered by the workers who experienced an increased cancer mortality rate except that they were exposed to dusty rather than to gaseous compounds (Morgan, 1958). Similarly, Mastromatteo (1967) referred to the association between furnace operations involving the inhalation of freshly treated insoluble dust or fumes and the increased risk of cancer, but he gave no exposure concentrations (Mastromatteo, 1967). No data are available on exposure levels in workers with an increased mortality experience of respiratory cancer, except that exposure to nickel-containing dusts was heavy in the past; thus, no observations can be made on a dose-response relationship.

The carcinogenic agent(s) in nickel refining is (are) likely to be related to dust exposure from the preliminary processes of calcination, but the exact identity of the carcinogenic nickel-containing species is open to speculation. Sunderman and Mastromatteo (1975) considered that fresh nickel dusts from sintering and roasting processes were especially carcinogenic, and focus on respirable particles of metallic nickel, nickel subsulfide, and nickel oxide as significant carcinogens. An increased lung, nasal, and laryngeal cancer risk was found in men who had worked in the roasting, smelting, and electrolysis departments in a Norwegian nickel refinery before 1953 (Pedersen *et al.*, 1973). This study adds to the evidence incriminating exposure to furnace fumes and dust from the high-temperature sintering or calcining of impure nickel mattes. However, a case-control study from New Caledonia showed increased lung cancer mortality not only in workers but also in residents in the vicinity of a refinery processing laterite, an ore-containing nickel oxide (Lessard *et al.*, 1978). Available data suggest that the cancer risk is greater in heavy cigarette

smokers, with an interaction between two carcinogens, as with asbestos and smoking (Kreyberg, 1978).

A long-term cohort study of 814 workers with substantial exposure to airborne metallic nickel at a gaseous diffusion plant showed no increase in mortality from cancer of the respiratory tract (Godbold and Tompkins, 1979). Similarly negative results were obtained in a mortality study of 1925 men employed for a minimum period of 5 years who were exposed to average concentrations of metallic nickel of between 0.5 and 0.9 mg Ni/m^3, half by weight being of respirable size (Cox *et al.*, 1981). From these studies, it seems less likely that metallic nickel is a human carcinogen; the active, carcinogenic agent must be a compound of nickel, probably nickel subsulfide.

An epidemiological report on cancer mortality in 10 cohorts of occupationally exposed workers aimed at identifying the chemical forms of nickel responsible for the elevated cancer risk. Mortality from lung and nasal sinus cancer was associated with exposure to high levels of oxidic nickel compounds, sulfidic nickel in combination with oxidic nickel, and exposure to water-soluble nickel alone or together with less soluble nickel compounds (Doll *et al.*, 1990). In a case-control study of Norwegian nickel-refinery workers, Grimsrud *et al.* (2002) examined dose-related association between lung cancer and cumulative exposure to four forms of nickel: water soluble, sulfidic, oxidic, and metallic nickel. The study positively correlated a dose-related association between lung cancer and cumulative exposure to water-soluble nickel compounds (Grimsrud *et al.*, 2002a).

The latent period from first exposure to nickel compounds to diagnosis of the cancer is long, averaging approximately 25 years, with a range of 10–40 years. In those cases for which information is available, epithelial tumors predominated, but anaplastic and pleomorphic tumors have also been observed (Sunderman, 1973).

Significant abnormalities have been found in nickel-refinery workers subjected to routine cytological screening. Nasal cancer and a high rate of epithelial dysplasia compared with controls were found after biopsy of the nasal mucosa. Sputum cytology has revealed epithelial dysplasia, and in 16 of a group of the 583 refinery workers from a nickel-sinter plant, malignant cells were found without clinical or radiological evidence of cancer (McEwan, 1978). The routine use of such screening procedures should be considered when any risk of nickel-induced cancer is believed to exist.

1.1.2 Animal Models

The experimental models that have been used to study nickel carcinogenesis have been reviewed by Sunderman (1973) and by the IARC (1976) (IARC, 1976; Sunderman, 1973). Carcinogenesis has been adequately demonstrated in several animal species after subcutaneous or intramuscular injection, but with the exception of nickel subsulfide, the results after inhalational exposure have been less conclusive. Nickel subsulfide, Ni_3S_2, has given rise to fibrosarcomas and to rhabdomyosarcomas in numerous experiments after subcutaneous or intramuscular injection. Gilman (1966) found a dose-response relationship with injections of 10 mg nickel subsulfide giving an 80% tumor incidence in Wistar rats (Gilman, 1966). Sunderman *et al.* (1975) also found a dose-response relationship, with 7 of 30 rats developing sarcomas after a dose of 0.63 mg, rising to 28 of 30 rats developing sarcomas with a dose of 2.5 mg nickel subsulfide. Heath and Daniel (1964a) injected powdered nickel suspended in fowl serum into the thigh muscle in rats, all of which developed rhabdomyosarcoma at the injection site within 17 to 41 weeks (Heath and Daniel, 1964a; 1964b). Tumors have also been produced after the intrarenal and intratesticular injection of nickel subsulfide (Damjanov *et al.*, 1978; Sunderman *et al.*, 1979).

Prolonged inhalation of nickel subsulfide, of fine particle size (70% smaller than 1 μm diameter), at a concentration of 1 μg/m^3, produced 14 malignant neoplasms of the lung in 226 exposed rats compared with only 1 neoplasm in 241 control animals (Ottolenghi *et al.*, 1975). Inhalation of nickel carbonyl resulted in only two pulmonary adenocarcinomas in a series of experiments on rats (Sunderman and Donnelly, 1965). The repeated intravenous injection of nickel carbonyl resulted in malignant tumors in 19 of 21 survivors, or 16% of the injected rats. In addition to the sarcomas, one cholangiosarcoma, one carcinoma of the kidney, one mammary carcinoma, and five pulmonary lymphomas were observed (Lau *et al.*, 1972).

Comparison of the relative poteny of the various nickel compounds tested is difficult because of the variation is experimental design is different studies(r) Potenic seems to be inversel related to solubility of the nickel compound is aqueoue media with the highly soluble salts suce as nickel chloride having no apparent carcinogeni activity(r). The carcinogenic activity of nickel subsulfide (α-Ni_2S_3) is greater than that of any other metallic compound so far investigated in animal bioassay. The variety of animal species, routes of administration, and high yield of tumors has made nickel subsulfide particularly useful for experimental tumor induction. However, bacterial mutagenesis tests for nickel compounds have been consistently negative. Nickel subsulfide and nickel sulfate induced dose-related morphological transformation and enhanced viral transformation in cultured hamster embryo cells

(Casto *et al.*, 1979; DiPaolo and Casto, 1979), and divalent nickel decreased the fidelity of DNA synthesis in the cell-free system of Sirover and Loeb (1976) (Sirover and Loeb, 1976b) and induced morphological transformation in cultured C3H/10T1/2 Cl 8 mouse embryo fibroblasts (Miura *et al.*, 1989). The laboratory of Landolph has found that in nickel compound–induced transformed 10T1/2 mouse embryo fibroblast cell lines, there are aberrations in the expression of 130 genes, with approximately 65 genes, including oncogenes, such as the ect-2 oncogene, being expressed at higher steady-state levels and 65 genes, such as the DRIP/TRAP80 gene, being reduced in expression or not expressed at all (Clemens *et al.*, 2005; Landolph *et al.*, 2002; Verma *et al.*, 2004).

Possible mechanisms of nickel carcinogenesis have been reviewed by Sunderman (1976, 1977) (Costa, 2002; Costa *et al.*, 2003; 2005; Sunderman, 1976; 1977). An inhibition by nickel carbonyl of RNA synthesis in hepatocytes has been demonstrated (Beach and Sunderman, 1970; Witschi, 1972). This is believed to be due to an inhibitory effect of nickel on RNA polymerase activity, thus altering the expression of genetic information. Manganese has been shown to inhibit the carcinogenicity of nickel subsulfide in the rat without altering the mobilization or excretion of nickel or the initial inflammatory reaction at the injection site. In keeping with the preceding hypothesis, manganese may antagonize the nickel inhibition of RNA polymerase activity (Sunderman, 1975). Nickel subsulfide also produces disordered patterns of cell proliferation in fetal cell cultures. This effect, too, can be prevented by the addition of manganese dust (Costa, 1978). The current issue of this handbook has a chapter on the mechanisms of metal carcinogenesis and toxicity and a separate chapter on nickel, and the reader is referred to this chapter for further information on mechanisms of nickel carcinogenesis.

1.1.3 Evaluation

The IARC (1990) concluded there is sufficient evidence in humans for the carcinogenicity of nickel sulfate and of combinations of nickel sulfides and oxides encountered in the nickel-refining industry. There is inadequate evidence in humans for the carcinogenicity of metallic nickel and nickel alloys (IARC, 1990).

There is sufficient evidence in experimental animals for the carcinogenicity of metallic nickel, nickel monoxides and hydroxides, and crystalline nickel sulfides. There is limited evidence in experimental animals for the carcinogenicity of nickel alloys, nickelocene, nickel carbonyl, nickel salts, nickel arsenides, nickel antimonide, nickel selinides, and nickel telluride. The data on the carcinogenicity of soluble nickel compounds such as nickel sulfate, is equivocal, although they are generally not carcinogenic in animals, it is difficult to get data on nickel sulfate alone in humans.

The overall evaluation of nickel compounds as a group as a basis of combined results of epidemiological studies, carcinogenicity studies, studies in experimental animals, and several types of other relevant data was supported by the underlying concept that nickel compounds can generate nickel ions at critical sites in their target cells. Overall, IARC classified nickel compounds to be carcinogenic to humans (group 1), and metallic nickel is possibly carcinogenic to humans (group 2B).

1.2 Chromium

1.2.1 Epidemiological Observations

Lung cancer in workers producing dichromates from the raw material, chromite, or chrome iron ore was first reported in Germany in the 1930s, and these reports have been reviewed by Baetjer (1950). The reader is also referred to the review of chromium by Costa (1997). The observation was confirmed in the United States by Machle and Gregorius (1948) and in Britain by Bidstrup and Case (1956). In the American series, the observed mortality for lung cancer was approximately 30 times the expected number from national mortality data of that time, but in the British series (a 6-year follow-up mortality study), the increased risk was computed at a little over three times that expected. Similar observations were made in other countries. An increased risk of dying from lung cancer with duration of exposure in the chromate industry was observed by Taylor (1966). In his cohort study, the death rate from respiratory cancer increased from 106 for men with 5–9 years' exposure, to 400 per hundred thousand for men with 20–24 years' exposure; this study provides the only evidence for a dose-response relationship (Taylor, 1966).

Langård and Norseth (1975) found three cases of bronchial carcinoma in a cohort study of 24 workers with more than three years' exposure to chromate pigments, including zinc chromate (Langard and Norseth, 1975). The average age of those with cancer was 50 years and the risk ratio, based on general population experience, was computed at 38. Exposure levels were estimated to have been between 0.5 and 1.5 mg Cr/m^3 over a 6- to 9-year period and occurred mainly in filling sacks and in the mixing of raw materials. By 1983, six cases of lung cancer had occurred among these 24 men, where only 0.13 had been expected, an excess large enough for smoking cigarettes not to be considered an important confounder (Langard and Vigander, 1983).

A mortality study in three English factories manufacturing chromate pigments showed a significant increase in lung cancer in the two factories producing zinc chromate pigments. The excess mortality was observed in those departments where exposure to dust occurred and was rated as high; it was also noticed in the less dusty, wet departments, where exposure was rated as being of medium intensity. Exposure for as little as 1 year was associated with a raised mortality, and induction times were found to be fairly short, with a ratio of observed deaths/expected deaths fairly constant at about 4/1 during 5–24 years after first exposure. This short induction time contrasts with the longer latent interval found in the dichromate-producing industry (Davies, 1978).

In an updated cohort mortality study of lung cancer in these three chromate pigment factories (Davies, 1984), workers exposed to lead chromate experienced normal mortality, even under conditions conducive to lead poisoning. Those workers who had low exposure to both lead and zinc chromate also had no increase in lung cancer. However, workers with high or medium exposure for at least 1 year to both lead and zinc chromate had significantly raised lung cancer mortality, with 32 observed deaths whereas 12 were expected. Again, latent intervals for the development of cancer were unusually short. The results indicate that moderate or heavy exposure to zinc chromate may give rise to a severe risk of lung cancer developing, but relatively mild exposure lasting less than 1 year may not constitute an effective risk.

In the industrial process, raw chromite is dried, crushed, mixed with soda ash, and roasted in a rotary furnace in an oxidizing atmosphere. The insoluble oxide is converted to a water-soluble monochromate and from this the dichromate and other chromium compounds are produced. The lung cancer hazard in the chromate production industry has been attributed to carcinogenic agents present in the primary ore or in one of the furnace products, either as an intermediate or as a component of the residue. Secondary users of chromates and chrome pigments may be at increased risk, but the epidemiological evidence is inconclusive. Exposed groups include welders and grinders and those engaged in chrome-alloy production, such as ferrochromium. An increased lung cancer risk in chrome-alloy workers was observed by Langård *et al.* (1980) but not by Axelsson *et al.* (1980) in a similar investigation (Axelsson *et al.*, 1980). The Federal Security Agency, U.S. Public Health Service (1963) did not demonstrate excess of lung cancer over a 14-year period in a refractory plant using chromite ore to make chromite brick. The investigators concluded that acid-soluble and water-soluble derivatives of chromite ore were the suspect carcinogens. With regard to the large population of platers exposed to soluble chromium compounds, the evidence for an increased lung cancer risk is again inconclusive (Norseth, 1981). A pilot cohort mortality study in nine small chrome-plating plants found 3 cases of lung cancer among hard-chrome platers who had heavy exposure, whereas 0.7 was expected (Franchini *et al.*, 1983). Because of the small size of the cohort, the effect of confounding variables could not be assessed, and further studies on the possible carcinogenicity of chromic acid are required. Thus, the epidemiological evidence at present shows a clear increased risk of lung cancer only in the primary chromate production and in the zinc chromate pigment production industries, consistent with the concept that slightly soluble hexavalent chromium compounds are carcinogenic.

In more than 50 epidemiological studies on chromium exposure and lung cancer, the major investigations have been performed on chromium production workers, chromate paint makers, and chrome platers (IARC, 1990). The overall relative risk for lung cancer in these studies has been estimated at 2.78 (95% confidence interval, 2.47–3.52). In those studies where asbestos, nickel exposure, and smoking were assessed, confounding was rejected as the major source of the observed elevated risk (Steenland *et al.*, 1996).

The time interval for lung cancer from first exposure to chromium compounds has varied from 4–47 years, with a mean latent interval from 10.6–21 years in different studies. Enterline (1974), reviewing the American data, concluded that the risk of respiratory cancer in chromate workers remained high in the period 1937–1960, with a higher risk in younger age groups; this suggests only a short latent period after exposure to a potent carcinogen (Enterline, 1974). Approximately 80% of the lung cancers examined were squamous cell carcinomas, but undifferentiated cancers and adenocarcinomas have also been seen. A few cases of nasal cancer have also been reported in chromate workers, but an increase in incidence has not been confirmed.

1.2.2 Animal Models

Animal models have so far not contributed to the precise identification of the carcinogen in the chromate-production industry, although they confirm the carcinogenic potential of chromium (VI). A small number of tumors have been observed in the rat after the intramuscular or intrapleural administration of chromite-ore roast (Hueper, 1958). The intramuscular implantation of pellets of 25 mg calcium chromate produced local sarcomas in 8 of 35 treated rats; and implantation of 25 mg sintered chromic trioxide gave rise to sarcomas in 15 of 35 rats (Hueper and Payne, 1959). The carcinogenic

activity of calcium chromate has been convincingly demonstrated by Roe and Carter (1969), who obtained a 75% tumor yield in 24 rats injected subcutaneously over a period of 20 weeks with 19 mg calcium chromate suspended in arachis oil (Roe and Carter, 1969). Laskin *et al.* (1970) introduced into the rat bronchus a small capsule saturated with the test material, thus providing continuous, long-term exposure to the bronchial epithelium (Laskin and Drew, 1970). Squamous cell carcinoma at the impaction site developed in six rats and adenocarcinoma developed in another 2 of 100 rats exposed to calcium chromate. Chromic chromate, chromic oxide, and chromic trioxide were without effect. Inhalational exposure to calcium chromate dust at a concentration of 13 mg/m^3 for 35 hours/week for life produced alveolar adenocarcinoma in mice in a single experiment (Nettesheim *et al.*, 1971). Levy and Venitt (1975) produced squamous cell carcinoma in the rat lung by implanting calcium chromate and zinc potassium chromate, both medium-soluble hexavalent chromium salts, into the bronchial tree. The tumor yield was low and the incubation period long, but adequate control groups were also examined, and the tumors were considered to be causally related to the chromium implants. In the animals without lung tumors, a significant increase in squamous metaplasia was seen in rats exposed to the pure hexavalent compounds only (Levy and Venitt, 1975). There has been considerable controversy over the carcinogenic activity of hexavalent chromium by ingestion. Hexavalent chromium resembles sulfate and phosphate and is able to penetrate all cells of the body. Thus, on the basis of molecular mimicry, human exposure to chromates has broad carcinogenic potential. Recent studies of hairless mice have demonstrated that when combined with ultraviolet (UV) exposure there was a dose-dependent induction of skin cancer in hairless mice that were given 0.5, 2.5, and 5 mg/l of chromate in the drinking water (Davidson *et al.*, 2004). These cancers were squamous cell carcinomas, and because this type of cancer is not recorded in humans, it is difficult to assess whether chromate can induce it in humans.

1.2.3 Short-Term Tests

Chromium in hexavalent form is the most active of the metals in giving rise to mutations in both the S. *typhimurium* and *E. coli* bacterial test systems, where it seems to act directly without prior activation. After reduction to trivalent chromium, the mutagenic effect was no longer observed (Nishioka, 1975). Soluble hexavalent chromium salts produce both chromosomal aberrations in mammalian cells *in vitro* and morphological transformation of hamster embryo cells in culture (DiPaolo and Casto, 1979). However, in the cell-free system of Sirover and Loeb (1976), trivalent chromium was shown to be more potent than hexavalent chromium in the induction of genetic miscoding (Sirover and Loeb, 1976b). A possible mechanism for chromium carcinogenesis has been proposed from these and other observations. In this model, hexavalent chromium readily traverses cell membranes and in the process it is reduced to the trivalent form. Within the cell, trivalent chromium binds to nucleotide bases in DNA to give rise to genotoxic effects (Levis *et al.*, 1978; Lofroth, 1978). Thus, although the cancer hazard seems to follow environmental exposure to hexavalent chromium, trivalent chromium seems to be the agent that initiates the changes within the cell. Of all the metals studied, chromium best fits the hypothesis that carcinogenesis entails mutagenic initiation of somatic cells as a result of direct interaction with DNA. The handbook has a separate chapter on mechanisms of metal toxicity and carcinogenicity and a separate chapter on chromium, and the reader is referred to these chapters for further information.

1.2.4 Evaluation

IARC (1990) concluded there was sufficient evidence in humans for the carcinogenicity of chromium (VI) compounds as encountered in the chromate production, chromate pigment, and chromium plating industries (IARC, 1990). There is inadequate evidence in humans for the carcinogenicity of metallic chromium and of chromium (III) compounds.

There is adequate evidence in experimental animals for the carcinogenicity of calcium chromate, zinc chromates, strontium chromate, and lead chromates. There is limited evidence in experimental animals for the carcinogenicity of chromium trioxide and sodium dichromate.

The IARC Working Group made its overall evaluation on chromium (VI) compounds on the basis of the combined results of epidemiological studies, carcinogenicity studies in experimental animals, and several types of other relevant data that support the underlying concept that hexavalent chromium ions generated at critical sites in the target cells are responsible for the observed carcinogenic action. Overall, the Working Group concluded that hexavalent chromium is carcinogenic to humans (Group 1). Metallic chromium and trivalent chromium compounds are not classifiable as to their carcinogenicity to humans (Group 3).

1.3 Arsenic

1.3.1 Epidemiological Observations

Evidence for the carcinogenicity of arsenic is based on observations made in occupational groups and also

in general population groups exposed to arsenic after absorption from food, water, or drugs. A number of different occupational groups with exposure to arsenic have shown a greater cancer risk. An increase in cancer deaths was found in chemical process workers engaged in the production of inorganic arsenicals (Ott *et al.*, 1974), in smelter workers (Lee and Fraumeni, 1969; Pershagen *et al.*, 1977; Pinto *et al.*, 1977; Tokudome and Kuratsune, 1976), and in miners (Osburn, 1969).

In Hill and Faning's study, a proportional excess of deaths mainly from lung and skin cancer was demonstrated (29% observed compared with 13% expected). Exposure to arsenical dust was heavy, ranging from a mean atmospheric concentration of 1034 $\mu g/m^3$ by the kibbler to 78 $\mu g/m^3$ in the packing rooms. A significant increase in lung cancer was found in another proportional mortality study in chemical workers producing a variety of inorganic arsenicals, mainly lead and calcium arsenate (Ott *et al.*, 1974). Lung cancer accounted for 16% of deaths in the exposed group compared with 6% in the controls. Exposure levels were calculated as the product of the time-weighted average concentration with months of exposure. A dose-response relationship was demonstrated, the ratio of observed to expected deaths from lung cancer increased from 0.6 in the lowest to 7.0 in the highest exposure category. In the study reported by Pinto *et al.* (1977), a time-weighted index of total lifetime exposure to arsenic trioxide in copper-smelter workers was linearly related to lung-cancer mortality, ranging from a standardized mortality ratio (SMR) of 111 at the lowest exposure to 833 at the highest. It has been estimated that 25 years' exposure to arsenic at a concentration of about 50 $\mu g/m^3$ could lead to a nearly threefold excess mortality in lung cancer. The exposure in the past was probably underestimated, however, and the conclusions with regard to a dose-response relationship should be regarded as tentative. Axelson *et al.* (1978), in a case-control study in a Swedish copper-smelter, found a fivefold increase in lung cancer mortality with a trend toward a dose-response relationship (Axelson *et al.*, 1978).

It should be noted that very high exposures to arsenic in copper smelters might have occurred in the past. Thus, Kuratsune *et al.* (1974) refer to their workers as having been engaged in the "dirtiest prewar operations" where arsenic trioxide was a major by-product of the smelter (Kuratsune *et al.*, 1974). Although occupational exposure to a variety of arsenic compounds may occur, smelter workers are predominantly exposed to airborne arsenic trioxide. Arsenic may be present in copper, lead, zinc, nickel, and other metal ores. Exposure patterns are, therefore, complex and may involve other suspected carcinogens. Sulfur dioxide is another pollutant with which arsenic exposure is frequently associated. Thus, Lee and Fraumeni (1969) found that deaths from lung cancer increased not only with increasing arsenic trioxide exposure but also with increasing exposure to sulfur dioxide (Lee and Fraumeni, 1969). Axelson *et al.* (1978) also referred to the high correlation between exposure to arsenic, sulfur dioxide, and copper and pointed out the difficulty in distinguishing the interaction in such mixed exposures (Axelson *et al.*, 1978). A case-control study among smelter workers examined the effect of both arsenic trioxide exposure and smoking cigarettes on mortality from lung cancer (Pershagen *et al.*, 1981). The age-standardized ratio for death from lung cancer was 3.0 for arsenic-exposed nonsmokers, 4.9 for non-arsenic–exposed smokers, and 14.6 for arsenic-exposed smokers in relation to subjects without exposure to these putative carcinogens, indicating a multiplicative effect of the two exposures. The authors drew attention to the preventive measures that could be taken with regard to smoking in such an exposure situation.

A cohort study of 1462 tin smelter workers potentially exposed to lead, arsenic, and cadmium showed a statistically significant excess mortality from lung cancer (Binks *et al.*, 2005). The mortality pattern was considered to be similar to that in copper smelter studies (Enterline *et al.*, 1987; Jarup *et al.*, 1989) in which lung cancer excess was attributed to arsenic exposure. Arsenic has been identified as a major causative factor for lung cancer mortality in the copper smelter cohorts (IARC, 1987).

German and French vineyard workers who used pesticide spray containing arsenic had chronic arsenic poisoning develop over a 12- to 14-year exposure period. Roth (1958) investigated 27 deaths and found among these workers 12 cases of lung cancer, one of which was bilateral, occurring 8–14 years after the pesticides had been banned. Exposure had been heavy, with intake estimated at >5.0 g over a 12-year period (Roth, 1958).

The total daily intake of arsenic is greatly influenced by the amount of seafood in the diet, usually <200 μg/day and normally <50 μg/day (WHO, 1981). The arsenic content of tobacco has fallen as a result of the decreased use of arsenicals in agriculture. Arsenic is usually present in its pentavalent form, although traces of trivalent arsenic derived from pesticides may also be found. Contamination of food and drinking water from natural and industrial sources giving rise to chronic arsenic poisoning has been widely reported (see Volume II, Chapter 3). In one such episode in Taiwan where artesian well water with a high arsenic content has been used for more than 60 years (Tseng, 1977), clinical manifestations of chronic arsenicism were seen in the exposed population, including Blackfoot

disease and hyperkeratotic skin lesions. Arsenic levels in drinking water were designated "low" (<0.3 mg/l), "medium" (0.3–0.6 mg/l), and "high" > 0.6 mg/l). The rate of skin cancer was very high, at 10.6 per 1000 persons, and evidence of a dose-response relationship was found in the form of a clearcut ascending gradient of the prevalence of skin cancer from low to high for both sexes in all age groups.

IARC (2004) evaluated the extensive evidence available for the carcinogenicity of arsenic in drinking water (IARC, 2004). The Working Group evaluated ecological studies in Taiwan, Chile, Argentina, Australia, cohort studies in Taiwan, Japan, and the United States, and case-control studies in Taiwan, the United States, and Finland. Studies that involved long-term high exposures showed substantial increases in the risk for bladder cancer, with evidence for a dose-response relationship. Similarly, an increased risk for lung cancer was observed, with evidence for a dose-response relationship. The potential confounding effect of cigarette smoking was excluded. It was considered that the findings for both bladder and lung cancer cannot be attributed to chance or confounding, are consistent, and demonstrate strong associations in populations with high exposure. Furthermore, ecological studies have indicated substantially elevated incidence, prevalence, and mortality rates for skin cancer associated with drinking water highly contaminated with arsenic, with evidence of a dose-response relationship.

An increased mortality from liver cancer and for kidney cancer in populations with high, long-term exposure to arsenic was also reported by the IARC Working Group; however, there was potential confounding for liver cancer, and relative risk estimates for kidney cancer were much lower than those for urinary bladder cancer.

As with nickel and chromium, the latent interval between first exposure to arsenic and development of cancer is long. Average latent intervals of 34, 39, and 41 years were estimated for heavy, medium, and light exposure categories (Lee and Fraumeni, 1969).

Chronic exposure to arsenic has been implicated in the etiology of hemangiosarcoma (hemangioepithelioma) of the liver, in some cases associated with hepatic cirrhosis (Lander *et al.*, 1975; Popper and Thomas, 1975). Cancers of the lymphatic and hemopoietic systems, bladder cancer, and cancers of other organs have also been reported to occur more frequently after arsenic exposure, both with and without skin cancer (IARC 2004).

1.3.2 Animal Models

Evidence is limited for carcinogenesis by arsenic in experimental animal studies. An increased incidence of lymphocytic leukemia and lymphoma was observed in female Swiss mice and their young after the subcutaneous injection of sodium arsenate during pregnancy (Osswald and Goerttler, 1971). Arsenic trioxide, sodium and potassium arsenite, and sodium arsenate have been extensively tested by oral administration and by skin application with negative results. A number of organic arsenicals have also been tested with similar findings. Lung tumors in rats have been produced after a single intratracheal instillation of a mixture of calcium arsenate, copper sulfate, and calcium oxide, a preparation similar to that formerly used in vineyards, indicating a carcinogenic potential at least for the mixture given (Ivankovic *et al.*, 1979). Multifocal bronchogenic adenocarcinomas and bronchiolar alveolar cell carcinomas were induced in 9 of 15 surviving rats, the mean induction time being 470 days. Intratracheal instillations with calcium arsenate alone induced only benign lung tumors in hamsters (Pershagen and Bjorklund, 1985). A small number of lung cancers developed in hamsters given arsenic trioxide together with charcoal carbon as a carrier dust. There was, in addition, a significant increase in adenomas and papillomas in the treated animals compared with the controls given only carbon dust.

Combined exposure to arsenic trioxide and benzo(a)pyrene by the same route markedly increased the incidence of pulmonary adenomas, papillomas, and adenomatoid lesions compared with benzo(a)pyrene alone (Pershagen *et al.*, 1984a). In a further study three carcinomas and a number of adenomas and papillomas were identified in 47 hamsters exposed to arsenic trioxide and charcoal, but no carcinomas and few other tumors in 53 hamsters without arsenic trioxide exposure (Pershagen *et al.*, 1984b). Benign pulmonary tumors and/or carcinomas have also been induced with arsenic trioxide in some other studies on experimental animals (Ishinishi *et al.*, 1977; 1980; 1983).

To explain the apparent contradiction between epidemiological and animal experimental data, it has been proposed that arsenic may act as a cocarcinogen or potentiator of the carcinogenic process rather than as a direct carcinogen. A number of studies have been performed on the effect of arsenic on tumor incidence and growth with contradictory results, and Squibb and Fowler (1983) have reviewed these.

1.3.3 Short-Term Tests

Chromosome aberrations have been observed in workers exposed to arsenic compounds (Nordenson *et al.*, 1978), and both aberrations and an increase in SCE have been noted after arsenic medication (Burgdorf *et al.*, 1977; Petres *et al.*, 1977). Although mutagenicity

could not be demonstrated with the *S. typhimurium* plate test (Lofroth, 1978), arsenic compounds gave positive results indicative of DNA damage in bacterial assay (Nishioka, 1975). Morphological transformation of hamster embryo cells in culture could be induced with sodium arsenate; and enhancement of viral transformation in the same system was obtained with arsenic trichloride (Casto *et al.*, 1979; DiPaolo and Casto, 1979). However, arsenic did not affect the fidelity of DNA synthesis (Sirover and Loeb, 1976a). Squibb and Fowler (1983) in their review concluded that arsenic compounds are not generally mutagenic, but that certain compounds have shown a potential for inducing DNA damage and inhibition of DNA repair enzymes (Squibb, 1983). Possible mechanisms to explain the carcinogenicity of arsenic have been discussed by Sunderman (Sunderman *et al.*, 1979). Again the reader is referred to the mechanism chapter in this handbook as well as the chapter on arsenic for further information.

1.3.4 Evaluation

The IARC (1980) concluded that there was sufficient evidence that inorganic arsenic compounds are skin and lung carcinogens, but the data suggesting an increased risk for cancer at other sites were inadequate for evaluation. They found the evidence for the carcinogenicity of arsenic compounds in animals to be insufficient.

The IARC (2004) concluded there is sufficient evidence in humans that arsenic in drinking water causes cancer in the urinary bladder, lung, and skin (IARC, 2004). It was considered that there is sufficient evidence in experimental animals for the carcinogenicity of dimethyl arsenic acid; limited evidence for the carcinogenicity of sodium arsenite, calcium arsenate, and arsenic trioxide; and inadequate evidence for the carcinogenicity of sodium arsenate and arsenic trisulfide. It was considered that, taken together, the studies on inorganic arsenic provide limited evidence for carcinogenicity in experimental animals. Overall, it was considered that arsenic in drinking water is carcinogenic to humans (Group 1).

1.4 Cadmium

1.4.1 Epidemiological Observations

Studies have been performed principally on industrial workers who have been exposed in the past to high concentrations, in particular, of cadmium oxide fume and dust.

Three of eight deaths in 74 nickel-cadmium battery workers with at least 10 years' exposure to cadmium oxide dust were noted by Potts (1965) to be from cancer of the prostate (Potts, 1965). In the past, exposure to cadmium oxide dust and fume had been heavy. For example, in 1949, concentrations in two work areas ranged from 0.6–2.8 mg Cd/m^3, and in a third up to 236 mg/m^3 (Potts, 1965). Improvements in ventilation reduced these levels to below 0.5 mg/m^3 by 1950 and to 0.1 mg/m^3 by 1956. Kipling and Waterhouse (1967) surveyed the records of nickel-cadmium battery workers exposed to cadmium oxide dust for a minimum period of 1 year. They found four cases of prostatic cancer for which the expected figure was computed at 0.58 from regional incidence rates. They concluded that the increase in prostatic cancer was highly significant, whereas the occurrence of other forms of cancer was close to that expected. However, the numbers involved were not large (they included the three cases noted by Potts), and they were unable to infer the existence of an industrial hazard.

Since then, another study has supported the preceding findings, a cohort mortality investigation of smelter workers heavily exposed to cadmium fume and cadmium oxide dusts (Lemen *et al.*, 1976). The study involved 292 smelter workers with a minimum of 2 years' exposure during a 30-year period starting in 1940. A significantly excessive risk was demonstrated for malignant neoplasms, with 27 cases observed and 17.6 expected. Twelve of the deaths were due to respiratory cancer, a significant increase, because only 5.11 were expected; and 4 were due to prostatic cancer, whereas 1.15 were expected, a difference that was not significant. The greatest risk for respiratory cancer was apparent 30 years after initial employment and for prostatic cancer 20 years after initial exposure, at which time the increased risk of prostatic cancer had attained significance. However, in this study there was concomitant exposure to arsenic, and smoking histories were not available.

Kjellstrom *et al.* (1979) have presented data on cancer mortality and cancer morbidity among 269 cadmium-nickel battery workers and 94 cadmium-copper alloy workers in Sweden (Kjellstrom *et al.*, 1979). All workers had at least 5 years' heavy past exposure to cadmium oxide dust or fume. Before 1947, the exposure levels in the battery plant were on average above 1 mg/m^3. An internal reference group of 328 alloy factory workers without cadmium exposure was also studied. The cumulative observed and expected number of prostatic cancer deaths in 1940–1975 among the alloy factory workers was 4 against 2.69, whereas the corresponding number in the reference group was 4 of 6.42. In the battery factory, there were two observed cases of prostatic cancer against 1.2 expected. In the combined cohort study, there were two cases of cancer of

the nasopharynx against an expected 0.2, with a risk ratio of 10; however, heavy exposure to nickel had also occurred. In a follow-up and an extension of the study in the nickel-cadmium battery workers, many of whom had been heavily exposed to both cadmium and nickel, in a subgroup exposed for more than 15 years, there were now 3 deaths from prostatic cancer with 1.6 expected and 3 deaths from lung cancer where 2.5 had been expected from Swedish male mortality rates. Thus, the small, no significant excess mortality from prostatic cancer in this cohort has been maintained (Andersson *et al.*, 1984). In a further analysis of the total cohort, the standardized mortality ratio for both prostatic and lung cancer increased with increasing dose and latency but did not attain statistical significance (Elinder *et al.*, 1985).

In copper-cadmium alloy workers in Britain, again with heavy past exposure exceeding 1 mg Cd/m^3, a total of 7 cases of lung cancer was observed compared with 3.9 expected in men exposed for more than 10 years (Holden, 1980). Among vicinity workers not directly involved in cadmium processes, 8 cases of prostatic cancer were observed compared with 3 expected, although only 3 of these 8 had had more than 15 years' exposure (Holden, 1980). With no evidence of a dose-response relationship, their association with past cadmium exposure might be questioned. No new evidence of any relation between occupational exposure to cadmium oxide dust and cancer of the prostate was found in a mortality study of more than 3000 nickel-cadmium battery workers in Britain, which included the 4 cases previously observed by Kipling and Waterhouse (1967). There was some indication of an increased risk from cancers of the respiratory system, because 89 deaths were observed, whereas only 70.2 were expected, although exposure to welding fumes and nickel hydroxide was considered an important confounding factor (Sorahan and Waterhouse, 1983).

In an extended follow-up study of the cohort described by Lemen *et al.* (1976), mortality experience has been investigated through 5 additional years and the cohort extended to include white men with at least 6 months' employment in a production area (Thun *et al.*, 1985). Since 1925, the plant had refined cadmium and a number of other trace metals from "baghouse" dust, a by-product of lead smelting. Previously, the plant had functioned as an arsenic smelter and initially as a lead smelter. Exposure to cadmium had been heavy; more than 80% of the workers measured had a mean urine cadmium concentration of at least 20 µg/l. Mortality from lung cancer was significantly greater among the workers employed for 2 years or more than would have been expected from US white male rates, with 20 observed deaths where 11.43 were expected. In addition, the study showed a significant dose-response relationship between lung cancer mortality and cumulative exposure to cadmium (Stayner *et al.*, 1992a; 1992b). No further deaths from prostatic cancer have been reported since the earlier study, thus weakening the original observation by Lemen *et al.* (1976) of a possible association between prostatic cancer and cadmium exposure. With regard to lung cancer, Thun *et al.* (1985) made certain assumptions from which they concluded that the two confounding factors, smoking cigarettes and past arsenic exposure, were unlikely to account for the observed excess lung cancer risk. However, arsenic exposures after 1926 may have been higher than estimated, and no account was taken of the possible combined effect of smoking cigarettes and arsenic exposure (Pershagen *et al.*, 1981).

To investigate mortality on a scale larger than available in earlier studies, the mortality of almost 7000 cadmium-exposed male workers from 17 cadmium processing plants in England, born before 1940 and exposed to cadmium for more than 1 year between 1942 and 1970, was examined initially until the end of 1979 (Armstrong and Kazantzis, 1983), followed up for an initial 5-year period from 1980–1984 (Kazantzis *et al.*, 1988), and for a second 5-year period to the end of 1989 (Kazantzis *et al.*, 1992). Jobs were assessed for each year as involving high, medium, or low exposure to cadmium. The years at risk of the study population were divided into three groups, "ever high" (minimum of 1 year), "ever medium" (minimum of 1 year), and "always low." Significant excess lung cancer mortality was observed in both the 5-year and the total study periods. The SMR increased with intensity of exposure in both the 5-year and the total study periods, being significantly raised in the "ever high" exposure groups. The increased risk was most marked in men first employed before 1940 with long exposure and a long period of follow-up. However, in attributing this apparent dose-response relationship to cadmium, account has to be taken of multiple confounding factors, including arsenic, in the study population (Ades and Kazantzis, 1988). No increased risk from prostatic cancer was observed in the overall cohort, with one case in the "ever high" exposure group where one was expected.

In a systematic review of studies since the IARC (1993) classification of cadmium as a human carcinogen, several cohorts of cadmium workers have been updated with additional data available regarding environmental exposure to cadmium and cancer risk (IARC, 1993). A lower relative risk of lung cancer in occupational groups exposed to cadmium in the absence of arsenic and nickel is indicated in some studies. No evidence was found that nonmalignant respiratory

disease represents a competing cause reducing mortality from lung cancer. The association between cadmium exposure and prostate cancer was not confirmed in the latest available updates. Although studies in environmentally exposed populations have not indicated an increased relative risk of cancer (Verougstraete *et al.*, 2003), the most recent study has shown that overall cancer risk, especially lung cancer, was significantly associated with the environmental exposure to cadmium (Nawrot *et al.,* 2006; Nordberg, 2006).

1.4.2 Animal Models

Finely divided cadmium metal suspended in fowl serum injected into the thigh muscle of the rat gave rise to rhabdomyosarcoma and fibrosarcoma (Heath *et al.*, 1962). Similar tumors followed the intramuscular and subcutaneous injection of cadmium sulfide and cadmium oxide suspensions (Kazantzis, 1963; Kazantzis and Hanbury, 1966). The tumors developed at the site of one of two subcutaneous injections of 25 mg cadmium sulfide or of cadmium oxide suspended in physiological saline, or after a single intramuscular injection of 50 mg cadmium sulfide in saline, after an interval of 4 months after subcutaneous injection and 9 months after intramuscular injection. The tumors were metastasizing and pleomorphic, and the tumor yield was high. In their series with cadmium oxide, this tumor yield was comparable with the nickel sulfide–induced tumors described earlier. Subcutaneous and intramuscular injection of soluble cadmium chloride or sulfate, in addition to producing sarcomas at the injection site, also induced hyperplasia interpreted as interstitial cell tumors of the testis (Haddow *et al.*, 1964; Reddy *et al.*, 1973; Roe *et al.*, 1964) and even after a single dose (Gunn *et al.*, 1963). However, the interpretation of the specific role of cadmium in initiating these changes in the testis is difficult. The initial effect of cadmium is a necrotizing one on the interstitial cells, probably acting through the vascular supply to the testis (Parizek, 1957), and it is possible that the hyperplastic or possible neoplastic change is secondary to this. However, zinc protects against the action of cadmium on the testis (Parizek, 1957). Zinc acetate given as three subcutaneous injections inhibited the development of both interstitial cell tumors of the testis and local sarcomas after the injection of cadmium chloride in rats (Gunn *et al.*, 1964).

In a study in which cadmium sulfate was given by subcutaneous injection or by stomach tube repeatedly over a period between 18 months and 2 years to rats and mice—in the latter by stomach tube only—no malignancy or other morphological change attributable to cadmium was seen in the prostate gland or in other systems. The dose range was based on exposure levels calculated to produce low-molecular-weight proteinuria in man. However, only the rats in the highest exposure group given cadmium sulfate parenterally had liver and kidney concentrations of the same order of magnitude as occupationally exposed workers (Levy *et al.*, 1973).

Striking results were obtained in an experiment by Takenaka *et al.* (1983), who exposed inbred male rats to cadmium chloride aerosols at concentrations of 12.5, 25, and 50 $\mu g/m^3$ continuously for a period of 18 months followed by 13 months' observation (Takenaka *et al.*, 1983). A dose-related incidence of lung cancer of varying histological types was seen, ranging from 15% in the low-exposure group to 71% in the high-exposure group.

1.4.3 Short-Term Tests

Chromosomal abnormalities have been noted in mammalian cells treated *in vitro* with cadmium chloride and with cadmium sulfate, but in high dosage. Observations on chromosome aberrations in cadmium-exposed persons are conflicting. Aberrations were noted in workers in a zinc smelter who had raised blood levels of cadmium and lead (Bauchinger *et al.*, 1976); and aberrations have also been seen in Japanese patients with *Itai-Itai* disease (Shiraishi, 1975). However, other studies on cadmium-exposed workers found no such abnormalities. Mutagenic effects are considered further in Section 1. Cadmium acetate was as potent as sodium chromate in producing morphological transformation of Syrian hamster embryo cells, and both compounds enhanced transformation of these cells by simian adenovirus (Casto *et al.*, 1979; DiPaolo and Casto, 1979). Cadmium also decreased the fidelity of DNA synthesis in the cell-free system of Sirover and Loeb (1976) (Sirover and Loeb, 1976a). The mutagenic effects of cadmium demonstrated in plants and insects, and the effects of cadmium on the metabolism of nucleic acids, have been reviewed by Degraeve (1981); a further review on mutagenic and carcinogenic effects of cadmium has been supplied by Kazantzis (Kazantzis and Armstrong, 1984). Again, the reader is referred to the mechanism chapter in this handbook and the chapter on cadmium for further information

1.4.4 Evaluation

An evaluation made by the IARC (1993) on the carcinogenic risk of cadmium may be summarized as follows (IARC, 1993). In two inhalation studies in rats, malignant lung tumors were produced by cadmium chloride, cadmium sulfide/sulfate, cadmium sulfate, and cadmium oxide fume and dust at low levels of

exposure for short durations. In several studies, single or multiple subcutaneous injections of cadmium chloride, cadmium sulfide, cadmium sulfate, and cadmium oxide caused local sarcomas in rats, as did cadmium powder, cadmium chloride, and cadmium sulfide after intramuscular administration. Excess zinc by inhalation, parenteral or oral routes, has been shown to reduce the carcinogenic potential of cadmium after exposure systemically or by inhalation.

After a report of the occurrence of prostate cancer in a small group of workers employed before 1965 in a nickel-cadmium battery plant in Britain, a series of cohort analyses did not confirm this excess, but increased lung cancer mortality was detected. Similar findings were reported in a small study in Sweden. A dose-response relationship was demonstrated between estimated cumulative cadmium exposure and lung cancer risk among workers in a U.S. cadmium recovery plant with excess lung cancer mortality. In the cohort of workers from 17 cadmium-processing plants in Britain, a decreased mortality from prostatic cancer was observed, whereas that for lung cancer was increased, with suggested trends with duration of employment and with intensity of exposure.

Although a number of early studies reported an increased risk for prostatic cancer, the results of later studies were not consistent, but both early and recent studies provide consistent evidence that the risk for lung cancer is increased in cadmium-exposed workers.

The IARC evaluation concluded there is sufficient evidence in humans and in experimental animals for the carcinogenicity of cadmium compounds, with limited evidence in experimental animals for the carcinogenicity of cadmium metal. The Working Group took into consideration the evidence that ionic cadmium causes genotoxic effects in a variety of types of eukaryotic cells, including human cells. Overall, cadmium and cadmium compounds are considered carcinogenic to humans (Group 1).

1.5 Beryllium

1.5.1 Epidemiology Observations

In an initial study, Mancuso (1970) found no overall evidence of an increased mortality in beryllium workers but encountered 6 cases of lung cancer in a group of 142 cases of beryllium-related bronchitis and pneumonitis (Mancuso, 1970). The age-adjusted lung cancer mortality rate was 284.3 per 100,000 compared with 77.7 for all white adult males employed in the same beryllium plant. Mancuso concluded that prior chemical pneumonitis influenced the subsequent development of lung cancer among beryllium workers. However, the lung cancer rate was higher in workers employed for a short duration, between 3 and 15 months, than in those employed for a longer period. Follow-up studies of subjects enrolled in the U.S. Beryllium Case Registry and in two primary beryllium-production works all showed excess lung cancer mortality. The Case Registry provided 7 lung cancer deaths against 3.3 expected (Infante *et al.*, 1980). However, 5 of these subjects had been exposed for less than 1 year, including 3 for less than 1 month. In a continuation of his earlier study, Mancuso (1980) again found evidence of excess lung cancer mortality in workers who had short but heavy exposures (Mancuso, 1980). A short exposure followed by a long latent interval before the development of lung cancer was confirmed by Wagoner *et al.* (1980), who also showed a significant excess, with 47 deaths observed and 34.3 expected. However, there is an overlap of the populations studied in these three investigations, as well as alleged statistical errors (Shapley, 1977), so that interpretation of the data presents some difficulties. The apparent lack of a dose-response effect was explained by the propensity for beryllium to be retained in the lung for long periods of time, thus demonstrating the inadequacy of length of employment as a measure of dose.

An extended analysis of mortality among those entered into the U.S. Beryllium Case Registry between July 1952 and the end of 1980 included 689 men of all races, as well as women. Mortality follow-up was extended through 1988. Excess mortality was found for all cancers, due primarily to an excess of lung cancer (SMR 2.00; 95% CI, 1.33–2.89) and also to nonmalignant respiratory disease (SMR 34.23; 95% CI, 29.1–40.0). The SMR for lung cancer was greater for those cohort members with acute beryllium pneumonitis. Taking account of the distribution of smoking habits, it was concluded that the study cohort smoked less than the U.S. reference population in 1965, and that if the 32% of the cohort members questioned were representative of the entire cohort, smoking was unlikely to be a confounder of the observed lung cancer excess (Steenland and Ward, 1991).

A cohort mortality study was carried out on 9225 male workers employed at seven beryllium plants in the United States (Ward *et al.*, 1992). A small, but significant, excess mortality from lung cancer was found in the total cohort, SMR 1.26 (95% C.1, 1.12–1.42). The risks for lung cancer were considerably higher in those plants in which there was also excess mortality from pneumoconiosis and other respiratory diseases, presumably indicative of higher exposure to beryllium. The risk from lung cancer increased with time since first exposure and was greater in workers first

hired in the period when exposures to beryllium in the workplace were relatively uncontrolled. Smoking was not considered to have been a confounding factor.

1.5.2 Animal Models

The carcinogenic activity of a number of beryllium compounds, in particular the oxide, sulfate, phosphate, and zinc beryllium silicate, has been confirmed in a number of investigations in rabbits, rats, mice, and monkeys. Intravenously administered beryllium oxide and zinc beryllium silicate have given rise to osteogenic sarcoma in mice and rabbits (Barnes and Denz, 1950). Inhalational exposure to beryllium oxide was followed by osteosarcoma in one of six rabbits (Dutra *et al.*, 1951).

Rats exposed to a beryllium sulfate aerosol at a mean concentration of 34 $\mu g/m^3$ for periods up to 56 weeks developed alveolar adenocarcinoma. All the 43 rats killed at the end of the exposure period had these tumors (Reeves *et al.*, 1967). Pulmonary tumors of various types also developed in rats, but not in monkeys or hamsters, after long-term exposure to beryl ore dust. After 17 months, 18 of 19 rats exposed to beryl ore at levels of 620 μg Be/m^3 had developed pulmonary tumors, but no control rats exposed to bertrandite dust did so (Wagner *et al.*, 1969).

Taking the various experimental observations together, a dose-response relationship for the production of malignant tumors in animal models becomes apparent, and the quantitative aspects of this are given in Chapter 21.

There are no reports of chromosome abnormalities in beryllium workers, but aberrations have been found in cultured mammalian cells. Beryllium has not been shown to be mutagenic in bacterial test systems (Simmon, 1979). However, beryllium sulfate induced morphological transformation of Syrian hamster cells and enhanced viral transformation of these cells (Casto *et al.*, 1979; DiPaolo and Casto, 1979). Beryllium chloride has been shown to decrease the fidelity of DNA synthesis in the cell-free system of Sirover and Loeb (1976a) (Sirover and Loeb, 1976b). Beryllium has been shown to block thymidine incorporation in hepatic DNA (Witschi, 1970) and of certain enzymes needed for DNA synthesis. It has been suggested that beryllium binding to nucleoproteins leads to specific inhibition of events leading to DNA replication (Sunderman *et al.*, 1979).

1.5.3 Evaluation

In summary, various beryllium compounds tested by inhalation in rats have produced lung tumors, mostly adenocarcinomas. Rabbits have developed osteosarcoma after intravenous injections. In the cohort mortality study of some 9000 workers involved in refining, machining, and producing beryllium metal and alloys, the significant excess in lung cancer mortality increased with time since first exposure, greater in workers first hired in the period when exposure to beryllium in the workplace was relatively uncontrolled. In the follow-up study of deaths among workers entered into the U.S. Beryllium Case Registry, the excess mortality from lung cancer was greater in those workers previously diagnosed with acute beryllium-induced pneumonitis. Potential confounding by smoking was addressed in several ways and did not seem to explain the increased risk for lung cancer.

In their evaluation, the IARC (1993) concluded there is sufficient evidence in humans and in experimental animals for the carcinogenicity of beryllium and beryllium compounds. Overall, beryllium and beryllium compounds are carcinogenic to humans (Group 1).

1.6 Lead

1.6.1 Epidemiological Observations

An early epidemiological study on lead workers showed no excess mortality from malignant neoplasms despite heavy exposure (Dingwall-Fordyce and Lane, 1963). In an update of this study, a small excess of malignant neoplasms of the digestive tract was found (observed 63, expected 48.6), but the excess mortality was not considered to be related to lead exposure (Malcolm and Barnett, 1982).

A case-control study of children with Wilms' tumor found an excess risk in children whose fathers had been employed in occupations classified as "lead related." However, the study could provide no evidence that actual lead exposure had occurred (Kantor *et al.*, 1979). A further case-control study found no evidence to support the hypothesis that paternal lead exposure is a risk factor for Wilms' tumor (Wilkins and Sinks, 1984).

An extensive cohort mortality study has been performed in the United States on a group of more than 7000 workers in lead-battery and lead-producing plants who had been exposed for a minimum period of 1 year (Cooper, 1976; Cooper and Gaffey, 1975). Lead absorption in many of these workers was greatly in excess of currently accepted standards, with a mean blood lead level of almost 80 $\mu g/100$ g in the production workers and 63 $\mu g/100$ g in the battery workers. In the initial study, a small excess mortality was found from cancer of the digestive organs and respiratory system (Cooper and Gaffey, 1975). In the succeeding 5-year period, however, the original mortality pattern was not maintained. In an update of this study, the mortality was examined

for the total 34-year period 1947–1980. Elevated standardized mortality ratios for malignant neoplasms have been found in both battery and production workers, the excess deaths being predominantly from gastrointestinal tract cancer and lung cancer. In the battery workers, there were 34 deaths from stomach cancer with 20.2 expected and 109 deaths from lung cancer with 87.8 expected, both standardized mortality ratios being significantly raised. A similar pattern was observed in the lead production workers. In interpreting these findings, account should be taken of the complex etiology of both lung and stomach cancer. The reference population used was U.S. white men, and smoking histories were not available. In the study, account could not be taken of the marked temporal and spatial variation of stomach cancer and of its close relationship to social class, and data were inadequate to seek a dose-response relationship. However, the possibility cannot be dismissed that heavy exposure to compounds of lead, perhaps in association with other chemical agents, has given rise to an excess of stomach and lung cancer; further epidemiological studies are required.

1.6.2 Animal Models and Short-Term Tests

Zollinger (1953) first observed renal adenoma and carcinoma in rats after the subcutaneous injection of lead phosphate (Zollinger, 1953). This observation has been confirmed, and several investigations have induced similar tumors after the administration in the diet of lead subacetate in rats and mice and lead acetate in rats (Boyland *et al.*, 1962; Van Esch and Kroes, 1969). Large doses of lead were used in these experiments, which caused gross morphological damage to the kidney, and which far exceeded doses tolerated by humans. Although tumors at other sites, including cerebral gliomas, have been reported, these have not been confirmed. Lead compounds have not given rise to mutagenic effects in bacterial test systems, but lead acetate has induced dose-related transformation in hamster embryo cells (DiPaolo and Casto, 1979), lead oxide enhanced transformation by simian virus (Casto *et al.*, 1979), and lead chloride has been shown to decrease the fidelity of DNA synthesis (Sirover and Loeb, 1976b). Epidemiological and experimental work from recent studies confirms that inorganic lead compounds are associated with increased risks of tumorigenesis (Silbergeld *et al.*, 2000). Several possible mechanisms by which lead compounds cause tumors have been shown that include direct DNA damage, clastogenicity, or inhibition of DNA synthesis or repair, and generation of reactive oxygen species that may cause oxidative damage to DNA. In addition, lead can also substitute for zinc in several proteins that function as transcriptional regulators and reduce the binding of these proteins to recognition elements in genomic DNA. This suggests that lead may alter gene expression through an epigenetic mechanism (Silbergeld *et al.*, 2000). Again the reader is referred to the mechanism chapter in this handbook as well as the chapter on lead for further information.

1.6.3 Evaluation

An IARC Working Group (2004) reevaluated the potential carcinogenic hazards to humans from exposure to inorganic and organic lead. The widespread occurrence of lead in the environment has been largely the result of anthropogenic activity since prehistoric times. Lead use has increased progressively with industrialization and rose dramatically with the use of lead auto batteries and leaded fuel. The predominant use of lead is currently in batteries, construction materials, and lead-based chemicals. It is being progressively phased out in pipes, paints, and gasoline. Recent human exposure has arisen predominantly from the widespread use of leaded gasoline and in areas near lead mines and smelters with high environmental concentrations of lead. Occupationally, the highest potential exposure to lead includes mining, primary and secondary smelting, production of lead acid batteries, pigment production, construction, and demolition.

To evaluate the epidemiological evidence of possible carcinogenic hazards, the Working Group considered six occupational cohort studies of highly exposed workers to be particularly informative, battery workers in the United States and the United Kingdom, and primary smelter workers in Italy, Sweden, and the United States. In their evaluation, the IARC concluded that inorganic lead compounds are probably carcinogenic to humans (group 2A) and organic lead compounds are not classifiable as to their carcinogenicity to humans (group 3). The Working Group noted that organic lead compounds are in part metabolized to ionic lead. To the extent that ionic lead generated from organic lead is present in the body, it will be expected to exert the toxicity associated with inorganic lead.

The remaining metals considered in this section have very little or no epidemiological evidence to incriminate them as carcinogens in human exposure situations. They have, however, been included here because of limited data in animal experimental studies. A more detailed review is given by Kazantzis (1981).

1.7 Cobalt

Finely divided cobalt metal powder, cobalt oxide, and cobalt sulfide have given rise to injection-site

fibrosarcoma after subcutaneous injection and to rhabdomyosarcoma after intramuscular injection in rats (Gilman, 1966). They were able to produce such neoplasms with fine particles from surgical prostheses made from a cobalt-chromium alloy. Cobalt chloride has been shown to decrease the fidelity of DNA synthesis *in vitro* (Sirover and Loeb, 1976a), to induce morphological transformation, and to enhance viral transformation of hamster embryo cells (Casto *et al.*, 1979; DiPaolo and Casto, 1979).

There have been few reports of cancer in cobalt workers despite past occupational heavy exposure. In certain nickel extraction plants in the U.S.S.R., an increased mortality from lung cancer was found in the cobalt-recovery shops and in the nickel-processing departments (Saknyn and Shabynina, 1973). However, exposure to arsenic-containing dust was heavy, and these workers may also have been exposed to nickel. There are no convincing reports of cancer having arisen in relation to surgical prostheses with cobalt-containing alloys, despite raised blood and urinary cobalt levels. IARC (1991) considered that cobalt and cobalt compounds are possibly carcinogenic to humans (group 2B) (IARC 1991). Again the reader is referred to the mechanism chapter in this handbook and the chapter on cobalt for further information.

1.8 Iron

Iron-dextran and certain other polysaccharide iron complexes have induced local sarcomas in mice, rats, rabbits, and hamsters after subcutaneous or intramuscular injection in large doses with evidence of a dose-response relationship (Haddow and Horning, 1960; Richmond, 1959; Roe and Carter, 1967). The significance of these observations arises in relation to iron therapy. Although many of these compounds have been administered parenterally in the treatment of anemia over the past 30 years or so, sarcoma at the injection site has been rarely reported. In a survey of all 72 soft tissue sarcomas found over a 2-year period in a defined area, no history of past parenteral iron therapy could be obtained (McIllmurray and Langman, 1978).

The repeated intratracheal installation, in hamsters, of ferric oxide suspended in normal saline did not give rise to lung tumors (Saffiotti *et al.*, 1968). However, given together with benzo(a)pyrene or with systemically administered diethylnitrosamine, an increased yield of lung tumors was obtained. Ferric oxide was thought to be a carcinogenic cofactor, possibly acting by retarding clearance of an inhaled carcinogen or by inducing cytopathological changes that make the cells of the respiratory tract more susceptible to carcinogens. Iron oxide did not produce morphological transformations in hamster embryo cells and only enhanced viral transformations in very high concentrations (Casto *et al.*, 1979). Iron did not decrease the fidelity of DNA synthesis in the cell-free system of Sirover and Loeb (1976) (DiPaolo and Casto, 1979).

There has been speculation on the role of iron oxide as a possible carcinogen common to hematite, asbestos, nickel, and chrome workers, but there is little evidence to support such a hypothesis. Boyd *et al.* (1970) observed a significantly increased mortality rate from lung cancer in hematite miners in Cumberland, England, but this excess risk could also be attributed to ionizing radiation from radon and radon daughters present in the atmosphere of the mine (Boyd *et al.*, 1970). Excess lung cancer mortality has also been observed in iron-ore miners from a number of other countries. Raised levels of ionizing radiation were found in the iron ore mines of Kiruna, Sweden, but measurements have not been made in all mines where a lung cancer hazard has been shown to exist. An increased mortality risk from lung cancer in iron and steel foundry workers has been attributed to exposure to polycyclic hydrocarbons. Exposure to high levels of iron oxide in the production of sulfuric acid from iron pyrites showed no excess mortality from cancer at any site (Axelson and Sjoberg, 1979).

On the basis of the experimental animal studies and on what they termed suggestible evidence in man, the IARC (1976) classified iron-dextran as "probably carcinogenic for humans." In regard to occupational exposure, the IARC concluded on the basis of epidemiological evidence that underground hematite mining does increase the risk of lung cancer in man, but that the evidence is inadequate to classify hematite as the carcinogen. A number of confounding factors have to be taken into account before the role of iron in human carcinogenesis can be adequately assessed. Again the reader is referred to the mechanism chapter in this handbook.

1.9 Manganese

In a single experiment, rats were given intramuscularly pure manganese powder, manganese dioxide, or manganous acetylacetonate suspended in trioctanoin in multiple treatments. Although no difference in tumor incidence between treated and control animals was found with manganese powder and manganese dioxide, a significant number of fibrosarcomas developed at the injection site in the rats given manganous acetylacetonate, with a mean latent interval of 17 months (Furst, 1978). Enhanced viral transformation of hamster embryo cells was obtained with manganese chloride (Casto *et al.*, 1979), and this compound also

decreased the fidelity of DNA synthesis *in vitro* (Sirover and Loeb, 1976b). When administered intranasally in rats, manganese is effectively taken up in the central nervous system (CNS) through the olfactory system (Henriksson and Tjalve, 2000). Studies have indicated that the astrocytes are the initial targets of manganese toxicity in the CNS and dysfunction of astrocytes possibly involved in neurotoxic action of manganese (Chen and Liao, 2002).

There are no clinical reports implicating manganese as a human carcinogen, and no epidemiological studies that have attempted to relate manganese exposure to cancer.

1.10 Platinum

The chance finding that certain coordination complexes of platinum have the property of inhibiting cell division in *E. coli* led to intense investigation of this group of platinum compounds. *cis*-Dichlorodiammine platinum(II) (*cis*-DDP) is a potent chemotherapeutic agent used in the management of, in particular, metastatic testicular and ovarian tumors. In a well-controlled study, DDP and analogues have been shown to significantly increase tumor frequency in rats and mice (Leopold *et al.*, 1979). Administered intraperitoneally in mice, DDP increased adenoma frequency, and together with topical applications of croton oil, produced skin papillomas and a few epitheliomas. Rats treated with *cis*-dichlorobiscycIopentylamine platinum (II) or *cis*-dichlorobispyrolidine platinum (II) developed a small number of injection-site and distant sarcomas. There were no tumors in the control animals. DDP and its analogues have been shown to be mutagenic in bacterial test systems without microsomal activation. DDP forms both intrastrand and interstrand cross-links with human DNA in cell culture (Roberts and Pascoe, 1972). The effects of DDP on chromosomes are described earlier in this chapter. Morphological transformation of hamster embryo cells and enhancement of viral transformation was produced with low concentrations of platinum, which together with antimony, arsenic, cadmium, and chromium, showed the highest activity in this respect (Casto *et al.*, 1979). Thus, these platinum complexes fit the model for electrophilic reactants binding to cellular nucleophiles, which are direct-acting mutagens and are also capable of cancer initiation. *Cis*-DDP is classified as probably carcinogenic to humans (group 2A) according to IARC (1987).

There are no reports of cancer related to occupational exposure to platinum compounds or of epidemiological studies of cancer mortality in platinum workers. However, because of the high risk of sensitization to soluble platinum compounds in the working environment, exposure has been consistently maintained at very low levels, making a cancer risk unlikely.

1.11 Titanium

In a long-term survival study, fibrosarcomas and lymphosarcomas were observed in a small number of rats injected intramuscularly with fine titanium metal powder suspended in trioctanoin. Tumors were observed with powdered nickel and powdered chromium metal but not with powdered copper or iron administered in a similar way. The organic compound titanocene, dichlorodicyclopentadienyl titanium, administered intramuscularly in rats and mice in a similar way also gave rise to injection-site fibrosarcoma, and a few animals developed hepatoma and malignant lymphoma, but details were not given (Furst and Haro, 1969).

Although titanium compounds such as titanium dioxide (TiO_2) are generally regarded as nontoxic mild pulmonary irritants, some studies have reported lung adenomas in rats exposed to high levels of TiO_2. As for human studies, a retrospective cohort mortality study was conducted among 4241 TiO_2 workers who were employed for at least 6 months, on or after January 1, 1960, at four TiO_2 plants in the United States. The result indicated that the exposures at these plants were not associated with increases in the risk of death from cancer or other diseases. Moreover, workers with likely higher levels of TiO_2 exposure had similar mortality patterns to those with less exposure, because internal analyses among workers revealed no increase in mortality by level of TiO_2 exposure (Fryzek *et al.*, 2003). A recent human study also did not suggest a carcinogenic effect of TiO_2 dust on the human lung (Boffetta *et al.*, 2004).

2 PRINCIPAL METALS SHOWING MUTAGENIC EFFECTS

Most of the toxic metals have very poor direct interactions with DNA and, in general, are weakly mutagenic. Even chromate, which can yield trivalent chromium DNA adducts, is not highly mutagenic in many systems. However, it is of interest that carcinogenic metals can interact with other environmental carcinogens. For example, metals can inhibit the repair of benzo(a)pyrene or UV-induced DNA adducts and potentiate the mutagenicity of benzo(a)pyrene or UV (Hartwig, 1998; Hu *et al.*, 2004a; 2004b). It is also of interest to note that there are poor animal models for metal-induced tumor. Many of the cancers that arise in humans occupationally exposed to carcinogenic

metals, such as nickel compounds and chromate, are associated with exposure to other environmental carcinogens such as cigarette smoking (Gibb *et al.*, 2000; Grimsrud *et al.*, 2002b; 2003). Recent success in inducing cancers with arsenite and chromate in mice was associated with the use of hairless mice exposed to both UV and these carcinogenic metals through their drinking water (Davidson *et al.*, 2004; Rossman *et al.*, 2001; 2004). Thus, in general, carcinogenic metals have strong interactions with carcinogens, such as PAH, UV, and the many carcinogens that are present in cigarette smoke. Some carcinogenic metals such as nickel compounds are able to induce cancers in animals exposed to these metals alone, although nickel also has a strong interaction with other organic carcinogens such as benzo(a)pyrene and UV (Schwerdtle *et al.*, 2002; Waalkes *et al.*, 2004; Wozniak and Blasiak, 2004).

2.1 Nickel

Nickel compounds are not very mutagenic in most of the tested assay systems (Coogan *et al.*, 1989; Costa, 1993b; 2002). However, some studies have shown that nickel compounds induce oxidative stress, genomic instability, and chromosome damage (Biggart and Costa, 1986; Conway and Costa, 1989; Costa, 2002; Kargacin *et al.*, 1993; Kasprzak *et al.*, 2003; Lin *et al.*, 1991; Sen and Costa, 1985). The Landolph laboratory has also shown that nickel compounds induce morphological cell transformation, but no mutation to ouabain resistance in C3H/10T1/2 cells (Miura *et al.*, 1989). Perhaps one of the most important effects of nickel in terms of effecting DNA is its ability to silence genes through DNA methylation and other epigenetic effects (Klein and Costa, 1997; Klein *et al.*, 1991). The epigenetic effects of nickel compounds are more potent with the water-insoluble forms of nickel compounds such as Ni_3S_2, but this effect is also evident when cells are exposed to soluble nickel compounds for long periods of time (Lee *et al.*, 1995). Nickel compounds can also induce oxidative stress *in vivo* (Coogan *et al.*, 1989; Doreswamy *et al.*, 2004; Kasprzak, 1995).

2.2 Chromium

Water-soluble chromate (chromium VI) compounds were tested for cell transformation, mutagenicity, and chromosomal damage and were found to be very active (Bianchi *et al.*, 1983). Analysis of the nature of mutations in a 104-base pair segment of the HPGRT gene exon 3 showed selectivity in substitutions for GC base pairs (Chen and Thilly, 1994). Treatment of a shuttle plasmid *in vitro* with a chromium (VI) reduction system with glutathione or cells containing the shuttle plasmid yielded mutations predominantly at GC sites (Liu *et al.*, 1999). Chromium (VI) is known to have a preference for binding to the phosphate backbone of DNA and with positively charged N7 of guanine (Huang *et al.*, 1995; Voitkun *et al.*, 1998; Zhitkovich, 2005). Chromium (VI) is also synergistic with other organic carcinogens, such as those present in cigarette smoke. One possible mechanism for these effects involves the inhibition of the repair of benzo(a)pyrene DNA adducts (Feng *et al.*, 2003; Hu *et al.*, 2004a). Chromate exposure has been shown to induce oxidative stress in cells, and these effects are greater with some insoluble chromium compounds (Leonard *et al.*, 2004; Martin *et al.*, 1998; Shi and Dalal, 1994). The oxidative stress, the reduction of chromium (VI) to chromium (III), and the binding of chromium (III) to DNA are thought to be involved in its mutagenesis and genotoxicity. The binding of chromium (III) to the DNA can yield a wide variety of DNA lesions, including ternary complexes of chromium (III) with its reducer (ascorbic acid or GSH), as well as DNA–DNA crosslinks, DNA–protein crosslinks, single-strand breaks, and formation of alkaline labile sites (Coogan *et al.*, 1991; Costa, 1993a; Huang *et al.*, 1995; Zhitkovich, 2005). It has been shown that lead chromate induced morphological and neoplastic transformation in C3H/10T1/2 Cl 8 mouse embryo cells, and chromium (VI) compounds induced mutation to 6-thioguanine resistance in cultured normal human diploid fibroblasts (Biedermann and Landolph, 1990; Patierno *et al.*, 1988).

2.3 Arsenic

In general, arsenic compounds show poor genotoxicity and mutagenicity. Some studies have found evidence of DNA damage by arsenite such as micronuclei formation (Liu and Huang, 1997; Schaumloffel and Gebel, 1998) and DNA protein crosslinks (Gebel, 1998), but others did not find DNA protein crosslink formation by arsenite (Costa *et al.*, 1997). Arsenic has been found not to be mutagenic in bacteria and cultured mammalian cells (Rossman *et al.*, 1980). However, a very consistent effect of arsenic compounds is being a comutagen, possibly by inhibiting the repair of DNA lesions produced by genotoxic agents such as UV (Rossman, 1981a; 1981b; Rossman *et al.*, 1977; 1986). Additional studies that support the comutagenic effects of arsenic are those studies showing that it is also a cocarcinogen (Rossman *et al.*, 2001; 2004). In addition, arsenic has been shown to inhibit the ligation step of DNA repair and thus can interact with many genotoxic insults (Hu *et al.*, 1998).

2.4 Cadmium

In general, cadmium compounds are weakly mutagenic in most assay systems (Filipic and Hei, 2004), but they have been shown to be genotoxic (Yang *et al.*, 2003). The mechanism by which cadmium may be genotoxic is by indirectly inducing oxidative stress in cells as a result of its inhibition of antioxidant enzymes and depletion of antioxidant molecules such as GSH (Stohs *et al.*, 2000). Cadmium has been reported to be very active in inducing apoptosis in a wide variety of cells, because of its induction of oxidative stress (Aydin *et al.*, 2003; Bagchi *et al.*, 2000; Jimi *et al.*, 2004; Poliandri *et al.*, 2003; Shin *et al.*, 2004; Tzirogiannis *et al.*, 2003; Watjen *et al.*, 2002). Cadmium has also been shown to inhibit DNA repair (Buchko *et al.*, 2000; Hartwig *et al.*, 2002a; 2002b; Waisberg *et al.*, 2003; Zharkov and Rosenquist, 2002).

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CHAPTER

11

Immunotoxicology of Metals

PER HULTMAN

ABSTRACT

The present understanding of the effect of metals (including ions and their compounds) on the immune system has evolved recently. These effects include suppression, unspecific stimulation, hypersensitivity, and autoimmunity. Many metals show a paradoxical dose-response relationship with stimulation of certain immune functions at low doses and suppression at high doses. The global immune function is often preserved because of the redundancy and the reserve capacity of the immune system, and clinically relevant effects are uncommon. Clinically relevant hypersensitivity reactions caused by metals are dominated by T-cell–mediated allergic contact dermatitis, mainly in connection with exposure to beryllium, cobalt, chromium, gold, mercury, and nickel. Immediate (type I) hypersensitivity reactions with dominating symptoms from the airways occur infrequently, and then most often with platinum, and rarely with nickel and chromium. Induction of metal-induced autoimmunity with immune-complex deposits is well documented in humans, but the recognized numbers of cases are few. Studies in rodents that used mercury and gold have increased the knowledge of mechanisms in metal-induced autoimmunity. Of special importance is the unravelling of genetic factors as regulating susceptibility to mercury-induced autoimmunity both by interaction with the immune system and metal toxicokinetics. Recently, mercury, lead, and cadmium have been shown to accelerate and/or aggravate development of autoimmunity in spontaneous animal models. The importance of metal exposure for induction and/or acceleration of autoimmunity in humans remains to be studied, and a better understanding of genetic factors will be needed.

1 INTRODUCTION

1.1 Development of the Concept Metal Immunotoxicology

The potential of metals, or more accurately "metallic elements, their ions and their compounds" (Duffus, 2003), to cause widely divergent effects on the body because of an interaction with the immune system is a recently discovered path in the history of metal toxicity. Disease conditions like occupational asthma and dermatitis were associated with exposure to metals such as platinum, chromium, nickel, beryllium, and mercury during the first part of the 20th century or earlier. However, a clear recognition of these conditions as immune mediated had to await the rapidly expanding knowledge in immunology during the 1960s and 1970s. At the same time, animal studies linked metal exposure with immunosuppression, as well as nonspecific immune stimulation (Koller, 1973; Vos, 1977). Metals as a mean of inducing autoimmunity began to be explored in the late 1970s. The state of the art was summarized at the International Symposium on the Immunotoxicity of Metals and Immunotoxicology held in Hanover in 1989 (Dayan *et al.*, 1990). More recently a number of reviews on the effect of metals on the immune system have appeared (Enestrom and Hultman, 1995; Kimber and Selgrade, 1998; Martin, 2004; Schuppe *et al*., 1998; Templeton, 2004; Zelikoff and Thomas, 1998).

1.2 Overview of Mechanisms in Immunotoxicology

Like all xenobiotics, metals (and their metabolites) may have a direct or indirect action on the immune system. This might take the form of morphological damage to immunologically active tissues such as the thymus, lymph nodes, spleen, and myelopoiesis (bone marrow and peripheral blood), which might be grossly or microscopically evident. Further studies may show these morphological alterations to be due to abnormalities in cell proliferation, necrotic and/or apoptotic cell death, causing, for example, alterations in populations of immunocompetent cells such as T and B cells and dendritic cells. Subpopulations such as T-helper type 1 and type 2 cells may also be altered by metals, disturbing the balance in finely tuned immune reactions. The functional effect of these morphological alterations may be assessed by measuring a number of endpoints related to innate, humoral, or cell-mediated immunity *in vitro* and *in vivo* using experimental animals or in humans. This forms the basis for defining a major and a minor effect of metals (and other xenobiotics) on the immune system, *immunosuppression* (Section 2) and *unspecific immune stimulation* (Section 8), respectively. However, metals may also interact with the immune system by causing an immunologically based host response to the metal (and/or its metabolites), which is exaggerated and/or inappropriate in relation to the situation, and leads to structural and/or functional damage to the tissues. These reactions include specific T and/or B cells, confer a memory to the immune system, and are defined as *hypersensitivity reactions* (Section 4). When the immune reaction is directed against self-antigens, *autoimmunity* (Section 9) ensues. The mechanisms causing autoimmune reactions are, in general, not well known, but metals may alter self-proteins both directly and indirectly (Sections 6 and 7). A common, final pathway may be exposure of parts of self-proteins not previously presented to the immune system, cryptic epitopes, which are, therefore, identified as non-self and may elicit an autoimmune reaction.

1.3 Dose-Response Considerations in Metal Immunotoxicology

Dose-effect and dose-response relationships are fundamental principles in metal toxicology (Chapter 6). Non-immune mediated toxicity is normally characterized by an increasing proportion of the exposed showing a specific toxic effect with increasing dose and a threshold below which there is no dose-related response. Immune-mediated toxicity shows many exceptions from these basic rules. For example, a low dose may *enhance* a specific immune function, a middle-range dose may have no effect, whereas a high dose causes *immunosuppression*. This type of dose-response curve has been observed for arsenic (McCabe *et al.*, 1983), cadmium (Marth *et al.*, 2000), chromium (Borella *et al.*, 1990), lead (Descotes, 1999), and selenium (Descotes, 1999; Kiremidjian-Schumacher and Stotzky, 1987). Furthermore, a specific immunological endpoint assessed may often be substantially reduced without affecting the ability of the host to sustain an immunological challenge, which demonstrates the *reserve capacity* of the immune system. This might, in turn, be due to a reserve capacity of the specific immunological endpoint, or to *redundancy*. Redundancy means that additional immunological functions are either present or induced, compensating for the reduction in the single endpoint measured. These observations are all accountable for by the fact that the immune system is a dynamic, integrated, interdependent system that is capable of reacting to changing circumstances. Furthermore, the timing between exposure to a metal and the challenge to which the immune system is confronted, such as an antigen or an infection, may affect the outcome (Laschi-Loquerie *et al.*, 1987).

Dose-response relationships are even more complicated in hypersensitivity reactions, where an initial phase of sensitization is necessary for the elicitation (effector) reaction to take place later on. Sensitization will only occur above a certain threshold dose of the metal. The dose required for sensitization may depend on the route of exposure. Furthermore, the ability to be sensitized depends on genetic factors, as demonstrated by using different guinea pig strains for contact sensitization (Polak, 1983). More recent observations in humans relate to hypersensitivity reactions occurring after exposure to gold (Section 5.4) or beryllium (Section 5.1). Once sensitization has taken place, the elicitation of a hypersensitivity reaction on repeated exposure to the metal requires a higher dose than sensitization. The dose-response curve for hypersensitivity reactions is often bell shaped, which means that a threshold dose exists, but also that a high dose might inhibit the reaction.

Finally, experimental studies have with few exceptions dealt with exposure to a single metal, despite the fact that the common environmental human exposure to metals always involves many metals, and simultaneous exposure to a set of metals is also very common in occupational settings.

2 IMMUNOSUPPRESSION INDUCED BY METALS

2.1 General Considerations

Immunosuppression is a reduction in the normal immune function, irrespective of the magnitude. This is uncomplicated for xenobiotics such as cytotoxic drugs that cause a severe reduction in immune functions. However, many metals (and other xenobiotics) cause only a mild-to-moderate reduction of the immune function, and the direct consequences for the host are usually marginal and self-limited. *Immunodepression* has been proposed as a term for such mild-to-moderate suppression of the immune function (Descotes, 1999). This concept is potentially very useful for metals but would necessitate an agreement on how to define immunodepression and immunosuppression, including dose considerations. Because such an agreement is lacking, the term immunodepression will not be used in the following text, although the concept is acknowledged.

2.2 *In Vitro* Studies

A large number of *in vitro* studies have demonstrated that metals may suppress the function of all immune cells (Lawrence, 1981), including T (Colombo *et al.,* 2004; Mattingly *et al.,* 2001; Shen *et al.,* 2001; Shenker *et al.,* 1992) and B (Gallagher *et al.,* 1995; Shenker *et al.,* 1993) cells, as well as monocytes/macrophages (Sakurai *et al.,* 2004; Wataha *et al.,* 2000). *In vitro* observations are critically dependent on the metal concentration, the cell type(s), the duration, and exact conditions of the culture, including the oxygen pressure and the oxidative status, as well as the strain and species from which the cells are derived. As a consequence, contradictory results have often been reported, and *in vitro* studies should be treated cautiously and as an indication of possible *in vivo* effects only.

2.3 *In Vivo* Studies

In vivo studies using animal models have established the ability of metals to act as immunosuppressants on various functions on the immune system in the intact organism. This is especially true for organotins (Battafarano *et al.,* 1998; Smialowicz *et al.,* 1990; Vos *et al.,* 1984), arsenic (Burns *et al.,* 1991), cadmium (Borgman *et al.,* 1986; Fujimaki *et al.,* 1983; Koller *et al.,* 1975; Krzystyniak *et al.,* 1987), lead (Lawrence, 1981; McCabe *et al.,* 1999), inorganic as well as organic mercury (Abedi-Valugerdi *et al.*, 1997; Christensen *et al.,* 1996; Ellermann-Eriksen *et al.,* 1994), and nickel (Dogra *et al.,* 1999; Harkin *et al.,* 2003; Smialowicz *et al.,* 1987). A special effect of some metals is skewing of the T-helper cell response toward type 1 or type 2. This was first observed for zinc as an adverse effect on Th2 cells (Prasad, 2000). A skewing favoring a Th2 type of response has been observed during lead and mercury exposure (Heo *et al.,* 1997). The latter finding correlates with a decreased resistance to intracellular pathogens after exposure to mercury (Bagenstose *et al.,* 2001; Silbergeld *et al.,* 2000) and lead (Dyatlov and Lawrence, 2002), which concurs with the observation that a Th1 response is needed to efficiently handle intracellular pathogens (Igietseme *et al.,* 2004).

2.4 Experimental Host-Resistance Challenge Systems

The perceived genuine functions of the immune system are to protect the host from pathogenic agents such as microorganism (including bacteria, viruses, parasites, and fungi); to suppress development of neoplastic cells and eliminate such cells; and finally to eliminate or at least contain other non-self components, for example, crystalline material like silica. The mere observation of a significant effect of a metal on a certain immune parameter *in vivo* should, however, not be interpreted as a significant immunosuppression. The reason is the redundancy and the reserve capacity of the immune system (Section 1.3). Redundancy is the multiplication of crucial mechanisms in the immune system: if a specific function is affected, other mechanisms may carry out the jeopardized function. Reserve capacity means that the integrated immune system is capable of carrying out its genuine functions although certain immune parameters are suppressed.

To better assess the functional importance of the immunosuppressive effects observed for several metals *in vitro* and in *vivo*, a number of experimental infectivity and neoplastic models—host resistance challenge models—have been developed. Perhaps the best-examined metals in this respect is lead, which causes enhanced susceptibility whether the challenge is with virus (Gainer, 1974; Gupta *et al.,* 2002; Youssef *et al.,* 1996), bacteria (Cook *et al.,* 1975; Dyatlov and Lawrence, 2002; Hemphill *et al.,* 1971), or tumors (Kerkvliet and Baecher-Steppan, 1982; Kobayashi and Okamoto, 1974). Another example is mercury, which causes reduced resistance to viruses (Ilback *et al.,* 1996), bacteria (Koller, 1975), and parasites (Bagenstose *et al.,* 2001; Silbergeld *et al.,* 2000).

2.5 Clinical Immunosuppressive Effects

Despite the evidence that certain metals have a significant effect on the ability of the immune system

to carry out its genuine functions in animal models, reports showing a significant clinical effect of metal exposure on the immune function in humans are relatively uncommon and often of uncertain significance. One explanation might be that early animal studies often used high doses that may never or only very rarely be relevant for the human situation. Even when more realistic doses were used in the animal studies, the reserve capacity and redundancy of the human immune system compared with animals (Cunningham, 2002) might prevent any clinical consequence. However, concurrent conditions like malnutrition or chronic infections may theoretically narrow the reserve capacity, allowing metals to have a clinical immunosuppressive effect that would not have developed in otherwise healthy individuals. However, data on this subject are not available.

3 ESSENTIAL METALS AND THE IMMUNE SYSTEM

The elements zinc, iron, copper, selenium, chromium, and cobalt are essential metals for the human body and the immune system. There is ample evidence that Fe deficiency comprises humoral immunity in both humans (Feng *et al.,* 1994; MacDougall and Jacobs, 1978) and animals (Kochanowski and Sherman, 1985), possibly because of the need for iron in early events associated with cell activation, including generation of second messengers (Kuvibidila *et al.*, 1999). In contrast, excess iron suppresses innate and cell-mediated immunity (Bowlus, 2003). Zn deficiency and the following increased corticosteroid levels cause apoptotic loss of precursor B and T cells in mice, leading to thymic atrophy and lymphopenia (Fraker and King, 2004). Zn deficiency also seems to be of clinical relevance in humans (Black, 2003). Excess of Zn has resulted in divergent effects on the immune function, including some reports of immunostimulation in humans. Copper deficiency is immunosuppressive and reduces IL-2 and T-cell proliferation (Percival, 1998), whereas an excess of copper seems to be of little importance for the immune system. Selenium has a beneficial effect on the immune function, strengthening primarily the cell-mediated immunity by upregulating the expression of the T-cell high-affinity IL-2 receptor (Kiremidjian-Schumacher and Stotzky, 1987) and, as a consequence, also stimulating antibody production (Hawkes *et al.,* 2001). Chromium (Anderson, 2000) and cobalt (Barceloux, 1999) are essentials metals but may cause suppression of T cells (Faleiro *et al.*, 1996) and B cells (Wang *et al.,* 1997), as well as hypersensitivity (Sections 5.2 and 5.3).

4 HYPERSENSITIVITY INDUCED BY METALS

4.1 General Considerations

Several metals may interact with the immune system causing a specific immune activation that damages the tissues of the body; the response is exaggerated and/or inappropriate in relation to the situation. The mechanisms involved in these hypersensitivity reactions were classified as type I–IV by Coombs and Gell in 1968. Although this classification has been criticized for not taking into account recent progress in the understanding of the immune system and reactions to xenobiotica, especially drugs (Descotes and Choquet-Kastylevsky, 2001), it is still widely used. However, it does not indicate the involvement of T cells in the induction of hypersensitivity reactions described as antibody-mediated in the classification (type II and III). Furthermore, it does not take into account so-called pseudoallergic (anaphylactoid) reactions, where xenobiotics activate complement or release mediators such as histamine without involvement of antibodies. Finally, a xenobiotic may well cause hypersensitivity by more than one mechanism in the individual patient and over time (Descotes and Choquet-Kastylevsky, 2001). Keeping these important comments in mind, the following description will follow the traditional Coombs and Gell classification.

4.2 Type I Hypersensitivity (Anaphylactic or Immediate Hypersensitivity)

In IgE-mediated hypersensitivity, the immune system has become sensitized to certain substances—allergens—and will on renewed exposure to these substances elicit a release reaction from mast cells armed with the allergen-specific IgE antibodies. The release of vasoactive amines (histamines, leukotrienes, platelet-activating factor), together with eosinophilic and neutrophilic chemotactic factors, is responsible for the clinical manifestations such as hay fever with rhinitis and conjunctivitis, asthma, and the calamitous systemic anaphylactic reaction. Symptoms of this type have been recognized since the 19th century after exposure to platinum compounds, now recognized to be platinum salts containing reactive halogen ligands (Merget *et al.,* 1988). Induction of asthma by chromium and nickel compounds has also been described (Haines and Nieboer, 1988; Novey *et al.*, 1983).

4.3 Type II Hypersensitivity (Antibody-Mediated—IgG or IgM—Reactions Against Cells or Matrix)

These reactions give rise to cytotoxic depletion of mainly red blood cells, thrombocytes, and granulocytes by means of complement activation. The type II reaction is associated with hemolytic anemia and thrombocytopenia during exposure to gold (Panayi, 1990). Development of autoantibodies to basement membrane components in rats after Hg exposure (Sapin *et al.*, 1977) also belongs to this type of hypersensitivity.

4.4 Type III Hypersensitivity (Immune-Complex Mediated Reactions)

Combination of antigens and antibodies in the circulation with subsequent deposition of the immune complexes in the tissues leads to complement activation and inflammation. Alternately, the formation of the immune complexes may develop *in situ* in the tissues when either the antigen or the antibody is planted in the tissues separately. Type III reactions are important in all spontaneous, systemic autoimmune diseases (Jancar and Sanchez Crespo, 2005). In connection with metals, type III reactions have mostly been associated with Au (Gunnarsson *et al.*, 2001) and Hg (Oliveira *et al.*, 1987; Tubbs *et al.*, 1982).

4.5 Type IV Hypersensitivity (Cell-Mediated Reactions)

This is by far the most common type of hypersensitivity that has been associated with metals. The sensitization of T cells to metal-modified self-antigens involves antigen-presenting cells (APC), such as dermal dendritic cells and epidermal Langerhans cells, which migrate with the modified antigen to the local lymph nodes, where the antigen is presented to T cells leading to clonal proliferation and maturation with aquisition of skin-homing receptors, cutaneous lymphoid-associated antigen (CLA). This process takes 1–2 weeks. On reexposure to the antigen, the elicitation phase ensues in which antigen-specific effector cells are rapidly produced in the local lymph nodes, and these cells home through their CLA-expression back to the skin. Both $CD4^+$ (mainly type 1) cells and $CD8^+$ cells are now recognized as effector cells, whereas $CD4^+$ type 2, IL-10–producing cells may act as regulatory cells (Kupper and Fuhlbrigge, 2004). The effector cells cause a release of various cytokines, the most important effector cytokine being IFN-γ, but proinflammatory cytokines such as IL-1, GM-CSF, and TNF-α also play a role in initiating a nonspecific cutaneous inflammatory reaction (Watanabe *et al.*, 2002), allergic contact dermatitis. Metals that cause allergic contact dermatitis with some frequency are Be, Co, Cr, Au, Hg, and Ni.

4.6 Relation Between Atopy and Metal Hypersensitivity

The relation between atopy, a condition with inherited predisposition to develop IgE-mediated hypersensitivity to small quantities of inhaled or ingested environmental agents, and metal immunotoxicology was discussed at the workshop of Immunotoxicity of Metals and Immunotoxicology in 1989 (Cattaneo, 1990). Although case reports of type I hypersensitivity caused by sensitization to chromium (Section 5.2), cobalt (Section 5.3), nickel (Section 5.6), and platinum (Section 5.7) have been published, only platinum allergy is sufficiently frequent to be assessed for the influence of atopy. A recent review found no association between atopy and type I allergy to platinum salts (Merget *et al.*, 2003). With regard to metal-induced type IV allergy, allergic contact dermatitis, no increased predisposition was reported as a result of atopy at the workshop in 1989 (Cattaneo, 1990). No conclusive new information has been published since then. For example, with regard to the most frequent clinical relevant sensitizing metal in the population, nickel, a few studies have indicated an increased incidence of allergic contact dermatitis in atopics (Dotterud and Falk, 1994), whereas other studies have not (McDonagh *et al.*, 1992). Recent studies have tried to discern different immune responses to nickel in atopic and nonatopic patients using *in vitro* techniques; in one study there were no differences (Szepietowski *et al.*, 1997), but another study reported an impaired immune response to nickel in atopics (Buchvald and Lundeberg, 2004).

5 METALS CAUSING HYPERSENSITIVITY REACTIONS

5.1 Beryllium

Beryllium (Be) is a durable and light metal that is widely used in a number of different industries. It has been recognized since the 1940s that a minority (2–16%) of workers exposed to beryllium, the exact fraction depending on the chemical type of Be, the dose, and the genotype of the exposed population, will develop berylliosis, a chronic granulomatous lung disease (Fontenot and Kotzin, 2003). A mutation of the gene for the human leukocyte antigen (HLA)-DPB1, with the amino acid glutamine in position 69, is found in 80–90% of patients with berylliosis and is

known to be directly involved in the presentation of Be to T cells (Lombardi *et al.,* 2001). Therefore, the DNA sequence coding for the HLA-DPB1 amino acid residue Glu69 is not only a supratypic marker but also the actual immune response gene of berylliosis. Patients with berylliosis exhibit a positive blood T-cell proliferative test to soluble Be. However, many more workers exposed to Be exhibit a T-cell response to soluble Be compared with the number of workers affected with berylliosis, but T-cell test-negative individuals seem to be resistant to development of berylliosis on prolonged exposure (Barna *et al.,* 2003). Recent studies have linked markers such as HLA-DRArg74 (HLA-DR3) to sensitization to Be, but not to berylliosis. The marker might be linked to low IFN-γ production, and IFN-γ is of prime importance for development of granulomatous inflammations. In addition, both sensitization to Be and berylliosis is associated with a gene for the cytokine TNF-α, the TNF-α-308*2 marker (Saltini *et al.,* 2001). These observations are in agreement with the concept that berylliosis is a cell-mediated, type IV immune reaction, with activation of $CD4^+$ type 1 cells recognizing Be when presented by a mutated MHC class II allele, leading to clonal proliferation of T cells, followed by release of cytokines, which leads to a granulomatous, destructive inflammation in the lung tissue.

5.2 Chromium

Chromium (Cr) is a widely spread metal with diverse use ranging from steel manufacturing to cement and fabrication of paints. Cr is an essential nutrient, enhancing the effect of insulin (Anderson, 2000), but it is also carcinogenic (Rowbotham *et al.,* 2000) and may cause hypersensitivity reactions (Shrivastava *et al.,* 2002). Cr is the second most frequent sensitizer in man, being the most common among males because of work-related exposure. The chemical form of immunotoxicological interest is Cr(VI), which is rapidly absorbed through the skin and penetrates the cell membrane, quickly reduced to Cr(III) by sulfhydryl group containing cysteine or methionine moieties, and finally binds to proteins. The Cr(III)–protein antigen complex is presented by APC to T cells in the lymph nodes, and memory as well as effector T cells are formed, but only memory cells remain in the immune repertoire of the host. On renewed exposure to Cr, a secondary response consisting of activation of the T-memory cells and elicitation of the inflammatory reaction by lymphokines takes place, a typical type IV reaction (Thomas *et al.,* 2000) leading to allergic contact dermatitis. Experimental studies have shown that among two inbred guinea pig strains, $K_2Cr_2O_7$ caused sensitization in 83% of the animals in one of the strains and in none of the animals in the other strain (Polak, 1983), indicating the importance of the genotype for sensitization to Cr. However, no information on genetic susceptibility is available in humans. There are some reports of asthma in relation to Cr exposure, which may be type I (Novey *et al.,* 1983) and type IV (Moller *et al.,* 1986) reactions.

5.3 Cobalt

Cobalt (Co) is an essential element necessary for production of vitamin B_{12} (Barceloux, 1999). Two valence states exist: Co(II) and Co(III); the former is often used in the chemical industry, whereas metallic Co is used in the metal industry to produce alloys, especially "hard metals" (Barceloux, 1999). Immunological reactions to Co include sensitization, which is quite unusual in the common population (<1%) but may occur concomitantly with sensitization to Ni in women and Cr in men. The most common hypersensitivity reaction to Co is allergic contact dermatitis, a type IV reaction (Garner, 2004), although occupational asthma with increased serum IgE antibodies to Co indicative of a type I reaction has also been reported (Shirakawa and Morimoto, 1997).

5.4 Gold

Gold (Au) is the most frequent sensitizer in the common population (15%), although clinically relevant hypersensitivity reactions are uncommon (0.5%) (Bruze *et al.,* 1994). A positive patch test to Au is significantly more frequent in patients with gold in dental restorations. Although the main manifestations of Au hypersensitivity after skin sensitization is allergic contact dermatitis, oral lesions such as lichenoid reactions and "burning mouth syndrome" have also been reported. The oral and dermatological conditions may coexist. Treatment with Au salts, especially sodium aurothiomalate, for inflammatory arthritides has been used for at least 70 years and with substantial effect in many patients. However, side effects are common, causing 15–45% of the patients to stop treatment. The side effects usually develop during the first 6 months of treatment and include skin rash, blood dyscrasia, mouth ulcers, proteinuria, colitis, hepatitis, and pneumonitis (Panayi, 1990). Although a genotype causing slow sulfoxidation increases the risk for toxic side effects nine-fold (Matzubara and Ziff, 1987), the adverse effects are mediated by immune-mediated hypersensitivity reactions. Thrombocytopenia is caused by antiplatelet antibodies similar to those in spontaneous autoimmune thrombocytopenia (Greinacher *et al.,* 2001) and represents a type II hypersensitivity reaction. The

immune-complex–mediated (type III) membranous glomerulonephritis (GN) (Gunnarsson *et al.*, 2001) that develops after Au treatment is linked to HLA-DR3, like the thrombocytopenia. The gold-induced GN virtually always resolves or improves considerably when treatment with gold is stopped. Sodium aurothiomalate injections in mice with certain mouse MHC class II (H-2) haplotypes induces, like Hg and Ag, antinucleolar antibodies (Robinson *et al.*, 1986; Schuhmann *et al.*, 1990), but not the immune-complex GN seen after Hg treatment in the same strains (Hultman and Pollard, unpublished observations). The development of Au-induced autoantibodies is delayed for 2–3 weeks compared with mercury and silver (Robinson *et al.*, 1986), which has been attributed to the need for oxidation of Au(I) to Au(III), which is the immunological active Au ion (Schuhmann *et al.*, 1990).

5.5 Mercury

Hg is a well-known inductor of allergic contact dermatitis, which was first recognized at the end of the 19th century (Boyd *et al.*, 2000). Heavy proteinuria, usually a nephrotic syndrome, has been described during Hg exposure since the 19th century. In modern times, Kazantzis *et al*. (1962) described four cases of occupationally mercury-exposed workers with a nephrotic syndrome among a cohort of similarly exposed individuals. Stewart *et al*. (1977) described proteinuria in 9 of 21 technicians occupationally exposed to mercury after spillage of corrosive sublimate and with a wide excretion rate of Hg. In these case reports, the heavy proteinuria dominated by albumin suggests a glomerular damage in contrast to the more modest, low-molecular weight proteinuria associated with the tubulotoxic effect of Hg. A number of case reports have described an IC-mediated membranous GN after occupational exposure to Hg, after use of skin-lightening creams and laxatives containing Hg, as well as other routes of Hg exposure (Barr, 1990; Charpentier *et al.*, 1981; Kibukamusoke *et al.*, 1974; Lagrue *et al.*, 1979; Oliveira *et al.*, 1987; Pairon *et al.*, 1992; Stewart *et al.*, 1977; Tubbs *et al.*, 1982). Exposure to Hg in adults has also been associated with systemic illness, including antinuclear antibodies (Schrallhammer-Benkler *et al.*, 1992), fulfilling criteria for undifferentiated connective tissue disease (Röger *et al.*, 1992).

Acrodynia (pink disease), described already in the 19th century, is restricted to a subset of infants and young children exposed to inorganic or organic mercury compounds in different ways but for prolonged periods (Clarkson, 1990). The patients show anorexia, irritability, photophobia, and exhibit painful, puffy, pink hands and soles with hyperkinetic circulation (Boyd *et al.*, 2000; Weinstein and Bernstein, 2003). Orlowski and Mercer (1980) emphasized the marked individual susceptibility to mercury in acrodynia and suggested that the mucocutaneous lymph node syndrome (Kawasaki's disease) might also be caused by Hg, on the basis of the similar clinical picture and increased exposure to Hg, but this view has later been disputed (Aschner and Aschner, 1989).

5.6 Nickel

Nickel (Ni) has been associated with type IV and to some extent type I hypersensitivity reactions. Ni is the most common skin sensitizer in the population, and totally dominating among women. This might be due to the prevalence of Ni-containing material in the modern environment, including jewelry, buttons, clip, coins, and many alloys, although a number of measures have recently been instated in many countries to lower the exposure. Ni dermatitis is an allergic contact dermatitis (Section 4.5) with itching, papular erythema beginning at the site of contact with Ni, but may on repeated exposure also occur on other parts of the body. The reaction becomes chronic if exposure cannot be eliminated, which is often difficult and may necessitate not only change of work but also restrictions in everyday lifestyle (Hostynek, 2002). A few cases of Ni-induced asthma believed to be due to type I hypersensitivity have also been reported (van Kampen *et al.*, 2003).

5.7 Multiple Metal Exposure Related to Prosthetic Devices

Joint replacement arthroplasties have reduced morbidity and increased life quality for millions of patients. Metals have been the main material in these prostheses, and they were initially considered biologically inert. However, the development of sensitivity to metals after implantation of prostheses is now well documented in case and group studies (Merritt and Rodrigo, 1996), which is not surprising considering the corrosion of all metals in contact with biological material with subsequent release of soluble and particulate material (Jacobs *et al.*, 1994). The metals of most concern have been chromium, cobalt, and nickel. Patients with metal implants demonstrate 25–60% incidence of metal reactivity compared with 10% in the general population (Hallab *et al.*, 2001). An elevated immune response to metals such as Co and Cr (Hallab *et al.*, 2005), and polyethylene particles has been demonstrated (Toumbis *et al.*, 1997). The periprosthetic tissue in metal (Co, Cr, Ni) containing arthroplasties with early recurrence of pain, joint effusion, and osteolysis showed signs of an ongoing, lymphocyte-dominated immune response (Willert *et al.*, 2005). In conclusion, implantation of devices containing

Co, Cr, and Ni induce metal sensitivity in a large proportion of the patients, but only few develop clinical significant hypersensitivity, which may, however, be an etiological factor for prosthetic failure.

5.8 The Platinum Group of Elements (Palladium, Platinum, Rhodium)

The recent development of automobile catalysts has lead to an increased exposure in the environment and in the manufacturing industries not only to platinum (Pt) but also palladium (Pd) and rhodium (Rh).

Pt is a highly corrosive-resistant, catalytic, and strong metal, which has been used for a long time in various applications, mainly in the form of complex salts, for example, platinum chloride, platinum dichloride, platinum dioxide, and platinum sulfate. Platinum complexes, in the form of platinum (II) compounds, cisplatin and carboplatin, are among the most important chemotherapeutic drugs. Biological reactivity, especially hypersensitivity, is limited to charged compounds that contain reactive ligands, halogenated Pt salts, and the sensitizing potential increases with the number of chlorine atoms. The sodium and potassium salts of hexachlorplatinic (IV) acid and tetrachlorplatinite (II) are the most potent sensitizers (Ravindra *et al.*, 2004). Symptoms of allergic-like reactions such as lacrimation, sneezing, rhinorrhea, cough, dyspnea, and frank asthma in workers exposed to soluble complex platinum salts have been observed since the early 19th century and were called platinosis but have been renamed as "allergy to platinum compounds containing reactive halogen ligands" (Jacobs, 1987). This reaction is now believed to be mainly a type I reaction, although a type IV reaction has been indicated in some patients. A direct interaction with basophilic cells with release of histamine may occur (Parrot *et al.*, 1969), which varies with the coordination number of the salt. It is also possible that Pt compounds by inhibiting Th1 cells may cause a Th2-skewed $CD4^+$ response favoring type I hypersensitivity (Dearman *et al.*, 1997). Metallic palladium (Pd) is a skin sensitizer causing allergic contact dermatitis; more than 90% of subjects with Pd sensitization are also sensitized to Ni (Finch *et al.*, 1999). Other hypersensitivity reactions caused by Pd or Rh are rare (Ravindra *et al.*, 2004).

6 INTERACTION BETWEEN METALS AND PROTEINS

6.1 Introduction

The way metals induce hypersensitivity conditions is beginning to be recognized especially for type IV and type I reactions. The mechanisms involved in, for example, systemic autoimmune diseases are less clear, but T-helper cells (Th-$CD4^+$ cells) are likely to play an important, but probably not exclusive, role in all these reactions.

6.2 Mechanisms of Interaction Between T Cells and Metal Ions

Conceptually T cells, $CD4^+$ and $CD8^+$ cells, will only react with antigens in the form of small peptides presented in context of the groove formed by the major histocompatibility complex (MHC) molecules on the APC. Superficially, the interaction of metals with proteins/peptides has been compared with that of classical haptens such as trinitrophenyl, which are recognized by T cells when associated with peptides. However, the chemically highly reactive haptens interact easily with proteins by a covalent binding (Cavani *et al.*, 1995), whereas metals often require highly defined coordination complexes (Williams, 1990). Metal–peptide interactions have mainly been studied from an inhibitory point of view. The reaction between Ni-specific T-cell clones and peptides was inhibited when the peptide-pulsed APC was treated with Ni. Ni interacted specifically with a histidine residue of the peptide, because the T-cell response was restored by substituting lysine for histidine, and radioactively bound Ni was identified in complex with histidine but not lysine (Romagnoli *et al.*, 1991). Au (I) forms a complex with MHC-binding peptides containing two or more cysteine residues that inhibit subsequent T-cell receptor binding—substitution of the cysteines by serines eliminated the inhibitory effect of Au (I) (Griem *et al.*, 1995).

However, there are reasons to believe that interactions between the T-cell receptor and the peptide–MHC complex may also enhance T-cell recognition. This was studied by presenting Ni^{2+} to HLA-DR4–restricted Ni^{2+}-sensitive T cells, where some DR subtypes stimulated IL-4/IL-5 secreting $CD4^+$Th2 cells by binding to endogenous peptides in the antigen-presenting groove of APC (Emtestam and Olerup, 1996). Ni may also lead to Th2 skewing, because a low density of Ni^{2+}-binding ligands favors Th2 cell activation (Del Prete, 1991).

Binding to the α- or β-chain of the MHC molecule in the groove of the APC has been demonstrated for Au and Ni. Exposure of APC to Au before the peptide inhibited proliferation of MHC class-II–restricted T-cell clones, and radiolabeled Au bound to a panel of MHC class-II positive, but not class-II negative, cell lines (Sinigaglia, 1994). In a more recent study Lu *et al*. (2003) demonstrated the existence of T-cell clones being stimulated by a preformed HLA-DR/Ni complex, in which Ni was bound to His81 on top of the

HLA-DR52c β-chain and probably two other coordination sites on a limited selection of DR52c-associated peptides.

In some studies, the exact way the metal interacts with the peptide-MHC structure to inhibit the reaction has not been explained. For example, Pb inhibits presentation of ovalbumin to the T-cell hybridoma DO11.10, allowing for an effect on either the ovalbumin peptide or the class II molecule (Smith and Lawrence, 1988).

Although these observations have increased the understanding of interactions between T cells and peptides-MHC, they fail to explain why, for example, Ni hypersensitivity is not linked to certain HLA class I or class II alleles (Emtestam *et al.,* 1993). T-cell receptor (TCR) families have, therefore, been intensely studied. In Ni^{2+} sensitization, the phenotype TCRVβ17 dominates the T-cell response *in vitro* (Budinger *et al.,* 2001), and this TCR type is associated with the clinical severity of the Ni^{2+}-induced contact dermatitis (Vollmer *et al.,* 1997). It has been proposed that Ni might enhance short-lived, nonproductive contacts between the TCR and the MHC–peptide complex, sometimes resulting in a sufficiently high avidity for activation of the T cell, a process that may also be facilitated by carriers of Ni such as serum albumin (Thierse *et al.,* 2004). A similar observation has been made for Be, because Vβ3 seems to be required in combination with HLA-DPB1Glu69 to elicit Be-responding $CD4^+$ clones (Fontenot *et al.,* 2001).

7 OTHER INTERACTIONS BETWEEN METALS AND PROTEINS—IMPLICATIONS FOR AUTOIMMUNITY

Apart from the possibility of a direct, but chemically promiscuous, interaction between TCR and metal-peptide-MHC complexes, metals may interact with proteins in other ways to alter presentation of the peptide to T cells. First, metals that possess a high redox potential (oxidizing capacity), such as Au (III), Pd(IV), and Pt(IV), may oxidize and denaturate sulfur-containing amino acid side chains of proteins. Second, metals such as Hg(II), Pt(II), and Au(I) make strong bindings with side chains in proteins, forming stable metal–protein complexes that may alter the protein unfolding taking place in antigen-processing. Third, metals may induce a change in the specificity of the proteolytic profile of APC. The effect of one or more of these mechanisms might be to display a hitherto unrecognized part of peptides, so called cryptic epitopes, which are presented to the T cells instead of the usual immunodominant epitopes. Because the T cells have not been made tolerant to the cryptic epitopes, this might lead to self-reactivity (Griem *et al.,* 1998).

These mechanisms have been studied for Au and Hg. Murine T-cell hybridomas specific for bovine RNase A treated with Au(III) recognize cryptic epitopes of non-Au–treated RNase. These T-cell hybridomas also reacted with RNase A pretreated with Pd(II), Pd(IV), Pt(IV), or Ni(IV), whereas metal ions such as Cu(II), Fe(II), F(III), or Zn(II) failed to elicit any cross-reactivity. The sensitizing potential of Au(III), Pd (II), Pd(IV), Pt(IV), and Ni(IV) may, therefore, be due to their ability to induce presentation of cryptic self-peptides (Griem *et al.,* 1996).

Mercury has been shown to interact with the major autoantigen in Hg-induced experimental systemic autoimmunity, the nucleolar 34-kDa protein fibrillarin. First, by binding to sulfhydryl groups, fibrillarin is physically altered (Pollard *et al.,* 1997). Second, nonapoptotic (necrotic) cell death caused by Hg modifies the proteolytic cleavage pattern for fibrillarin, resulting in neopeptides of fibrillarin (Pollard *et al.,* 2000). Because exposure to Hg creates a 19-kDa immunogenic fragment of fibrillarin even without physical modification of fibrillarin, a new cleavage pattern of fibrillarin seems to be of prime importance for induction of autoimmunity development (Pollard *et al.,* 2000). In another experiment, T-cell clones obtained from genetically Hg-susceptible mice that had been given repeated injections of Hg for 1 week reacted with Hg-carrying self-proteins, including Hg(II)-complexed fibrillarin (Kubicka-Muranyi *et al.,* 1993; 1995). After 8 weeks Hg treatment, the T-cell clones preferentially reacted with untreated fibrillarin, suggesting a shift of the T-cell specificity from self-proteins modified by Hg to unaltered self-proteins. This process is known as a determinant spreading (McCluskey *et al.,* 1998) and makes tracing of the original insult-inducing autoimmunity difficult.

8 NONSPECIFIC IMMUNOSTIMULATION INDUCED BY METALS: THE EXAMPLES OF PB AND HG

Stimulation of cells in the immune system by metals that do not primarily induce antigen-specific reactions or generate metal-specific lymphocytes is considered nonspecific immunostimulation. An example of this is the lead-induced increase of plaque-forming (B-) (Stiller-Winkler *et al.,* 1988) cells and increased expression of class II molecules on B cells (McCabe *et al.,* 1991). Mercury stimulates proliferation of T cells in guinea pigs, rats, and rabbits (Pauly *et al.,* 1969); humans (Carron *et al.,* 1970); and mice (Jiang and Moller, 1995; Pollard and Landberg, 2001; Reardon and Lucas, 1987), although the stimulatory concentration range is very narrow in humans and mice. However, distinct

differences have been reported between the species with regard to the exact cells involved. For example, human (Nordlind and Henze, 1984) but not murine (Pollard and Landberg, 2001; Reardon and Lucas, 1987) thymocytes proliferate *in vitro* in response to mercury. Murine T cells show an absolute requirement for adherent cells (Jiang and Moller, 1995; Pollard and Landberg, 2001), whereas they are not needed (Caron *et al.*, 1970; Nordlind and Henze, 1984), or may even be dementrial (Shenker *et al.*, 1992), for the proliferative effect of Hg on human lymphocytes. Mercury caused after injection a significant proliferation of T cells in 22 of 23 mouse strains as assessed by the popliteal lymph node test (Stiller-Winkler *et al.*, 1988), demonstrating the ability of Hg to cause immune stimulation also *in vivo*. However, the dependency on MHC class II molecules (Hu *et al.*, 1997) and costimulatory molecules, especially IL-1 (Pollard and Landberg, 2001), in combination with the oligoclonal murine T-cell response *in vitro* (Jiang and Moller, 1996) as well as *in vivo* (Heo *et al.*, 1997) makes it possible that the Hg-induced T-cell response is antigen-dependent, although the antigen(s) is (are) unknown. Secondary effects of a polyclonal activation of T cells by mercury are B-cell activation and Ig isotype switching because of cytokines such as IL-4 and IFN-γ.

However, a number of nonantigen-specific proliferative effects of Hg have been reported *in vitro*: increase in intracellular calcium (Tan *et al.*, 1993), aggregation of transmembrane CD4, CD3, CD45, and Thy-1 receptors on T cells with increased tyrosine kinase $p56^{lck}$ (Nakashima *et al.*, 1994), and attenuation of lymphocyte apoptosis (Whitekus *et al.*, 1999) because of interference with the *Fas–Fas* ligand interaction *in vitro* (McCabe *et al.*, 2003).

9 METAL-INDUCED AUTOIMMUNITY

This is a seemingly paradoxical area of metal immunotoxicology. Ample evidence from experimental studies demonstrate that certain metals, most notably Hg and Au, may cause autoimmune diseases in several species (Fournie *et al.*, 2002; Hultman *et al.*, 1995; Pollard *et al.*, 2005; Schuhmann *et al.*, 1990), but there are relatively few reports of metal-induced autoimmunity in humans. Some authors (Descotes, 1999) have, therefore, questioned whether metal-induced autoimmunity is a relevant issue for humans. Some factors must, however, be taken into consideration when trying to assess the potential of metals for inducing autoimmunity.

First, induction of autoimmune diseases is heavily dependent on genetic susceptibility, and neither the susceptible genotype(s) in humans for metal-induced autoimmunity nor the number of humans that may carry such susceptibility is known. Because the genetic susceptibility to mercury-induced autoimmunity in rodents is, to a large extent, determined by MHC class II genes, which are also well known for their association with spontaneous autoimmune diseases in humans, there are strong theoretical reasons to assume the existence of humans genetically susceptible to mercury. Furthermore, genetic factors outside MHC determine the accumulation of Hg in mice given the same dose of Hg (Nielsen and Hultman, 1998), and non-MHC genes also determine the internal dose of Hg required to elicit an autoimmune response in mice with susceptible MHC genes (Hultman and Nielsen, 2001).

The largest cohort of humans exposed to inorganic mercury is dental amalgam bearers. Studies of dental amalgam implanted in the peritoneal cavity of mice (Hultman *et al.*, 1994) and inserted in the teeth of rats (Hultman *et al.*, 1998), have clearly demonstrated the potential of dental amalgam to cause systemic autoimmune diseases in genetically (MHC) susceptible rodents. However, it seems likely that the dose required for *de novo* induction of autoimmunity is not met in most humans with dental amalgam fillings. However, to what extent some dental amalgam bearers may develop *de novo* autoimmunity or acceleration of spontaneous autoimmune diseases because of a genotype causing high accumulation of Hg in the body and/or a low thresholds for induction of autoimmunity is unknown.

Historically, large cohorts have been exposed to mercury in environmental disasters (Bakir *et al.*, 1973; Harada, 1995), but autoimmune conditions have not been reported. This would indicate either that the frequency of such susceptibility genes is low or absent in the affected populations or that the dose was insufficient to elicit a reaction. However, in the Hg disasters, the main exposure was to organic mercury compounds, especially methylmercury but also ethyl mercury. Both these compounds are able to induce systemic autoimmunity in genetically susceptible mice (Havarinasab *et al.*, 2004; Hultman and Hansson-Georgiadis, 1999), although probably because of transformation of the organic compounds to inorganic mercury (Havarinasab and Hultman, 2005), which is, however, incomplete and, at least for MeHg, a rather slow process (Haggqvist *et al.*, 2005; Havarinasab *et al.*, 2004). Therefore, the exposure to autoimmunogenic inorganic Hg might have been too low in many cases to induce autoimmunity. Another explanation for the lack of reports on autoimmune diseases during the environmental Hg disasters might have been a limited attention to autoimmune diseases in those early years and not least that symptoms of autoim-

mune diseases might have been overlooked because of the drastic effects of organic mercury on the nervous system.

10 ACCELERATION AND AGGRAVATION OF AUTOIMMUNITY BY XENOBIOTICS

10.1 General Considerations

Environmental and other agents may induce various phenotypic expressions of autoimmune diseases because of interaction with different genotypes. This includes the possibility of not only *de novo* autoimmune disease but also an acceleration and aggravation of autoimmune conditions with other primary genetic or nongenetic etiologies. For example, in experimental models, the polyclonal B-cell activating agent lipopolysaccharide-lipid A portion (Hang *et al.,* 1983), UV radiation (Ansel *et al.,* 1985), halothane (Lewis *et al.,* 1982), and polyinosinic/polycytidylic acid (Carpenter *et al.,* 1970) accelerate spontaneous autoimmune disease manifestations. For metals, important observations on this subject have recently emerged from studies in rodents.

10.2 Acceleration of Spontaneous Autoimmune Diseases by Hg

Hg is a potent *de novo* inductor of a systemic autoimmune disease condition in rodents with certain MHC (H-2) class II genotypes (Fournie *et al.,* 2002; Pollard and Hultman, 1997). Although these rodent models continue to be very useful for explaining immunological mechanisms in systemic autoimmunity, dose-response studies applied to humans make it likely that *de novo* induction of autoimmunity by these agents will require exposure levels occurring only in specific hazardous work places or during accidental exposure or voluntary ingestion. However, uncertainty prevails regarding the existence and prevalence of especially susceptible genotypes (Chapter 33).

Recently, inorganic Hg was shown to accelerate the spontaneous autoimmune manifestations in the NZBWF1 mouse strain as evidenced by lymphoid hyperplasia (Pollard *et al.,* 1999), polyclonal B-cell activation (al-Balaghi *et al.,* 1996), hyperimmunoglobulinemia and antichromatin antibodies (Pollard *et al.,* 1999), as well as immune-complex deposits (Abedi-Valugerdi *et al.,* 1997; Arnett *et al.,* 1996; Pollard *et al.,* 1999). Hg treatment of the MRL-$^{+/+}$ strain and the autoimmune-prone, *Fas*-deficient MRL-$^{lpr/lpr}$ strain caused severely and slightly accelerated autoimmune manifestations, respectively (Pollard *et al.,* 1999). However, recent studies have shown that the autoimmunity may be severely aggravated also in the MRL-$^{lpr/lpr}$ strain, provided that a lower dose of Hg is administered (Pollard *et al.,* 2005). By use of the AKR strain, which is H-2 congenic with the MRL strains, aggravation of the autoimmune disease was linked to non-MHC genes (Pollard *et al.,* 1999). Studies in the autoimmune-prone BXSB and the nonautoimmune C57BL/6 strains, which share the H-2^{b} haplotype, showed that Hg triggers the *Yaa*-gene dependent lupus-like autoimmune disease in BXSB mice by aggravating lymphoid hyperplasia, antichromatin antibodies, and glomerulonephritis, whereas Hg had little effect on the MHC-congenic C57BL/6J strain, linking the genetic susceptibility for acceleration of autoimmunity by Hg to non-MHC genes also in this disease model (Pollard *et al.,* 2001). Interestingly, a short course of Hg to BXSB mice was sufficient to cause a lifelong increase in the autoimmune response. Furthermore, a dose of Hg that was comparatively lower than the dose accepted in the occupational setting accelerated the spontaneous autoimmune disease in the BXSB mice (Pollard *et al.,* 2001). Acceleration of spontaneous autoimmunity has also been observed in NZBWF1 mice after very low doses of organic Hg (Havarinasab and Hultman, 2006).

Although the preceding observations may give the impression that a sufficient dose of Hg will always accelerate spontaneous autoimmune diseases, we have found (Hultman *et al.,* 2006) that Hg treatment for more than a year in the spontaneous autoimmune (SWRxSJL)F1 mouse model (Vidal *et al.,* 1994) neither accelerated the onset nor increased the severity of the systemic autoimmune manifestations. The proposed explanation is that the SWR strain possesses non-MHC genes that can suppress Hg-induced exacerbation of autoimmunity. Therefore, the effects of autoimmune inductors such as Hg, Ag, and Au need to be examined in all available spontaneous models of autoimmune disease, because a specific interaction takes place between the genetic factors (especially non-MHC genes), the spontaneous autoimmune conditions, and the specific metal.

These studies on the accelerating effect of Hg exposure were all performed in models with a distinct and known genetic predisposition. Recently, Silbergeld *et al*. examined whether an autoimmune disease induced by a primarily nongenetic mechanism might also be accelerated by Hg (Via *et al.,* 2003). A lupus-like chronic graft-versus-host disease (GVHD) was induced using F1-hybrids of two strains resistant to Hg (C57BL/6 and DBA/2) and DBA/2 donor cells. A 2-week exposure to host and donor mice of low-dose Hg (20 μg/kg every other day) ending 1 week before GVHD induction, aggravated the lupus-like GVHD.

10.3 Acceleration of Spontaneous Autoimmune Diseases by Cadmium and Lead

Recent studies using Cd and Pb have expanded the results obtained by Hg. Cd in a high dose increased the ANA in autoimmune-prone NZBWF1 mice during the first months of treatment, whereas a lower dose increased the IgG2a serum level and the proteinuria, although without affecting the IC deposition (Leffel *et al.*, 2003). As mentioned previously, Pb enhances the autoimmune manifestations in autoimmune-prone NZBWF1 hybrid mice (Rudofsky and Lawrence, 1999). A recent study on lead in four New Zealand mixed strains revealed a complex relation between the spontaneous autoimmune disease, Pb exposure, genetics, and gender, leading to different phenotypic outcome: both acceleration and inhibition of the autoimmune condition were observed (Hudson *et al.*, 2003).

10.4 Comments on the Autoimmune Effects of Metals

The ability of metals to accelerate and aggravate genetically, as well as nongenetically, determined autoimmune disease processes in rodents is an important new observation in the immunotoxicology of metals. Although a few of these studies included some dose-response data, the potential importance of these findings for human conditions and the variation in phenotypic expression of the effect of metals warrant further dose-response studies in all autoimmune models. In addition, the mechanism(s) by which the metals are able to accelerate the autoimmune process should be examined, which will also improve the understanding of the general mechanisms in autoimmune diseases.

Therefore, new findings in rodents such as major genetic factors regulating susceptibility to induction of autoimmunity by mercury, silver, and gold; the existence of genetic factors determining uptake and retention of metals, as well as the threshold of metals for elicitation of autoimmunity; and the accelerating/aggravating effect of metals such as mercury, cadmium, and lead on spontaneous autoimmune conditions are all reasons why metals may be of importance for autoimmune conditions in humans. Although case reports in humans are evidence for the ability of metals like mercury and gold to induce autoimmune conditions, determining the importance of metal exposure for human autoimmune disease will require further studies.

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CHAPTER

12

Reproductive and Developmental Toxicity of Metals

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ABSTRACT

Metals exert a wide variety of adverse effects on reproduction and development including influence on fertility, intrauterine growth retardation, abortions, malformations, birth defects, and developmental effects, mainly those on the nervous system. Metals may affect reproduction or development directly, at relatively low doses, or indirectly through systemic toxicity, generally at higher doses. More recent, important mechanisms of action are those related to endocrine disruption and oxidative stress. The effects produced by metals depend on several factors, such as the timing and duration of exposure, their distribution and accumulation in various organs (e.g., the nervous system), and on the ability to interfere with specific developmental processes. Interesting interactions between environmental and congenital factors have been documented; genetic polymorphisms can affect fetal susceptibility to teratogens or maternal ability to detoxify and excrete xenobiotics.

Male reproductive effects of high doses of many metals have been observed in animals, whereas there is still insufficient evidence to the human male concerning a quantitative dose-response relationship. This could be partly explained by considerable interspecies differences in susceptibility to reproductive toxicity and in the route, level, and duration of metal exposure. Human studies have provided substantial evidence of effects for only a few metals. These include lead-induced effects on male fertility and certain parameters of semen quality, even at moderate exposure levels, effects by cadmium on prostate impairment and serum testosterone, and less well-documented male reproductive effects of mercury, manganese, chromium, nickel, and arsenic at levels significantly lower than those that cause systemic toxicity.

The effects of metals on female reproduction may arise from their action on several stages beginning in fetal life, during early development or maturity, and include manifestations such as subfertility, infertility, intrauterine growth retardation, spontaneous abortions, malformations, birth defects, postnatal death, learning and behavior deficits, and premature aging; evidence is usually limited to animal data or studies on fertility and spontaneous abortions The clinical and epidemiological findings related to metal-induced effects on female reproduction are often difficult to interpret, because many other factors may influence the outcome such as age, ovarian reserve, hormonal imbalance, behavior, genetics, male fertility factors, and sexually transmitted diseases.

Exposure to metals during organogenesis may cause fetal anomalies, whereas exposure during other periods of development may result in embryo or fetal lethality or other developmental effects. Teratogenesis bioassays in rodents (hamsters, mice, or rats) have yielded positive results for the compounds of many metals (Al, As, Bo, Cd, Co, Cr, Cu, Ga, Hg, Li, Mn, Ni, Pb, Se, U, V, and Zn), producing fetal and early postnatal deaths, as well as malformations such as anencephaly, eye defects, cleft palate, and skeletal anomalies. The relevance of these findings to human exposure is, however, still unclear.

Neonatal and early postnatal periods are lifespan segments during which sensitivity to metals is high, and lead toxicity on the developing organism represents an illustrative example for related problems and questions. In the last decade, children's blood lead (PbB) levels have declined significantly in a number of countries, and current prevalent mean levels in developed countries are <50 μg/L. Despite this reduction, childhood lead poisoning continues to be a public health problem for certain high-risk groups. Recent studies have extended the association of blood lead and intellectual impairment to PbB levels <100 μg/L and now challenge the safety of this level that for more than a decade has served as a community alert level for preventive action. The recent studies cast doubt whether a toxicological threshold and no-effect value exists for lead-related developmental toxicity. This chapter focuses on the reproductive and developmental effects by metals of traditional interest for toxicologists, such as lead, mercury, and cadmium, but also provides information, where available, on chromium, arsenic, manganese, and nickel, and on metals of more recent interest such as aluminum, platinum, vanadium, lithium, and uranium.

1 INTRODUCTION

Adverse effects of metals on human reproduction and development continue to be a demanding challenge for researchers. It is often difficult to identify the metals involved, or their sources; a wide range of adverse events must be investigated; and there are limited data on the mechanisms of action and damage. For most metals, there is little information about quantitative dose-response relationships and no-adverse-effect exposure thresholds. Furthermore, most published studies have considered effects by a single metal, with few studies addressing combined exposure to toxic and/or essential metals—as is typical for human exposure. The possibility of synergistic or antagonistic interactions of metals and other factors (see also Chapters 6 and 7) should be given much more prominence in the future.

Metals can produce a wide variety of adverse effects on reproduction and development, influencing fertility, intrauterine growth retardation, abortions, malformations, birth defects, and developmental effects, including those on the nervous system. Although considered severe in general, the relevance of these outcomes is, however, differently perceived in various social, economic, and cultural contexts. Abortion or malformations are generally more easily identified, whereas subtle effects on fertility may be less obvious. There is also a worldwide tendency to delay parenthood, thus lengthening the preconception period for potential exposure, with possible effects on both male or female reproductive ability, as well as increasing prenatal and early postnatal cumulative exposure to toxic metals.

The long-term assessment of reproductive and developmental effects of metals is sometimes complicated by reduced exposure to certain metals, resulting from technological improvement and preventive measures, and by the presence of other occupational or environmental factors possibly affecting reproduction. The effective exposure to metals should be evaluated as close as possible to the "target time"—the period during which the metal may be toxicologically most active. The target time may be specific for each animal species, organ, or tissue, kind of effect, and route of exposure.

Metals may affect reproduction or development directly, at relatively low doses, or act indirectly through systemic toxicity, generally at higher doses. The direct toxicity can be identified as a "critical effect" on reproduction or development. With regard to occupational and environmental exposures, in contrast to experimental conditions, it is difficult to correlate specific exposures to specific effects because of the complexity of such exposures and the need to measure metals at very low levels in biological specimens.

In the last years, there has been increasing interest in two mechanisms of action on reproduction and development because of chemical hazards, including some metals, on endocrine disruption, and oxidative stress.

Endocrine disruptors (EDs) have been defined as "exogenous chemical substances or mixtures able to alter the structure or function of the endocrine system and to cause adverse effects on organisms or their progeny" (Medical Research Council, 1995). The importance of EDs is related to the biological hypothesis that low-level exposure to certain chemicals may contribute to endpoints such as lowering of age at menarche, impairment of semen quantity and quality, decreasing male-to-female sex ratio at birth, increasing rates of hypospadias and testicular cancer, infertility, spontaneous abortions, and structural and functional congenital malformations. However, linking specific exposures to effects is often difficult because of multiple exposures, the latency of effects, and the subtle nature of some outcomes (Davis *et al.*, 1998; Kaplowitz *et al.,* 2001; McKiernan *et al.*, 1999). Some effects of EDs may be receptor-mediated and may directly interact with cellular targets (Kavloc *et al.*, 1996). Cadmium, for example, may affect steroidogenesis by mimicking or inhibiting the actions of endogenous estrogens (Henson and Chedrese, 2004). The experimental and human investigations on EDs

were reviewed by Sharpe and Irvine (2004); they concluded that few definitive studies link reproductive disorders with exposure to environmental chemicals; this may either reflect the difficulty in obtaining such data or a genuine lack of effects.

The toxicity of several metals, including iron, copper, cobalt, and lead, may be mediated through oxidant-based and free-radical–based mechanisms, leading to oxidative stress. These metals increase the production of reactive oxygen species, decrease the levels of glutathione and other antioxidants (including selenium and zinc), affect the protective antioxidant enzymes by interfering with the metabolism of the specific metal(s) essential for the enzyme activity (such as Cu, Zn-dependent superoxide dismutase, Se-dependent glutathione peroxidase, Fe-dependent catalase), enhance cell membrane lipid peroxidation, cause apoptosis, and contribute to oxidative DNA damage.

Many factors including animal species and strains, gender, age, stress, inherited disorders, nutritional status, smoking, alcohol consumption, use of medications, and concomitant exposure to other chemicals or physical factors can influence both metabolism and effects, as well as dose-response relationships for reproductive and developmental toxicity of metals.

In risk assessment, the ideal situation combines adequate indicators of exposure with sensitive and specific effect indicators. Indicators of exposure are frequently based on blood concentrations or levels of metals in other body fluids, which may not adequately reflect body burden. New methods for assessing cumulative exposure, such as the application of X-ray fluorescence for measuring metals in bone, have the great potential of increasing the accuracy of risk assessment and evaluation of dose-response relationships. Examples of effect indicators for investigating reproductive damages include the time to pregnancy (TTP) and the sperm chromatin structure assay (SCSA).

TTP is defined as the number of months of unprotected intercourse that elapse before conception occurs. It is a composite measure that includes libido, ovulation, sperm or semen quality, and conceptus survival (Joffe, 1997). Regarding metals for which exposure may decline with time, TTP values could be causally associated with earlier, higher exposures; the resulting bias would overestimate the association of exposure and fertility (Weinberg *et al*., 1994).

The SCSA is a measure of the spermatozoal abnormal chromatin, defined as increased susceptibility to acid-induced denaturation *in situ* and quantified by flow cytometric measurement of denaturated DNA and native DNA (Spano *et al*., 1999). A higher proportion of single-stranded DNA occurs in altered cells. The metachromatic shift from normal to altered cells, expressed as alfaT, represents the ratio of altered to total fluorescence (Evenson *et al*., 2002).

The informative value for both effect and dose indicators is strictly related to the quality of the data. The main requirements of analytical quality assurance are related to the control of all the known sources of variations (Aitio and Apostoli, 1995) and compliance with international clinical and laboratory standards. Good laboratory practices, internal quality assurance, and participation in external quality control programs should be encouraged. In addition, to enhance interpretation of the results of effect indicators, validation studies and dose-response relationships are commonly required.

This chapter focuses on metals of traditional interest for toxicologists, such as lead, mercury, and cadmium, but also provides information, where available, on chromium, arsenic, manganese, and nickel, and on metals of more recent interest such as aluminum, platinum, vanadium, lithium, and uranium.

2 MALE REPRODUCTIVE EFFECTS

Mammalian male reproductive function can be affected through a direct effect on the testis, resulting in decreased or altered sperm production, through impairment of the accessory sex gland secretions, and/or indirectly through the neuroendocrine system, causing hormonal imbalance. Adverse effects on male fertility include altered genetic material of sperm, contributing to altered spermatogenesis, pregnancy loss, or genetic disease in offspring. Common endpoints for assessment of male reproductive function include size of testis, semen quality, secretory function of the prostate and seminal vesicles, reproductive endocrine function, impotence or reduced libido, and fertility. When evaluating reproductive effects of a certain metal on human males, one must take into account possible influences of concomitant exposures to other toxic and essential metals; these may act additively, synergistically, or antagonistically. Moreover, the influence of age and lifestyle factors particularly stress, smoking habits, and alcohol consumption must be considered. In addition, certain toxic metals such as lead and cadmium are pervasive in the human environment and accumulate in the human body over a lifetime; biomarkers of lead and cadmium exposure commonly correlate with age, smoking habits, and/or alcohol consumption. Quantitative assessment of the body status of certain essential elements, especially zinc and selenium, is relevant, because they can reduce or protect from the adverse male reproductive effects of several metals.

Recent evidence indicates that the human male reproductive capacity has deteriorated considerably during the past five decades. In industrialized countries, a substantial number of couples seek *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI) because of poor semen quality.

Furthermore, large differences in mean sperm concentration between countries, and between different locations within a country, have been observed. The possible role of environmental and lifestyle factors in contributing to these trends and differences has been suggested and is widely debated, because consideration must be given to many potentially confounding factors. Because the underlying causes are likely to be multiple and complex, it is important to identify the factors contributing to the deterioration of human male fertility to prevent further decline in fertility potential.

The human male has a relatively low fertility potential compared with other mammals. For example, the number of sperm per human ejaculate is typically only twofold to fourfold higher than the number at which fertility is significantly reduced, whereas the number of sperm in rat, rabbit, or bull ejaculate is many times (up to 1400-fold) the number that will produce maximum fertility (Working, 1988). Human males have markedly smaller relative testis size and the lowest rate of daily sperm production per gram of testis, by a factor of more than 3, compared with mouse, rat, or monkey. The percentages of progressively motile sperm and morphologically normal sperm in human semen are also considerably lower than in experimental animals (Working, 1988). Certain rodent species and strains, commonly used in experimental studies, seem to be resistant to the male reproductive toxicity of lead (Apostoli *et al*., 1998) and cadmium (Gunn *et al*., 1965; Liu *et al.,* 2001). The human male may be more susceptible than the rat to metal toxicity, possibly because of poorer efficacy of the antioxidant defense system and greater vulnerability to oxidative damage to sperm DNA and sulfhydryl (–SH) groups required for the maintenance of sperm maturation and motility. Because of differences among species in reproductive endpoints and in the route, level, and duration of metal exposure, the experimental animal data may be useful for estimates of allowable human exposure.

Although experimental animal and *in vitro* studies have indicated adverse reproductive effects of high doses of many metals and beneficial or protective effects of some essential metals (particularly zinc, selenium, and magnesium), the internal metal dose was often not measured, and relatively few studies have evaluated the effects of long-term moderate oral exposure. For most metals, data relevant to humans are scanty and are usually limited by inadequate controls and adjustments for the influence of potentially confounding variables.

2.1 Lead

Since the mid-19th century, many studies have shown the toxicity of lead on male reproductive function in humans and experimental animals, although the impact of low to moderate levels of chronic lead exposure is still controversial. A recent comprehensive review presents the relevant literature (Apostoli *et al*., 1998), and, therefore, the subsequently published evidence is emphasized here.

Several studies of men occupationally exposed to lead have shown that blood lead levels equal to 400 μg/L were associated with significantly reduced semen quality, whereas reproductive endocrine function in the same subjects was either not affected or was only marginally affected (Alexander *et al*., 1996b; Assennato *et al*., 1986; Cullen *et al*., 1984; Lancranjan *et al*., 1975; Telišman *et al*., 2000). Other studies that measured only sex hormones have generally shown no relevant lead effect on male reproductive endocrine profile (Erfurth *et al.,* 2001; Gennart *et al.,* 1992a; Gustafson *et al.,* 1989; McGregor and Mason, 1990; Ng *et al*., 1991). For example, a Swedish study of 62 active and 15 retired lead workers and 26 control subjects (Erfurth *et al.,* 2001) showed no significant association between either blood lead, serum lead, or fing er bone lead levels and serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone, and sex-hormone binding globulin; the median and range blood lead values in the subgroups were 332 (83–932) μg/L, 186 (104–497) μg/L, and 41 (8–62) μg/L, respectively.

The following lead-related effects on human semen quality have been reported: a decrease in semen volume (Fisher-Fischbein *et al*., 1987; Lerda, 1992; Wildt *et al*., 1983), a decrease in sperm concentration and sperm count (Alexander *et al*., 1996a; Assennato *et al*., 1986; Braunstein *et al*., 1978; Cullen *et al*., 1984; Fisher-Fischbein *et al*., 1987; Lancranjan *et al*., 1975; Lerda, 1992), a decrease in sperm motility (Chia *et al*., 1992; Cullen *et al*., 1984; Lancranjan *et al*., 1975; Lerda, 1992; Viskum *et al*., 1999), and in the quality of motility (Fisher-Fischbein *et al*., 1987; Viskum *et al*., 1999), an increase in abnormal sperm morphology (Cullen *et al.,* 1984; Fisher-Fischbein *et al*., 1987; Lancranjan *et al*., 1975; Lerda, 1992; Wildt *et al*., 1983), particularly at the head of the sperm (Fisher-Fischbein *et al*., 1987; Lerda, 1992; Wildt *et al.,* 1983), and impairment of prostate secretory function as indicated by decreased seminal plasma zinc level (Wildt *et al*., 1983). Most of these

studies have one or more of the following shortcomings: a small number of subjects examined, no control group or an inadequate control group because of relatively high blood lead levels, the inclusion of subjects with current or recent urogenital tract infections, and/or a lack of control for other common factors capable of affecting reproductive parameters. The findings were mainly related to dose-response trend(s) on a group basis for different exposure categories, so that insufficient evidence was presented for establishing a quantitative dose-response curve and no-adverse-effect exposure threshold, particularly concerning the range of very low to moderate blood lead levels.

Some data suggest that the reproductive effects of lead in men are reversible; a trend toward normalization was found in subjects treated with a lead-chelating agent (Cullen *et al.*, 1984; Fisher-Fischbein *et al.*, 1987) or after cessation of occupational lead exposure (Viskum *et al.*, 1999).

A cross-sectional study of 149 industrial workers with low to moderate chronic lead exposure (Telišman *et al.*, 2000) showed significant lead-related reproductive effects even at blood lead levels <400 µg/L. The median (range) of current blood lead values were 103 (67–208) µg/L in 51 control subjects and 367 (119–659) µg/L in 98 lead workers; their long-term average blood lead values were <400 µg/L. Changes in reproductive parameters were more pronounced at relatively low-level lead exposure (<350 µg/L) compared with higher levels. For example, the dose-response relationship between sperm count and blood lead in the 149 subjects showed a decrease of approximately 65×10^6 sperm in the blood lead range 50–350 µg/L. A decrease in sperm count was significant even at blood lead levels of 240 µg/L compared with <100 µg/L. After adjusting for age, smoking, alcohol, blood cadmium, serum zinc, and serum copper, an increase in blood lead was significantly associated with decreasing sperm concentration, counts of total sperm and progressively motile sperm, seminal plasma levels of zinc, acid phosphatase and citric acid, and increasing percentage of pathological sperm with abnormal sperm head morphology. A better correlation of reproductive parameters was found with respect to delta-aminolevulinic acid dehydratase (ALAD) activity in blood, indicating that the reproductive effects were in part mediated through lead interference with zinc metabolism (Telišman *et al.*, 2000). In a similar study, Alexander and coworkers (1996a) showed that sperm concentration and sperm count in lead workers were inversely related to both current blood lead and long-term average blood lead, even at levels <400 µg/L.

In contrast, an international cross-sectional study of 362 lead workers and 141 control subjects from 10 companies in Belgium, Italy, and the United Kingdom (Bonde *et al.*, 2002) reported that, although median sperm concentration was reduced by 49% in men with blood lead >500 µg/L, a likely threshold as high as 440 µg/L was found for this parameter of semen quality. The mean (range) blood lead values were 44 (0–198) µg/L in the control subjects and 310 (46–645) µg/L in lead workers. Abnormal sperm chromatin structure was not significantly related to blood lead levels. However, it seems possible that differences in some lifestyle factors and the body burden of other relevant metals, not controlled for in this study, might have contributed to variations in reproductive parameters among the study population, thereby weakening or masking the relationship between blood lead and reproductive effects.

Recent data indicate that lead can adversely affect human semen quality even at blood lead levels <150 µg/L (Jurasovic *et al.*, 2004; Telišman *et al.*, 2003). In a study of 123 men who had never been occupationally exposed to metals, the median (range) blood lead values were 57 (range 25–149) µg/L. After adjusting for confounding variables (age, smoking, alcohol, blood cadmium, and serum copper, zinc, and selenium), an increase in blood lead was significantly associated with decreasing percentages of morphologically normal and subnormal sperm and with increasing percentages of slow sperm and overly wide sperm. A decrease in ALAD activity was significantly associated with decreasing size of testes and seminal plasma levels of the lactate dehydrogenase isoenzyme LDH-C_4. In another study (Benoff *et al.*, 2003), the seminal plasma lead levels of subjects not occupationally exposed to lead were found to inversely correlate with fertilizing capacity of sperm (sperm acrosome reaction) and the fertilization rate when using IVF technique, but also with seminal plasma zinc levels. Taken together, these studies suggest that lead may significantly reduce human semen quality even at low-level lead exposure that is common for general populations worldwide.

Several studies of lead workers have indicated that paternal blood lead levels of approximately 300–400 µg/L are a most likely threshold for increased rate of spontaneous abortions (Lindbohm *et al.*, 1991), reduced rate of live births (Gennart *et al.*, 1992b; Lin *et al.*, 1996), and prolonged TTP (Sallmén *et al.*, 2000; Shiau *et al.*, 2004), although inconsistent findings (Bonde and Kolstad, 1997; Coste *et al.*, 1991; Joffe *et al.*, 2003) or a minor incompatibility were also reported.

An Italian study (Apostoli *et al.*, 2000) reported that, although shorter TTP was found in lead workers compared with control subjects, within the group of lead

workers a longer TTP was associated with higher blood lead levels and significantly longer TTP was observed at blood lead levels equal to 400 µg/L.

A recent study from Taiwan (Shiau *et al.*, 2004) showed a clear dose-response trend of prolonged TTP with respect to increasing paternal blood lead levels; the fecundability ratios (inversely related to TTP) were 0.90, 0.72, 0.52, and 0.40 for blood lead categories <200, 200–290, 300–390, and equal to 400 µg/L, respectively, compared with unexposed men, indicating that even blood lead levels <300 µg/L may prolong TTP.

A significantly increased rate of congenital malformations was found in children of occupationally lead–exposed fathers at paternal blood lead levels of 200 µg/L (Sallmén *et al.*, 1992). A reduced sex ratio (reduced male proportion) among offspring of lead-exposed fathers has been observed in some studies (Dickinson *et al.*, 1994), but not in others (Min *et al.*, 1996).

Several experimental studies in rats, mice, rabbits, or monkeys have indicated that chronic lead exposure for at least 30 days, resulting in current blood lead levels equal to 400 µg/L, was associated with decreased intratesticular or epididymal sperm counts, sperm production rate, sperm motility, and serum testosterone levels, although mainly without significant effect on male fertility, whereas several other studies have shown no significant reproductive effect at comparable blood lead levels (Apostoli *et al.*, 1998). A recent experimental study in rabbits (Moorman *et al.*, 1998) showed an estimated threshold for reduced sperm count at a blood lead level of 240 µg/L and even lower for several other semen characteristics.

A study on the effect of combined exposure to lead and cadmium on the testes of rats (Saxena *et al.*, 1989) showed that animals orally exposed to the combination exhibited significantly more pronounced pathological testicular changes, with a reduction in sperm counts, compared with animals exposed to either of the metals alone. A protective effect of zinc against the lead-induced and cadmium-induced testicular injury in rats was reported (Batra *et al.*, 1998; Saxena *et al.*, 1989).

With regard to possible mechanisms for a male-related transgenerational effect of lead, an *in vitro* study (Quintanilla-Vega *et al.*, 2000) has shown that lead can compete with or replace zinc in human protamine P2 (HP2), a zinc-containing protein that protects sperm DNA by binding to it during spermatogenesis. Exposure of HP2 to lead resulted in a dose-dependent decrease in the extent of HP2-DNA binding, although lead effects on sperm DNA also contributed to this effect. This may affect sperm chromatin integrity, thereby reducing sperm fertilizing capacity and causing sperm DNA damage.

2.2 Mercury

Male reproductive toxicity of organic and inorganic mercury has been observed in animals mainly at dose levels that are otherwise toxic; evidence of a causal relationship in humans is limited because of a small number of studies performed and insufficient control for potentially confounding variables.

In workers exposed to mercury vapor (urinary mercury levels 71.2 µg/g creatinine), sex-hormone binding globulin level in serum inversely correlated with duration of exposure, whereas no correlation was observed with serum levels of FSH, LH, and testosterone neither with respect to duration of exposure nor mercury levels in blood and urine (McGregor and Mason, 1991). A significant positive correlation between serum total testosterone, but not free testosterone, and cumulative mercury exposure was found in workers exposed to mercury vapor for an average of 10 years (mean urinary mercury 27 µg/g creatinine and mean blood mercury 9.5 µg/L (Barregärd *et al.*, 1994).

No effect on fertility as assessed by the rate of live births was observed in male workers chronically exposed to mercury vapor (urinary mercury levels, 5.1–271.2 µg/g creatinine) (Lauwerys *et al.*, 1985). An increased rate of spontaneous abortions among wives of workers exposed to mercury vapor was, however, noted at paternal urinary mercury levels >4000 µg/L; the effect was not significant after controlling for previous miscarriage history (Alcser *et al.*, 1989). In another study of workers exposed to mercury vapor (Cordier *et al.*, 1991), a trend of increasing rate of spontaneous abortions was associated with paternal urinary mercury levels of 1–19 and 20–49, µg/L; however, the study did not address confounding factors such as smoking and alcohol consumption.

In studies of men with suspect infertility, compared with fertile men, nonsignificant association was found between parameters of semen quality and mercury levels in blood (Chia *et al.*, 1992; Leung *et al.*, 2001), urine, or ejaculate (Hanf *et al.*, 1996). In another study, an increased risk of subfertility was found to be associated, in a dose-dependent manner, with increasing levels of mercury in hair (Dickman *et al.*, 1999).

Both methylmercury and inorganic mercury can accumulate in animal testes, although the uptake and clearance of methylmercury were faster. Exposure of mice to methylmercury or inorganic mercury (a single intraperitoneal injection of 1 mg Hg/kg body weight) resulted in adverse effects on spermatogenesis, testicular morphology, and fertility, whereas DNA synthesis

in spermatogonia was depressed by methylmercury and, to a lesser extent, by inorganic mercury (Lee and Dixon, 1975). In monkeys dosed with methylmercury (25 μg/kg/day orally for 20 weeks), decreased sperm motility and increased abnormal sperm tail morphology were observed at subneurotoxic levels; there was no change in testicular morphology and serum testosterone (Mohamed *et al*., 1987). In rats exposed to methylmercury (0.8, 8.0, or 80 μg/kg twice weekly in the diet for 19 weeks), significantly decreased intratesticular testosterone and somewhat lowered epididymal sperm count were found in the high-dose group, whereas inverse correlation was observed between fertility and testicular mercury content (Friedmann *et al.*, 1998).

In rats, mice, guinea pigs, and hamsters exposed to inorganic mercury (mercuric chloride, intraperitoneally, 1, 2, or 5 mg/kg/day for 1 month), the highest dosage caused testicular degeneration and cellular deformation of the seminiferous tubules and the Leydig cells in all species, whereas the lowest dosage caused testicular degeneration only in the hamster; partial degeneration was observed in the rat and mouse, and no change was noted in the guinea pig (Chowdhury and Arora, 1982). In rats orally exposed to mercuric chloride (9 mg/kg/day, for 60–180 days), testicular morphological changes and decreased testosterone levels were found (Agrawal and Chansouria, 1989). In mice exposed to inorganic mercury through drinking water (4 ppm mercuric chloride, for 12 weeks), degenerative testicular changes, decreased absolute and relative testicular weight, and decreased epididymal sperm count were found; a protective effect of zinc was reported (Orisakwe *et al.*, 2001). Vitamin E, administered with mercuric chloride (1.25 mg/kg/day) by gavage for 45 days in mice, was protective against reduced epididymal sperm count, sperm motility and viability, and resulted in lower concentrations of mercury in the testis, epididymis, and vas deferens (Rao and Sharma, 2001).

2.3 Cadmium

Although various cadmium-associated effects on the male reproductive system have been described, there is little conclusive published evidence of cadmium-related effects on semen quality, sex hormones, or fertility in human males on which to base a reliable estimate of quantitative dose-response relationship(s) or no-adverse-effect exposure threshold(s). Most studies have failed to adjust for the influence of potentially confounding variables such as age, smoking habits, alcohol consumption, and the body status of the relevant metals, particularly lead, zinc, and selenium.

In men not occupationally exposed to cadmium, a significant increase in cadmium levels in blood and seminal plasma has been related to smoking habits (cigarette-years, or cigarettes/day) (Chia *et al*., 1994; Telišman *et al*., 1997, 2000). However, in addition to increasing individual exposure to cadmium, smoking itself may adversely affect male reproductive function by mechanisms of oxidative stress involving other compounds present in cigarette smoke. Published data on men with suspect infertility (including nonsmokers and smokers) showed a significant positive correlation between abnormal sperm morphology and blood cadmium levels (Chia *et al*., 1992, 1994; Telišman *et al*., 2000), but not with seminal plasma cadmium levels (Telišman *et al*., 2000).

An inverse correlation has been reported between testis size and blood cadmium (Jurasovi *et al*., 2004), between sperm motility and blood cadmium (Telišman *et al*., 2000), between semen volume and either blood cadmium (Chia *et al*., 1992) or seminal plasma cadmium (Chia *et al.*, 1994; Xu *et al*., 1993), and between sperm concentration and sperm count with respect to blood cadmium levels (Chia *et al*., 1994; Xu *et al.*, 1993). Several other studies reported no significant correlations between semen quality and cadmium levels in semen, seminal plasma, or blood. Nonsmoking patients with varicocele had significantly higher cadmium and lower zinc levels in the seminal plasma compared with fertile subjects (Benoff *et al*., 1997). In nonsmokers and nonconsumers of alcohol, a significant positive correlation was found between sperm DNA oxidative damage (8-OHdG level in sperm DNA) and the seminal plasma levels of both cadmium (range, 0.5–1.3 μg/L) and lead (range, 5–14 μg/L), whereas an inverse correlation was found with seminal plasma selenium levels, suggesting that cadmium may contribute to sperm DNA oxidative damage and thereby affect semen quality (Xu *et al*., 2003); however, the study did not evaluate the combined effect or interaction of cadmium, lead, and selenium concerning these endpoints.

A study of 297 Chinese men environmentally exposed to cadmium showed a significant dose-response trend of increasing urinary cadmium (range, 0.1–43.0 μg/g creatinine) and the prevalence of cases with abnormal levels of prostate specific antigen in serum. Furthermore, subjects with abnormal findings on digitorectal examination of the prostate had significantly higher blood cadmium (blood cadmium range, 0.1–44.7 μg/L), suggesting that increased chronic cadmium exposure can cause injury to the human prostate (Zeng *et al*., 2004a). In the same study population, a significant dose-response trend of increasing urinary cadmium and the prevalence of abnormally high serum testo-

sterone levels was found, although the relationship was not significant after adjusting for age, smoking, alcohol, and body mass index (Zeng *et al*., 2004b).

In a study of 123 men with no occupational exposure to metals, no significant relationship was found between parameters of semen quality and blood cadmium levels (range, 0.2–11.9 µg/L) after adjusting for age, smoking, alcohol, and biomarkers of lead, copper, zinc, and selenium by multiple regression, whereas significant relationships were found between increasing serum levels of estradiol, FSH, and testosterone with respect to blood cadmium (Jurasovic *et al*., 2004). Another study of men not occupationally exposed to cadmium also showed a significant relationship between increasing serum testosterone and blood cadmium levels (range 0.2–13.3 µg/L) after adjusting for similar confounding variables (Telišman *et al.*, 2000).

In a study of 161 men, including smelter workers exposed to cadmium and control subjects, significantly increased serum testosterone was found in men with urinary cadmium of 10–20 µg/g creatinine compared with 0–2 or 2–5 µg/g creatinine, no significant difference in serum testosterone was found for men with urinary cadmium levels >20 µg/g creatinine, but significantly increased serum LH was found in this group (Zeng *et al*., 2002). Other studies of cadmium-exposed male workers showed no significant effect of cadmium on reproductive endocrine function (Mason, 1990; McGregor and Mason, 1991).

No significant influence of occupational exposure to cadmium was found on the fertility of 83 male workers (mean urinary cadmium was 6.9 µg/g creatinine and mean duration of exposure 24 years) on the basis of the rate of live births compared with unexposed workers and in the cadmium-exposed workers before or after the onset of exposure (Gennart *et al*., 1992b).

Male reproductive toxicity after acute exposure to cadmium is well documented in experimental animals, but there is considerably less information on the effects of low to moderate levels of chronic oral cadmium exposure. In most experimental studies, a single, large dose of cadmium was administered by injection (intraperitoneally, subcutaneously, or intramuscularly); the acute effects seen in animals are not likely to occur in humans, because the exposure to cadmium is different in both industrial and environmental settings.

Many studies in experimental animals have shown that the mammalian testis is highly vulnerable to cadmium, which can cause germinal cell damage and testicular necrosis, possibly through a direct effect on the testicular vasculature, which may exert a secondary action by lowering testosterone production and thereby also affecting accessory genital organs, including the prostate. The following acute effects have been reported in experimental animals injected with soluble cadmium salts: decreased serum testosterone, a decreased size and weight of the testes, epididymis, vas deferens, prostate and seminal vesicles, decreased sperm production and motility, and suppressed libido and reproductive capacity (Laskey *et al*., 1984; Lau *et al*., 1978; Nordberg, 1971, 1975; Parizek, 1960; Saksena and Lau, 1979; Waalkes and Rehm, 1994). Testicular atrophy and necrosis and decreased fertility have been observed in animals at nearly fatal doses of cadmium (Bomhard *et al.*, 1987). In rats, long-term exposure to cadmium through the drinking water (10 mg/L for 52 weeks) led to pathological testicular changes, as well as liver and kidney damage, whereas reproductive capacity was reduced in 40% of animals (Saygi *et al*., 1991).

A synergistic effect of lead and cadmium on testicular injury in rats has been reported (Saxena *et al*., 1989). In contrast, a protective effect against male reproductive toxicity of cadmium was observed in animals treated with zinc (Gunn *et al*., 1966a; Niewenhuis, 1980; Parizek, 1960; Saxena *et al.*, 1989), selenium (Jones *et al*., 1997; Niewenhuis and Fende, 1978), or with sulfhydryl (–SH) containing compounds such as BAL, cysteine, glutathione, and metallothionein (Gunn *et al*., 1966b; Nordberg, 1971). Neither small chronic doses of cadmium, which induce metallothionein biosynthesis, nor injections of cadmium partially bound to metallothionein cause testicular necrosis (Nordberg, 1971).

Both the route and duration of cadmium administration is relevant for male reproductive effects. In rats exposed to 50, 100, or 200 ppm cadmium in drinking water for 3 or 6 months, significantly increased (rather than decreased) serum testosterone was found in all cadmium exposure groups after 3 months and in the 200 ppm group after 6 months (Zeng *et al*., 2003). Prostate zinc content decreased, and there was significant downregulation of the metallothionein gene. As previously mentioned, elevated concentrations of serum testosterone and its correlation with blood cadmium levels have been reported in chronically exposed men. In view of the fact that testosterone augments the progress of prostate cancer in its early stages, it is possible that cadmium-induced changes may contribute to initiation and development of prostate cancer in both rodents and humans.

2.4 Manganese

In men occupationally exposed to manganese dust, clinical signs of manganism were commonly accompanied with impotence or reduced libido (Emara *et al*., 1971; Mena *et al*., 1967; Rodier, 1955), indicating that manganese-related effects were in part neurological in

origin. A study of male workers moderately exposed to manganese dust (Lauwerys *et al*., 1985) showed a significantly reduced rate of live births compared with a control group (39 births observed vs. 70.5 expected); in a similar study, no effect of manganese was found (Gennart *et al*., 1992b). This may be due to higher levels of manganese in the former study (12.0 vs 8.2 µg/L mean blood levels; 1.50 vs. 0.84 µg/g creatinine in urine).

A high single dose of manganese (160 mg MnO_2/kg by intratracheal instillation) caused degenerative testicular changes and sterility in rabbits (Chandra *et al*., 1973; Seth *et al.,* 1973). Chronic dietary exposure of young rats to manganese Mn_3O_4 for 224 days resulted in no effect on male fertility at manganese doses <1100 ppm, whereas at a dose of 3500 ppm decreased testicular weight, sperm count, and serum levels of FSH and testosterone were noted together with general toxic effects (Laskey *et al*., 1982). In mice orally exposed to manganese acetate for 43 days, a significant decrease in sperm count and motility was observed at doses of 15.0 and 30.0 mg/kg/day, whereas there was no effect on fertility and testicular pathology (Ponnapakkam *et al*., 2003).

2.5 Chromium

In male workers exposed to chromium (VI) for 1–15 years in an electroplating factory, compared with unexposed workers (Li *et al*., 2001), significantly decreased sperm concentration (by 47%), sperm motility, and seminal plasma levels of zinc, lactate dehydrogenase, and the lactate dehydrogenase isoenzyme LDH-C_4 were found, whereas serum FSH was significantly increased; serum chromium levels in exposed workers were 1.40 ± 0.01 µmol/L. A study of male welders, whose chromium levels in postshift spot urine samples ranged from 0.17–4.74 nmol/mmol creatinine and those in blood ranged from 6.0–46.4 nmol/L, showed no significant difference in parameters of semen quality compared with nonwelding workers (Bonde and Ernst, 1992). Another study of male welders, whose blood chromium levels were 131.0 ± 52.6 µg/L, showed significantly decreased sperm concentration (by 67%) in exposed workers compared with controls and an inverse correlation between sperm concentration and blood chromium levels in the exposed workers (Dandevi *et al*., 2003).

Animal exposure to high doses of chromium (III or VI) has been shown to adversely affect spermatogenesis. Chromium (VI) is considerably more toxic (Ernst, 1990; Ernst and Bonde, 1992; Li *et al*., 2001) and may involve oxidative stress. This is evidenced by increased lipid peroxidation in the testes, decreased sperm count, and increased abnormal sperm morphology of mice exposed to chromium (VI), each of which was partially preventable by the supplementation with the antioxidants, vitamin E and especially vitamin C (Acharya *et al*., 2004).

2.6 Nickel

In a study of male welders, whose blood nickel levels were 123.3 ± 35.2 µg/L, a significant positive correlation was found between the percentage of morphologically abnormal sperm with sperm tail defects and the blood nickel levels (Dandevi *et al.,* 2003).

Experiments in animals have shown testicular toxicity involving oxidative stress after high doses of nickel. This is evidenced by increased lipid peroxidation, DNA damage, and apoptosis in the testes, morphological sperm head abnormalities, and decreased fertility in mice (Doreswamy *et al*., 2004); decreased DNA, RNA, and total protein in testes, and decreased sperm count and motility in rats (Das and Dasgupta, 2000); and decreased absolute and relative weights of testes, epididymides, seminal vesicles and prostate gland, decreased sperm count and motility, and increased abnormal sperm morphology in mice (Pandey *et al*., 1999). Other reports provide some evidence that nickel may be essential for male reproduction in rats (Nielsen *et al*., 2002; Yokoi *et al*., 2003).

2.7 Arsenic

No relevant evidence with regard to dose-response relationship for the human male is available because of a small number of studies performed and insufficient control for potentially confounding variables. Exposure of mice and rats to high doses of inorganic arsenic can adversely affect spermatogenesis and can decrease testicular and accessory sex organ weights and serum levels of LH, FSH, and testosterone (Pant *et al*., 2001; Sarkar *et al*., 2003). Long-term oral exposure of mice to sodium arsenite in drinking water at 4 ppm arsenic for 365 days, which is a dose level similar to human exposure through drinking water, resulted in significant accumulation of arsenic in the mouse testis, epididymis, seminal vesicles, and prostate gland, a decrease in the absolute and relative testicular weight but not of epididymal and accessory sex organ weights, a decrease in sperm count and motility, increase in abnormal sperm morphology, and changes in the activities of testicular enzymes (Pant *et al.,* 2004). Exposure of mice to the same dose of sodium arsenite in the drinking water (4 ppm arsenic) for only 35 days produced no significant effects (Pant *et al*., 2001).

3 FEMALE REPRODUCTIVE EFFECTS

Effects of metals on female reproduction may arise from their action on several stages beginning in fetal life, during early development or maturity, and include

manifestations such as subfertility, infertility, intrauterine growth retardation, spontaneous abortions, malformations, birth defects, postnatal death, learning and behavior deficits, and premature aging. Because it is difficult to obtain detailed information concerning effects on the reproductive system in humans, evidence is usually limited to animal data or studies on fertility and spontaneous abortions. Pregnancy loss is the endpoint most frequently used to monitor effects on female reproductive function, starting from early losses, which contain a large proportion of chromosomal abnormalities and may represent 35–40% of human pregnancies. The remaining 10–15% later abortions are clinically manifest, and some have been linked to environmental factors (Miller *et al*., 1980; Runnebaum *et al*., 1997). Thus, there seems to be a remarkably high background rate of fetal loss in humans.

The clinical and epidemiological findings related to metal-induced effects on female reproduction are often difficult to interpret, because many other factors may influence the outcome such as age, ovarian reserve, hormonal imbalance, behavior, genetics, male fertility factors, or sexually transmitted diseases. In addition, timing, duration, and intensity of exposure are important in assessing reproductive adverse effects. Ernhart (1992) pointed out some critical aspects in measuring the dose indicators, such as sampling time and the matrix (maternal or fetal blood, cord blood, or placenta) in which indicators of prenatal exposure should be determined. To better assess early exposure to metals and the trend of absorption through pregnancy, at least one sample should be taken during the first trimester and another during the last 6 weeks of the pregnancy. This will facilitate the assessment of effects on the various stages of development and organogenesis.

Knowledge about pathogenetic mechanisms of female reproductive damage is limited. Effects may be direct, when environmental or occupational metals interact with specific reproductive target organs, or indirect, when metals act on endocrine or other systems. The ovaries and ova are susceptible to direct damage by metals for an extended period of time, from meiosis through ovulation. Some experimental studies suggest an increased risk of miscarriage, fetal malformation, placental insufficiency, and premature birth because of metal exposure (Fagher *et al*., 1993; Laundanski *et al*., 1991). Metals such as lead may interfere with the hypothalamic-pituitary-ovarian axis at different levels by modifying the secretion of prolactin, adrenocortical steroids, or thyroid hormones; vascular effects on the placenta have also been suggested (Gerhard *et al*., 1998; Hertz-Picciotto and Croft, 1993; Piasek and Laskey, 1994).

Current evidence provides warning signals: the female reproductive system is vulnerable to toxic agents, and the number of working women potentially exposed to metals is increasing worldwide. It is estimated that most of working women are in reproductive age; about half of working women are used during pregnancy, and about 20% are exposed to chemicals of potential concern (Sharara *et al.,* 1998). Reviews on female reproductive effects include Gold and Tomich (1994), Gardella and Hill (2000), Foster (2003), and Kumar (2004).

3.1 Lead

The adverse effects of lead on both male and female reproduction have been known for as much as a century. Infertility, spontaneous abortions, and fetal and neonatal death have been reported after either male or female occupational exposure to lead (Mattison, 1983; Rom, 1976). In the early studies, such effects were usually associated with high exposure levels. The evidence for effects resulting from low to moderate exposure is less clear. In one investigation, 831 pregnant women living near a lead smelter in Port Pirie, Australia, were studied prospectively for lead exposure and absorption, assessed by blood lead and pregnancy outcome (McMichael *et al*., 1986). The exposed women had a mean blood lead concentration (PbB) of 100.6 µg/L, and control women 76 µg/L. Preterm delivery was significantly associated, in a dose-response manner, with maternal blood lead concentration. No association was detected for spontaneous abortion, low birth weight (for births at term), intrauterine growth retardation, premature rupture of the membranes, and congenital anomalies.

Falcon *et al*. (2003) assessed the relationship between placental lead concentration and outcomes of pregnancy. Higher placental lead levels were measured in premature rupture of membranes and preterm pregnancies (gestational age ≤37 weeks) compared with term pregnancies. The proportion of abnormal pregnancy outcome associated with placental lead concentrations above 120 ng/g was 40.6% versus 8.8% in placentas below this concentration. Higher placental lead levels were not generally related to reduced size at birth.

The literature on high lead exposure and abortion provides consistent evidence, starting with historical surveys carried out in Milan by Torelli (1930). In this study, spontaneous abortions were reported in 24% of women exposed to lead, with a relative risk of 5.3, and an infant mortality more than double the Italian national rate at that time.

In a survey carried out by a questionnaire in more than 500 women, who had worked at a smelter and

were born between 1930 and 1959, the spontaneous abortion rates were highest when the mother was employed during pregnancy (13.9%) or had been employed before and was living close to the smelter (17%). The frequency rate was higher (19.4%) when the father worked at the smelter. Because the smelter produced copper and lead in addition to other metallurgical and chemical products, the effects reported may not necessarily be attributable to lead alone (Nordstrom *et al*., 1978a). Other reports provide virtually no evidence that low-to-moderate lead exposure is associated with an increased risk of spontaneous abortion (Laundanski *et al*., 1991; Murphy *et al*., 1990).

Hu *et al*. (1991) provided interesting data on the pregnancies of women who experienced lead poisoning during their childhood between 1930 and 1944. Because lead is stored in bone for decades, it was hypothesized that demineralization of the skeleton might occur during pregnancy. The proportion of pregnancies ending in spontaneous abortion or stillbirth was approximately 30% among cases and 15% among controls. No increased risk of spontaneous abortion was seen for pregnancies fathered by lead-poisoned men.

Murphy *et al*., (1990) compared the rates of spontaneous abortion among 304 women living in the vicinity of a lead smelter with those of 335 nonexposed women. The geometric mean PbB concentrations in the sample were around 150 μg/L in exposed versus 50 μg/L in the not exposed group. The rates of spontaneous abortions in first pregnancies were similar, suggesting that the reported low levels of exposure from the general environment were not associated with increased risk of abortion.

The risk of spontaneous abortion of spouses of male occupationally exposed to lead seems less consistent. Anttila and Sallmén (1995) summarized the epidemiological studies on the possible impact of parental occupational exposure to lead or other metals on spontaneous abortion. They stated that no clear conclusion could be reached for maternal exposure. The investigation by Borja-Aburto *et al*. (1999) on pregnant women environmentally exposed in Mexico City concluded on the contrary that lead exposures in the range 100–250 μg/L could have adverse effects on pregnancy and that some of the effects were noted close to 100 μg/L. Other developmental effects, different from the neurodevelopmental, have been associated with very low maternal blood lead levels, as discussed in detail later.

In a review, Hertz-Picciotto (2000) examined studies conducted among populations with low-to-moderate exposures, most of which provided little evidence of an association with pregnancy loss or spontaneous abortions. However, these studies were hampered by small sample sizes, problems in definition or ascertainment of outcome, lack of controls for confounding variables, and/or deficiencies in the exposure assessment and evaluation of accumulated dose. The author concluded that exposures comparable to U.S. general population levels during the 1970s and to many populations worldwide today (i.e, far lower than many occupational exposures) may increase the risk for spontaneous abortion. Further research is needed to confirm the association, to delineate the role of maternal versus paternal exposures, and to assess increases in menstrual variability as an explanation for these findings.

In experimental animals, lead has been shown to reduce litter size, weight of offspring, survival rate, and to alter maturation of the female reproductive system or to interfere with function in the sexually mature animal (WHO, 1977).

Examination of 48-hour embryos of dams exposed to lead in their diet revealed delays in early cell divisions, with fewer embryos reaching the 8-cell stage. Although blastocyst formation seemed to be relatively resistant to lead, implantation of blastocysts was impaired. Lead-exposed blastocysts were able to implant when transferred to a lead-free environment, although neither estrogen nor progesterone levels in lead-treated mice were significantly altered; the maximum binding capacity of uterine cytosolic receptors for estradiol was increased in lead-treated animals. Coadministration of estrogens and progesterone with the lead treatment prevented implantation failure. The normally occurring increase in estrogen and progesterone after implantation was not observed in lead-treated mice. These data suggest that lead interferes with ovarian steroid stimulation of the endometrium (Jacquet, 1977; Wide, 1983; Wide and Nilsson, 1977; Wide and Wide, 1980).

The onset of puberty was delayed in female rats receiving lead in drinking water from the time of weaning. Delays in vaginal opening were also observed in their offspring exposed continuously from conception to doses of lead from 25–250 ppm. No other effects on fertility or reproductive performance were noted (Grant *et al*., 1980; Kimmel *et al.,* 1980).

Several studies have examined the importance of the exposure period. For example, Epstein *et al*. (1999) evaluated mice exposed at premating, prenatally, and postnatally. Compared with controls, prenatally and postnatally exposed mice had slowed brain weight development, lowered brain weight, and decreased DNA per brain, but there was no effect on proteins per brain. In contrast, premating lead exposure significantly increased brain weight and protein but significantly lowered DNA per brain. The latter effects could be due to action of lead on the developing maternal reproductive systems or on ovulation-fertilization. Time of exposure was also investigated by Ronis *et al*. (1996) when

rats were exposed to lead acetate in drinking water *in utero*, prepubertally or postpubertally. The most severe effects were observed in the "*in utero*" exposed group, with delayed vaginal opening and disrupted estrous cycling. These effects suggest lead actions on the hypothalamic pituitary axis and on gonadal steroid biosynthesis directly.

Mc Givern *et al*. (1991) administered lead acetate in drinking water to Sprague–Dawley rat dams. Female offspring from lead-treated dams had significantly delayed vaginal opening, and 50% of them exhibited prolonged and irregular periods of diestrous, accompanied by an absence of observable corpora lutea. The release of gonadotropins revealed irregular patterns of both FSH and LH. Alterations in pubertal progression and hypothalamic-pituitary-ovarian-uterus functions have been confirmed in female monkeys prenatally or postnatally exposed to lead. Blood levels of approximately 350 µg/L resulted in subclinical suppression of circulating LH and FSH and estradiol without producing overt effects on general health and menstruation (Foster and Younglai, 1991). The overall results of these investigations suggest that different levels of the hypothalamic-pituitary-gonad axis can be affected by exposure to lead, mainly when structures are undergoing rapid proliferation.

3.2 Mercury

Limited data are available from epidemiological studies showing that mercury disrupts female reproductive function. One recent study in girls examined the relationship of mercury and other toxicants to timing of menarche (Denham *et al*., 2005). No effects of mercury were found, although mercury levels in the study population of girls were at or below background levels observed in the general population. However, earlier studies had noted menstrual cycle changes in women who were exposed to higher levels of mercury vapor in the workplace (De Rosis *et al.*, 1985; Sikorski *et al*., 1987). Decreased fertility was noted in some dental health care workers with increased exposure to mercury vapor (Rowland *et al*., 1994). In contrast, a number of effects have been described in experimental animals exposed to mercury, including alterations in ovulation and estrous cycle. Mercury vapor exposure resulted in prolonged estrous cycles and alterations in progesterone and estradiol levels, but primarily in animals with weight loss; morphological changes in corpora lutea were also observed. However, no adverse pregnancy outcomes (rate or number of implantation sites) were observed (Davis *et al.*, 2001).

Exposure of nonhuman primates to 50 or 90 µg/kg/day methyl mercury did not alter menstrual cycles or menses length through four cycles. However, reproductive effects, including failure to conceive and resorptions were related to increased mercury blood levels (Burbacher *et al*., 1984). In female macaques, blood levels greater than 1 ppm were associated with decreased pregnancy rates and increased abortion rates (Mottet *et al.*, 1985).

Mercury was detected by photochemical techniques within ovarian follicles and in the corpora lutea of rats after chronic ingestion of mercuric chloride (1 mg /day) for 12 weeks. Estrous cycles were prolonged in these animals (Stadnicka, 1980). In golden hamsters, mercury was also localized to the corpora lutea after inorganic mercury administration (Lamperti and Printz, 1974). Decreased ovulation was observed at the third estrous cycle in these animals, which received 1 mg $HgCl_2$/kg on the four days of the estrous cycle. Progesterone levels in mercury-treated animals were also different from controls (Lamperti and Printz, 1973). Watanabe and coworkers (1982) reported similar decreases in ovulation in golden hamsters injected (subcutaneously) with 6.4 or 12.8 mg Hg/kg but found no alterations in ovulation in animals treated with the same amounts of methylmercury chloride. In a more recent study, reproductive performance was evaluated for mice, males and females, exposed to inorganic mercury premating, during mating and during gestation and lactation (females only). Fertility and offspring survival were significantly reduced, although litter size was not affected (Khan *et al*., 2004).

There are few mechanistic studies related to mercury effects on female reproduction. Morphological alterations in the actuate nucleus of the hypothalamus were associated with changes in pituitary levels of FSH and LH in hamsters treated with mercuric chloride by injection (Lamperti and Niewenhuis, 1976). The role of these alterations in regulation of ovulation is not clear.

Early development of mouse embryos was disrupted after exposure to methylmercury *in vitro* (Matsumoto and Spindle, 1982). Treatment of female mice with 0.5 or 1 mg Hg/kg of methylmercury chloride or mercuric chloride on day 0 of gestation did not result in increased abnormalities in embryos recovered on day 3.5 of pregnancy. Exposures to higher levels of mercury (up to 20 mg Hg/kg methylmercury and 2.5 mg Hg/kg inorganic mercury) resulted in some abnormalities. However, embryos exposed *in vivo* were less sensitive to damage than those exposed *in vitro* (Kajiwara and Inouye, 1986).

3.3 Cadmium

Few data are available on the effects of cadmium on human female reproductive function. Epidemiological

studies have not revealed reproductive effects in contrast to experimental evidence in animals. Studies are needed to determine whether cadmium at low doses may act as reproductive and developmental toxicant and whether the fetal toxicity is related to placental defects or to accumulation of cadmium in the fetus.

In vitro studies have shown effects on calcium and oxytocin activities in myometrial strips from term pregnant women (Sipowicz *et al*., 1995) and on steroid genesis in granulose cells obtained from ovarian follicular aspirates (Paksy *et al*., 1997). However, the lowest cadmium concentration that reduced progesterone production was about 3.5 times higher than levels reported in the ovary of a female smoker.

After acute and at high-dose administration in rats, cadmium has been shown to affect various female reproductive endpoints, resulting in hemorrhagic changes in the ovary and uterus or in persistent estrous and ovulation; these effects can be prevented by coadministration of selenium (Saksena, 1982; Watanabe *et al.,* 1979).

The effects of cadmium vary as to the sensitivity of early embryos, and studies on cadmium acetate exposure indicate no effects on fertilization, but only at the blastocyst stage or *in vitro* reduction of development to the morula stage (Schmid *et al*., 1983; Storeng and Jonsen, 1980).

Exposure of experimental animals to cadmium increased uterus weight accompanied by proliferation of the endometrium and induction of progesterone receptors, mimicking the effects of estrogens. Female offspring experienced an earlier onset of puberty and growth of the mammary gland. In rats, exposure to cadmium oxide dusts increased the duration of the estrous cycle, decreased the preovulatory luteinizing hormone levels in blood, and inhibited ovulation. These effects were generally observed after high-dose, acute exposures and, therefore, provide little information for current human exposures (Baranski and Sitarek, 1987).

Cadmium given before mating may lead to sterility in a dose-dependent fashion, because of anovulation resulting from reversible pituitary dysfunction. However, animals may develop tolerance and remain fertile despite cadmium treatment, with normal fetal outcome and postnatal development (Paksy *et al.*, 1996).

Piasek *et al*. (1999) evaluated the direct effects of *in vitro* cadmium exposure on steroidogenesis in Sprague–Dawley rat ovaries; production of progesterone and testosterone was affected in proestrous rats and pregnant dams, whereas estradiol was not affected. They concluded that cadmium interferes with the ovarian steroidogenic pathway at more than one site. These effects may be mediated by interference with DNA-binding zinc in steroidogenesis or by estrogen-like activity (Henson and Chedrese, 2004; Johnson *et al*., 2003).

3.4 Manganese

Reports in humans on the association between manganese exposure and adverse reproductive outcomes in females are limited. A survey carried out in Australian general population found more frequent stillbirths than expected in the group exposed to manganese (Tsuchiya *et al*., 1987).

In rats, manganese exposure reduced the number of ovarian follicles and induced persistent corpora lutea (Laskey *et al*., 1982). In mice, exposure during gestation led to fetal growth retardation and anencephaly (Sanchez *et al*., 1993).

3.5 Chromium

Few studies in human females have addressed potential adverse effects of chromium and chromium compounds. The effect of chromium (VI) on human pregnancy outcomes was examined in females working in manufacturing facilities in Russia (Shmitova, 1978; 1980). Complications during pregnancy and childbirth (not described further) were reported in women with higher levels of chromium in blood and urine than the control group. However, the quality of the data and reporting limits their interpretation regarding the potential ability by chromium to produce reproductive effects.

Bonde (1993) studied the spouses of stainless steel welders exposed to chromium (VI) for spontaneous abortions and congenital malformations. The author concluded that the weak indications of an increased risk of spontaneous abortion among partners of stainless steel welders (odds ratio [OR], 1.9; 95% confidence interval [CI], 1.1–3.2) needed to be corroborated. In a subsequent investigation of a similar population by Hjollund *et al*. (2000), information was collected on exposure and outcomes for 245 clinically recognized pregnancies. Male welding of stainless steel was associated with an increased risk of spontaneous abortion in spouses (adjusted relative risk, 3.5; 95% CI, 1.3–9.1). The mutagenic effect of chromium (VI) previously found in both somatic and germ cells could be responsible for the abortions, which in this case would be a male-mediated effect. In an earlier study, the same group (Hjollund *et al.,* 1995) examined outcomes in 2520 pregnancies of spouses of Danish metal workers exposed to chromium (VI) from 1977 to 1987. The number of spontaneous abortions was not higher for pregnant women whose spouses worked in the stainless steel welding industry compared with

controls (OR, 0.78; 95% CI, 0.55–1.1). At present, the effects of chromium on female reproductive function in humans remains unclear.

Reproductive effects have been observed in the offspring of mice exposed to chromium (III) after oral maternal exposure. Significant decreases in the relative weights of reproductive tissues (ovaries and uterus) were observed in the offspring of exposed BALB/c mice. A significant delay in timing of vaginal opening was also noted (Al-Hamood *et al*., 1998). Cultured mouse embryos were sensitive to chromium (VI) exposure *in vitro*; incubation of blastocysts with potassium dichromate inhibited edinner cell mass growth and differentiation, whereas hatching, attachment, and trophoblast outgrowth were not affected (Iijima *et al*., 1983).

Murthy *et al*. (1996) reported a number of reproductive effects (reduced number of follicles at different stages of maturation, reduced number of ova/mice, increased estrous cycle duration and histological alterations) in the ovaries of female mice exposed to potassium dichromate in drinking water for 20 days. The severity of the reproductive effects seemed to be dose related. Other experimental studies reported increased preimplantation losses and resorptions in rats and mice exposed to chromium (VI) (Kanojai *et al*., 1998; Trivedi *et al*., 1989). A decrease in the number of pregnant females was observed after the mating of unexposed females with male mice exposed to chromium chloride, whereas an increase in relative ovarian weight was observed in female mice exposed to potassium dichromate. Impaired fertility was observed in females exposed to chromium (III) mated with unexposed males. In females of different species fed with potassium dichromate (VI), microscopic examination of the ovaries revealed no treatment-related effects (Elbetieha and Al-Hamood, 1997).

Additional studies were carried out in rats to assess reproductive effects from exposure to potassium dichromate in drinking water. At the highest level of exposure, there were a decreased number of implantation sites, number of live fetuses, and fetal weight. There were also increases in the number of resorptions and the number of preimplantation and postimplantation losses. There was also a significant reduction in ossification of fetal bones (Junaid *et al*., 1996a). In a second study (Kanojia *et al*., 1996), female rats were exposed to potassium dichromate for 20 days before mating. Similar effects were observed on gestational weight, postimplantation loss, number of live fetuses, and fetal ossification (in fetal caudal bones). In a third study, female rats were exposed to potassium dichromate for 3 months premating (Kanojia *et al*., 1998). Reduced maternal gestational weight gain, increased preimplantation and postimplantation loss, reduced fetal weight, fetal subdermal hemorrhagic thoracic and abdominal patches, and increased incidences of reduced ossification in fetal caudal bones were observed in all treatment groups. In addition, the highest dose group exhibited increased resorptions, reduced numbers of corpora lutea and fetuses per litter, reduced implantations, reduced placental weight, and reduced fetal length. No treatment-related gross visceral abnormalities were seen in these studies.

3.6 Nickel

The effects of nickel exposure on female reproductive function remain unclear, and available information is sparse in both human and experimental studies. An investigation carried out in the arctic region of Russia, showed a spontaneous abortion rate of 15.9% in 356 women from a nickel refining plant compared with 8.5% in a control group of 352 females working in construction industry. Exposure concentrations were 0.08–0.196 mg Ni/m^3, primarily as nickel sulfate, and nickel concentrations in the urine ranged from 3.2–22.6 µg/L (Chashschin *et al*., 1994).

Nickel exposure is reported to affect early embryonic events in mice. Injection of nickel chloride on the first day of pregnancy led to decreased implantation frequency and reduced litter size, whereas the administration on days 2–6 significantly reduced litter sizes but did not modify implantation. *In vitro* incubation inhibited growth of two-cell-stage embryos and development to blastocysts (Storeng and Jonsen, 1980; 1981).

In more recent animal studies, no effect was demonstrated on the length of the estrous cycle or microscopic changes in the reproductive organs in mice or rats exposed to air concentrations of nickel sulfate, nickel oxide, or nickel subsulfide, ranging from 3–0.11 mg Ni/m^3, respectively (NTP, 1996a,b,c). Fertility was not adversely affected in female rats exposure to nickel chloride in drinking water (Kakela *et al.,* 1999). Other studies on histological alterations in reproductive tissues of rats exposed to nickel or to nickel sulfate in drinking water failed to show relevant effects (Obone *et al.,* 1999).

3.7 Arsenic

The effect of arsenic on female reproductive function has not been studied sufficiently and is not clear. The effect on human reproduction of arsenic was investigated in females exposed by means of air or drinking water. Tabacova *et al*. (1994) examined the relationship between arsenic exposure from a copper smelter area in Bulgaria and oxidative damage during pregnancy.

Placental levels of arsenic were highest in areas with the highest environmental contamination, and exposed pregnant women were at higher risk of oxidative damage during pregnancy. Aschengrau *et al*. (1989) investigated the relationship between community drinking water quality and spontaneous abortion. Type and concentration of trace elements were gathered from routine analyses of public tap water supplies of areas where the women resided during pregnancy. After adjustment for potential confounders, an increase in the frequency of spontaneous abortion was associated with high levels of arsenic.

In a case-control study of stillbirths, Ihrig *et al*. (1998) included the assessment of environmental arsenic exposures and analysis of confounders (race, ethnicity, maternal age, median income, and parity). There was a statistically significant increase in the risk of stillbirth in the group with highest exposure to arsenic. Further analysis showed that the increase was limited to Hispanic people, possibly because of a genetic impairment in folate metabolism. However, this study had a small number of cases in the high exposure group, lacked data on smoking, and did not consider potential confounding exposures to other chemicals. As a whole, the studies on arsenic reproductive effects have been criticized because they did not adequately measure exposure to arsenic and other metals and did not evaluate other potential confounding factors (Golub *et al*., 1998, NRC, 1999).

In the multigenerational experimental study by Schroeder and Mitchener (1971), female rats continuously exposed to arsenate in drinking water did not show decreased fertility. Two other studies demonstrated that reproductive functions (included precoital interval, mating index, and fertility index) were not affected in female rats orally exposed to trivalent arsenic by gavage from 14 days before mating through gestation (Holson *et al.*, 1999; 2000b). Some effects were demonstrated in female mice exposed to monomethyl arsonic acid before mating and during pregnancy with production of fewer litters than normal, but this effect was attributable mainly to decreased fertility of the males (Prukop and Savage, 1986). Also, rats treated with arsenic by daily gavage (before mating and continuing through gestation) had significantly reduced fetal body weights and significantly increased skeletal malformations that the researchers considered to be consequences of growth retardation.

In mice treated with arsenic acid, there was a significant increase in the number of resorptions per litter and significant decreases in the number of live pups per litter and mean fetal weight. However, overt maternal toxicity (including death) was found at the same or lower doses as those leading to developmental effects (Holson *et al*., 2000a; Nemec *et al*., 1998; Stump *et al*., 1999).

3.8 Platinum

After the introduction of automobile catalytic converters, platinum, palladium, and rhodium have been emitted with exhaust fumes, and increasing levels have been found in different environmental matrices such as road dusts, soils along heavily frequented roads, and sediments of urban rivers. Compared with other heavy metals, the biological availability of platinum, palladium, and rhodium in some experimental studies on road dusts ranged between that of cadmium and lead (Zimmerman and Sures, 2004). As stated by the same researchers, chronic effects on the biosphere cannot be excluded because of (1) their cumulative increase in the environment, (2) their unexpected high biological availability and bioaccumulation, and (3) their unknown toxicological and ecotoxicological potential.

Many clinical cases of pregnant patients affected by cancer treated by chemotherapy with platinum complexes have been reported in the obstetrical and gynecological scientific literature, with births at term and normal infants.

Male-mediated effects on Sprague–Dawley rats treated with a single intraperitoneal injection of cis-platinum were studied by Kinkead *et al*. (1992). Significant preimplantation loss was seen in the treated groups. The weights of the fetuses were also significantly lower than those of the control group. These results suggested that cis-platinum has a deleterious effect on the female reproductive system.

Morphological and functional effects on rat ovaries (Borovskaja *et al*., 2004), embryotoxic effects in rat (Chug *et al*., 1998), embryo lethality, and teratogenic effects (Ognio *et al*., 2003) have been demonstrated after exposure to platinum complexes.

3.9 Mixed Metal Exposure

For humans, there are few recent studies on mixed exposures to metals. Nordstrom and coworkers (1978a,b; 1979a) reported on the frequency of spontaneous abortions and birth weights of children born near a copper smelter in northern Sweden that emitted lead, arsenic compounds, and sulfur dioxide. The comparison of women working at the smelter with women not occupationally exposed suggested increased frequencies of abortions and depressed birth weights related to exposure. Because of the low levels of exposure in the nonoccupationally exposed population and the inclusion of smelter workers in the regional analysis, great care must be taken in interpreting the results. Similar results relating metal exposure to spontaneous abortions have been reported by Hemminki and coworkers (1980). Hospital records, union memberships,

and census data were used to identify groups with potential metal exposure. In women belonging to the union of metal workers, the rate of abortions was slightly higher (7.82%, number of abortions; $n = 195$) than the national average (7.34%; $n = 24{,}107$). The rate of abortions for women belonging to union branches with possible exposure to metals such as zinc, cobalt, and arsenic was compared for pregnancies during membership or for pregnancies before or after membership. The rate of abortions for conceptions during membership was again slightly higher than for nonmembership periods.

In a community study in which the major site of employment was a factory producing zinc and cobalt, increased abortions were noted for economically active women compared with all women in the community and for wives of men employed at the metallurgy factory compared with the wives of all industrial workers. The number of pregnancies among the female workers at the metallurgical factory was too low to be evaluated. However, data gathered from census and hospital records showed that the rate of abortions among welders (9.5%; $n = 28$) was higher than for all industrial workers (8.2%; $n = 2260$) (Hemminki *et al.,* 1983).

The effects of coexposure to metals on female reproductive function was demonstrated in a study carried out by Belles *et al*. (2002), focusing on the developmental toxicity in mice of lead nitrate (25 mg/kg, subcutaneously), methylmercury chloride (12.5 mg/kg, orally), and sodium arsenite (6 mg/kg, subcutaneously). Metals were administered on gestation day 10 separately or in their binary and ternary combinations. Maternal toxic effects were more remarkable in the group concurrently exposed to Pb, Hg, and As than in those given binary combinations of the elements and in those given the metals separately. With regard to developmental toxicity, the most relevant effects, namely decreased fetal weight and cleft palate, corresponded to the Hg-treated groups. These data suggest that at the current doses, the interactive effects of Pb and As on Hg-induced developmental toxicity were not greater than additive. In contrast, exposure of pregnant mice to Pb and As at doses that were practically nontoxic to dams, but administered concurrently with organic Hg at a toxic dose, caused supraadditive interactions in maternal toxicity.

4 DEVELOPMENTAL EFFECTS OF PRENATAL EXPOSURE

Exposure to metals during organogenesis may give rise to fetal anomalies, and exposure during other periods of development may result in embryo or fetal lethality or other developmental effects. Although methylmercury is clearly teratogenic for humans, other metals such as nickel, arsenic, and lead have less severe effects. Teratogenesis bioassays in rodents (hamsters, mice, or rats) have yielded positive results for the com pounds of many metals (Al, As, Bo, Cd, Co, Cr, Cu, Ga, Hg, Li, Mn, Ni, Pb, Se, U, V, and Zn), producing fetal and early postnatal deaths, as well as malformations such as anencephaly, eye defects, cleft palate, and skeletal anomalies (Sunderman, 1998). However, the relevance of these findings to human exposure is uncertain.

The effects produced by metals depend on the timing and duration of exposure, on their distribution and accumulation in various organs such as the nervous system, and on the ability to interfere with specific developmental processes. Effects may be enhanced by biochemical, physiological, and anatomical changes occurring during development, which may result in a modified metabolism of the metal itself (Rice and Barone, 2000). Interesting interactions between environmental and congenital factors have been documented; genetic polymorphisms can affect fetal susceptibility to teratogens or maternal ability to detoxify and excrete xenobiotics (Garcia, 2000).

Mechanisms of damage during development include proliferation (cell division), cell death, cellular differentiation, biosynthesis, cell–cell or tissue–tissue interactions, and cellular movements. Damage may be related to these kinds of epigenetic injury or to a direct genetic action on developing tissues (Mottet and Perm, 1983).

The primary etiological factors and pathogenetic mechanisms contributing to developmental effects caused by metal exposures are only partly known. Neurodevelopmental impairment may range from serious mental retardation and other overt clinical syndromes to subtle subclinical deficits as sensory, motor, and cognitive impairment that are difficult to identify. Subtle effects, although less severe at an individual level, may be important for public health policy. The damage may not become apparent until after some latency period; therefore, the critical periods of sensitivity and latent effects may complicate the interpretation of many experimental and epidemiological studies.

The symposium on Health Risks Associated with Prenatal Metal Exposure held in 1995 was designed to evaluate the health risks for mother and developing offspring associated with *in utero* exposure to metals commonly found in the workplace and/or ambient environment. Epidemiological and toxicological evidences of these effects were demonstrated, focusing on the mechanisms associated with exposure to arsenic, lead, and methylmercury (Zelikoff *et al*., 1995). Reviews by Carpenter (2001) and Mendola *et al*. (2002) may provide further background information.

4.1 Lead

Developmental effects of lead have been predominantly associated with postnatal exposure; however, evidence is emerging that both prenatal and postnatal exposures are important, and occur, for example, in areas where drinking water is contaminated by the metal.

Several studies have documented placental transfer of lead and lead levels in maternal blood, placenta, and cord blood. Environmental exposure in areas with water concentrations of lead >50 μg/L was associated with increased lead concentrations in cord blood and placenta, as well as in maternal blood. Lead levels in maternal blood were generally higher than in cord or neonatal blood, despite the higher affinity of fetal hemoglobin for lead (Alexander and Delves, 1981; Hubermont *et al*., 1978; Miller and Shaikh 1983; Ong and Lee, 1980; Roels *et al.,* 1978). Wibberley *et al*. (1977) studied placental lead levels in a series of Birmingham births classified as stillbirth, neonatal death, or survival beyond 1 week. Despite a wide range of lead levels even in normal births (15–356 μg/L) the results showed a pronounced excess of lead in those who failed to survive both birth and the neonatal period. There was no association of placental lead with impaired birth weight among survivors but, in agreement with other authors, seasonal variation was noted: with higher levels during the summer months. A study in Cassandra, Zambia, near a lead mine and smelting factory found mean blood lead (PbB) levels of 412 μg/L and 370 μg/L for mothers and infants, respectively, with a significant correlation ($r = 0.77$; $P < 0.001$) (Clark, 1977). The increased lead transfer, however, did not seem to have an adverse effect on the newborn's birth weight or red cell values.

The main current human data indicate that a direct association between moderate low-level lead exposure and malformations has not yet been proven, and there is a predominance of negative results. In particular, no evidence of association of low lead exposure with major congenital malformations has been reported.

However, Nordstrom *et al*. (1979a,b) investigated the frequencies of congenital malformations in the offspring of female employees at a smelter in northern Sweden and in the population living nearby. The frequency of single and multiple malformations was significantly higher (approximately twofold and fourfold, respectively) in the offspring of the women who had worked at the smelter during pregnancy. A significantly increased risk was found only in pregnancies where the mother had worked at the smelter. The numbers of malformations in the offspring of female employees were low; therefore, the results, although statistically significant, should be interpreted with caution. These results combined with those from studies carried out in seven different countries provide little evidence that low or moderate lead exposure is associated with an increased risk of major malformations. Effects of higher exposures are more equivocal. Needleman *et al*. (1984) examined 5183 consecutive deliveries of at least 20 weeks' gestation, correlating the congenital anomalies and lead concentration in umbilical cord blood. Lead was associated, in a dose-related fashion, with an increased risk of minor anomalies but not with the risk of malformations. However, an increased risk of neural tube defects was observed in an English study of mothers exposed to lead from the general environment (Bound *et al.*, 1997).

An effect on growth (stature about 2 cm shorter at 15 months of age) was noted for children whose mothers had a PbB concentration exceeding 77 μg/L, and the infant PbB increased to 100 μg/L during the first 3–15 months of life (Shukla *et al*., 1989). Bellinger *et al*. (1991) evaluated the relationship between prenatal low-level lead exposure and fetal growth in a sample of 4354 pregnancies. The authors concluded that the risk of adverse fetal growth is not increased at cord blood lead levels <150 μg/L but that modest increases in risk may be associated with levels ≥150 μg/L. Factor-Litvak *et al*. (1991) tested the hypothesis that exposure to lead during pregnancy from residence in a lead smelter town is associated with reduced intrauterine growth and an increase in preterm delivery. Mean PbB at midpregnancy was around 192 μg/L in the exposed women and 60 μg/L in the control group. After adjustment for the effects of potential confounders, no significant relationships were found between maternal PbB measured at midpregnancy, at delivery, or in the umbilical cord and birth weight or length of gestation or preterm delivery.

Dietrich *et al*. (1986; 1987) correlated prenatal, cord, and 10-day neonatal PbB levels and performance tests at 3 and 6 months. PbB levels during prenatal periods were inversely related to a series of sensorimotor developmental indices at 6 and 12 months of age, as well as lower birth weight. Further analyses by structural equation modeling showed that the effect of prenatal lead exposure, all less than 300 μg/L, was in part mediated through its effects on birth weight and gestational age.

Bellinger and coworkers (1994) investigated the association between prenatal and postnatal lead exposure and behavioral problems in 8-year-old Boston children. Prenatal and postnatal lead exposure was assessed by umbilical cord PbB (mean, 68μg/L) and dentin lead of a shed deciduous tooth (mean, 3.4μg/g).

The results showed that cord PbB level was not associated with the overall prevalence of problem behaviors. However, tooth lead was significantly associated with total problem behavior scores in both crude and adjusted analyses, suggesting that information about accumulated dose is important.

Some neurobehavioral effects, decreased ability to self-quiet and to be consoled, were associated with a rise in maternal blood lead levels from 36 weeks of gestation to birth (Rothenberg *et al.*, 1989). A longitudinal investigation of prenatal lead exposure and juvenile delinquency (Dietrich *et al.*, 2001) showed an increase in antisocial behavior with low-level lead exposure, even after adjusting for a variety of medical and social covariates. There is, however, still insufficient information on the role of potential confounding, as well as issues related to the transferability of experimental models, developmental periods of particular vulnerability, and dose-response relationships (Winneke, 1996).

A review (Bellinger, 2005) focused on the impacts of lead exposure on reproductive health and outcomes. Maternal blood lead levels of approximately 100 µg/L have been linked to increased risks of pregnancy hypertension, spontaneous abortion, and offspring neurobehavioral effects. Higher maternal lead levels have been linked to reduced fetal growth and congenital malformations, although considerable uncertainty remains regarding the specific malformations and the dose-response relationships. Of special concern is the likelihood that the fetus might be exposed to lead mobilized from bone stores as a result of pregnancy-related metabolic changes, making fetal lead exposure the result of exposure to a composite of exogenous lead during pregnancy and endogenous lead accumulated by the woman before pregnancy. Studies during recent years have confirmed that the risk to the fetus and long-term sequelae is discernible at progressively lower blood lead levels. In experimental animals, maternally administered lead is transferred to the conceptus. High concentrations of lead were found in fetal skeleton, liver, and blood but not in kidney (Dencker *et al.*, 1983).

Teratogenic effects may vary with the forms of lead and time of administration. Perm and Carpenter (1967) first observed specific tail and sacral vertebral anomalies in hamsters after administration of lead; these malformations persisted when lead was administered at different times during gestation; the histogenesis of these lesions is thought to involve localized edema, blisters, and hematomas (Carpenter and Perm, 1977; Perm and Perm, 1971). In mice, malformations occur in the anterior portion of the axial skeleton (Jacquet and Gerber, 1979); lead has also produced spina bifida and anencephaly in mice (Kruckenberg *et al.*, 1976). In rats, a single intravenous injection of lead nitrate (50–70 mg/kg) on day 9 of gestation caused urorectocaudal malformations; treatment on day 10–15 caused fetolethality, but administration after day 16 had little effect (McClain and Becker, 1975). Lead-chelator complexes were also found to be less teratogenic than free lead ions (McClain and Siekierka, 1975a,b).

Retarded fetal growth or fetal death is a consistent result of lead exposure during development. Resorptions and decreased fetal weights were noted in rats and mice after lead exposure (Kennedy *et al.*, 1975). Other effects have included depressed body length, brain, and body in neonatal pups (Carmichael *et al.*, 1981); increased resorptions in rats with no effects on fetal length or weight (Wardell *et al.*, 1982); and small (10%) decreases in the body weight of postpuberty pups (Ronis *et al.*, 1998).

Several behavioral parameters seem to be altered by prenatal exposure to lead. Discrimination learning is widely reported to be affected in animals at levels that did not alter growth (reviewed by Bornschein *et al.*, 1980); social behavior in mice was also altered (Cutler, 1977). By contrast, open-field activity, passive avoidance, and Rotorod performance were unaltered in surviving rats after maternal injection (intravenous) of 5 or 25 mg/kg lead nitrate on day 17 of pregnancy; the higher dose, however, produced increased mortality, hydrocephalus, and decreased weight in the pups (Minsker *et al.*, 1982). Grant *et al.* (1980) also reported no effect in most behavioral tests on rats after chronic exposure to lead.

4.2 Mercury

Several compounds of mercury are documented as affecting development in humans or experimental animals. Of these, methylmercury is the most thoroughly studied and best-documented example of a metal compound that disrupts normal development. Similar to lead, it exemplifies both the qualitative and quantitative differences in the response of adult and developing tissues. Reference reviews have been published by Castoldi *et al.* (2001), Clarkson (2002), Clarkson *et al.* (2003), and Davidson *et al.* (2004).

Methylmercury crosses the human placenta to the fetus. Mercury levels in the blood of prenatally exposed infants and in cord blood were higher than in maternal blood (Amin-Zaki *et al.*, 1974a; Miller and Shaikh, 1983). Although organic mercury bound preferentially to fetal erythrocytes, the highest concentrations of inorganic mercury were found in the placenta (Kuhnert *et al.*, 1981). The higher levels of mercury in cord and infant blood may reflect the

higher hematocrit of the fetus (White and Rothstein, 1973).

Prenatal exposure of humans to methylmercury can cause damage to the CNS, resulting in cerebral palsy and mental retardation in the infant. Engelson and Herner (1952) first described a human case of prenatal methylmercury poisoning, resulting from accidental contamination of food. An infant born to an exposed woman, with only minor signs of poisoning, suffered severe mental retardation. A similar case of family poisoning occurred in the United States in the late 1960s (Snyder, 1971).

Two major outbreaks of methylmercury poisoning, one in Japan in the mid-1950s and one in Iraq in the winter of 1971/72, included cases of prenatal poisoning. In Minamata, Japan, 21 cases of cerebral palsy in infants were associated with prenatal exposure (reviewed by Harada, 1968; 1977; 1995). The mothers generally experienced only mild, transient symptoms, whereas the effects on the children were severe and permanent. More subtle effects of prenatal exposure to methylmercury were described after a large outbreak of poisoning in Iraq in the winter of 1971/72 (Bakir *et al*., 1973). Families in rural Iraq consumed bread prepared from grain contaminated with a methylmercury fungicide. A number of children were severely affected (Amin-Zaki *et al*., 1974a). In a follow-up study, children with milder cases of psychomotor retardation were identified after prenatal exposure to methylmercury (Amin-Zaki *et al*., 1976; Marsh *et al*., 1977; 1980). The severity and frequency of effects in children correlated closely with maximum hair concentrations (marker of maternal exposure) in mothers during pregnancy (Clarkson *et al*., 1981; Marsh *et al*., 1980; 1981). Comparisons of dose-response relationships for the prenatally exposed children and nonpregnant adults in the Iraq outbreak indicated that the prenatal stage was roughly three to four times more sensitive to methylmercury damage than the adult (Clarkson *et al*., 1981). This dose-response relationship for prenatal effects was the first to be described for human exposure to any environmental chemical. Other studies have also used cord blood as an indicator of exposure to methylmercury (Grandjean *et al*., 1999a).

More recently, two large studies have examined the effects of low-level prenatal methylmercury exposure from consumption of fish or other seafood. The study based in the Faroe Islands examined neurodevelopmental outcomes in children whose mothers had consumed contaminated whale meat and fish (Grandjean *et al*., 1997; 1998; 1999b; Murata *et al*., 1999a). Subtle effects on brain function (motor function, visual spatial perception, language, attention, and memory) were found in children (age 7) exposed prenatally to low levels of mercury (mean 4.0 ppm mercury in maternal hair). In addition, prenatal methylmercury exposure was also associated with heart rate and blood pressure affects (Sorensen *et al*., 1999). The findings of delayed brainstem auditory evoked potential latency (Murata *et al*., 2004) and heart rate variability, but not blood pressure (Grandjean *et al*., 2004), have persisted in children now evaluated at 14 years of age. An additional study on infants in the same population associated methylmercury exposure with adverse neurological outcomes at 2 weeks of age (Steuerwald *et al*., 2000).

A second series of studies conducted in the Seychelles assessed global IQ in children with prenatal exposure because of maternal fish consumption (mean 6.0 ppm mercury in maternal hair); children were tested five times after birth up to age 9. In these studies, no detrimental effects of methylmercury exposure were found, except for one endpoint in boys only at 107 months (Davidson *et al*., 1995a,b; 1998; 2000; Myers *et al*., 1995). In fact, several endpoints were positively associated with mercury levels and fish consumption. A number of articles based on the Seychelles study, as well as Peru and Iraq, are found in a special issue of *Neurotoxicology* (Spyker Cranmer, 1995).

Possible explanations for the differing results include the patterns of exposure (episodic versus continuous), ethnic/genetic differences in responses to methyl mercury, types of neurodevelopmental measures, and possible confounders, such as diet other than fish and other chemicals found in seafood (Mahaffey, 1998; Nakai and Satoh, 2002).

Additional studies have examined children exposed prenatally to methylmercury in the Brazilian Amazon (Grandjean *et al*., 1999a), French Guiana (Cordier *et al*., 2002), Greenland (Weihe *et al*., 2002), Madeira (Murata *et al*., 1999b), New Zealand (Kjellstrom *et al*., 1986; 1989), Northern Quebec (McKeown-Eyssen *et al*., 1983), Peru (Marsh *et al*., 1995), and the Philippines (Ramierez *et al*., 2000; 2003). These studies reviewed by Castoldi *et al*. (2001) and Davidson *et al*. (2004) generally have small sample sizes, various designs and endpoints, and show differing results.

The effects of methylmercury on the developing and mature CNS are qualitatively different. In the adult, damage is selective in certain areas, whereas prenatal exposures produce more generalized damage. After prenatal exposure, the brain and skull may be abnormally small, and brain cytoarchitecture may be grossly disturbed (Huter and Russell, 1954). In two Iraqi children cell migration was disrupted, with Purkinje cells found in the white matter of the developing cerebellum (Choi *et al*., 1978).

Animal models provide additional information on the distribution of mercury compounds. As in humans,

methylmercury was readily distributed to fetal tissues in experimental animals (Berlin and Ullberg [1963c]; reviewed by Chang *et al*. [1980] and Stern *et al*. [2001]). Reported mercury concentrations in fetal brain were higher than in other fetal tissues and often exceeded maternal brain levels (Lee and Han, 1995; Matsumoto *et al*., 1967). Studies in nonhuman primates demonstrated greater movement of methylmercury from maternal to fetal circulation than back from fetus to mother, suggesting a mechanism for the accumulation of methylmercury in fetal tissues (Reynolds and Pitkin, 1975). Maternal mercury levels and infant blood kinetics were also described for a group of nonhuman primates that received between 0 and 90 μg methylmercury/kg/day orally during pregnancy (Stinson *et al*., 1989).

Developmental effects of methylmercury have been extensively studied in animals. Specific malformations have been induced by administration of a single dose of methylmercury during organogenesis. The sensitivity and types of malformations vary with time of treatment and strain or species of animals used. Examples include cleft palate (Gale and Ferm 1971; Olson and Massaro, 1977; Spyker and Smithberg, 1972; Su and Okita 1976a), limb malformations (Harris *et al*., 1972), and facial and brain defects (Inouye and Murakami, 1975; Spyker and Smithberg, 1972). Other developmental effects include increased fetal death and malformation (Nolens *et al*., 1972); decreased fetal weight, fetal death, and maternal toxicity (Mottet, 1974); and depressed birth weights (Curle *et al*., 1983).

Experimental models have been developed for neural damage caused by methylmercury. The original studies on experimentally caused Minamata disease were summarized in Murakami (1972). Cerebral damage has been reported in several types of animals (Fuyuta *et al*., 1978; Harada, 1977; Khera, 1973; Murakami, 1972). Cerebellar alterations have been detected in animals well after birth after *in utero* exposure (Chang *et al*., 1977a,b; Kakita *et al*., 2000a,b; Markowski *et al*., 1998; Reuhl *et al*., 1981a,b; Sakamoto *et al*., 2002).

Nonhuman primates have been used extensively to study the developmental toxicology of methylmercury. A series of studies have been conducted in a cohort of macaques exposed to relatively low levels of methylmercury *in utero* (0, 50, 90 μg/kg/day to the mother). Effects on birth weights and reproduction were seen in this group exposed to 90 μg/kg/day (Burbacher *et al*., 1984; 1988). Deficits in juvenile and adult macaques after methylmercury exposure *in utero* included visual recognition memory (Gunderson *et al*., 1986; 1988), social behavior (Burbacher *et al*., 1990), and growth (Grant-Webster *et al*., 1992). Tests of cognitive function yielded equivocal results; spatial memory was not affected (Gilbert *et al*., 1993), and only mild effects on fixed interval performance were noted in males after *in utero* exposure to methylmercury (Gilbert *et al*., 1996).

Behavioral changes have also been documented in other animal models exposed to methylmercury *in utero*. These include differences in motor coordination (Sakamoto *et al*., 2002), locomotor activity (Dorè *et al*., 2001; Geyer *et al*., 1985; Rossi *et al*., 1997; Spyker *et al*., 1972; Su and Okita 1976b), passive avoidance (Hughes and Annau, 1976; Kakita *et al*., 2000a; Sakamoto *et al*., 2002; Zanoli *et al*., 1994), operant conditioning (Musch *et al*., 1978), audiogenic seizure response (Meashi *et al*., 1982), and ultrasonic vocalizations (Adams *et al*., 1983). Studies have also documented sex-related differences on some behavioral measures (Goulet *et al*., 2003) and no effects on other behavioral measures (Colomina *et al*., 1997; Kakita *et al*., 2000b; Roegge *et al*., 2004; Rossi *et al*., 1997).

The mechanisms of damage that lead to the selective action of methylmercury on the developing nervous system are not well understood, although a number of cellular and molecular mechanisms have been proposed. See reviews by Castoldi *et al*. (2001, 2003), and Costa *et al*. (2004) for detailed discussions and references.

A few studies have documented effects on systems other than the nervous system, including impaired natural killer cell (NKK) function in (Ilback *et al*., 1991; Wilde *et al*., 1997); altered thymocyte development in mice (Thuvander *et al*., 1996); and structural changes in the kidney (Chang and Sprecher, 1976), although little persistent functional damage was noted (Smith *et al*., 1983); and ultrastructural changes in the liver (Ware *et al*., 1974; Fowler, 1983).

No human studies have documented adverse effects from prenatal exposure to elemental mercury or mercury vapor. In the general population, exposure of pregnant women to mercury vapor results primarily from mercury amalgam fillings in teeth. Mercury levels in autopsy tissues from infants and fetuses are reported to correlate with the number of mother's dental amalgams (Drasch *et al*., 1994). In another study, levels of inorganic mercury in blood and urine during early pregnancy correlated with amalgams, as did cord and maternal blood (Vahter *et al*., 2000). Removal of amalgam fillings during pregnancy may be an additional source of mercury vapor exposure, leading to potential fetal exposures (Molin *et al*., 1990). However, in a study that compared mercury levels in maternal and infant hair, no differences were found in woman who acquired amalgams during pregnancy as opposed to before pregnancy; both groups had higher levels of mercury in infant and maternal hair than those without amalgam fillings (Lindow *et al*., 2003). To date, the consequences of this prenatal exposure are not known.

Relatively few animal studies have been done with inorganic mercury or mercury vapor. After exposure

of pregnant rats to radiolabeled mercury vapor during late gestation, mercury accumulated in the placenta and the fetus in about equal ratios (Clarkson *et al*., 1972). There was substantial deposition of mercury in fetal tissues after exposure to mercury vapor in mice (Khayat and Dencker, 1982), guinea pigs (Yoshida *et al*., 1986), and squirrel monkeys (Warfvinge *et al*., 1994). High levels of mercury were found in fetal rat brain after prenatal exposure, but no adverse effects were noted except in the presence of maternal toxicity (Morgan *et al*., 2002). When pregnant rats were given dental amalgam restorations, mercury accumulated in fetal tissues, but was lower than in maternal tissues (Takahashi *et al.,* 2003). Combined mercury vapor and ethanol exposure increased mercury levels in fetal tissues (Dencker *et al*., 1983; Yoshida *et al*., 1997). In guinea pigs, mercury vapor was oxidized in the fetal liver and bound to metallothionein (Yoshida *et al*., 1987). Recent studies have identified a protective role for metallothionein in preventing transplacental transfer after mercury vapor exposure (Yoshida *et al*., 2002).

Some studies have demonstrated behavioral effects of prenatal exposure to mercury vapor (Danielsson *et al*., 1993; Fredriksson *et al*., 1992), whereas others in nonhuman primates showed no difference in various behavioral measures after prenatal exposure to fairly high levels of mercury vapor (Newland *et al.,* 1996).

Earlier studies using several species of animals showed little transport of inorganic mercury to the fetus (Berlin and Ullberg, 1963a–c; Clarkson *et al*., 1972; Suzuki *et al*., 1967). However, a more recent study using rats exposed to mercuric chloride during pregnancy and until weaning (postnatal day 20) reported measurable levels of mercury in fetal tissues, with the highest mercury levels in the kidney; levels of mercury were higher in fetal brains than maternal samples (Feng *et al*., 2004). Studies in BALB/c mice have also shown that prenatal exposure to mercuric chloride resulted in persistent alterations in immune function. Effects included inhibitory effects in females on cytokine projection by thymocytes, lymph node cells, and splenocytes; in contrast, stimulatory effects were observed in males (Silva *et al*., 2005). Distribution of mercury after administration of phenyl mercury exposure was similar to that of mercuric mercury (Berlin and Ullberg, 1963b; Garrett *et al*., 1972; Suzuki *et al*., 1967).

4.3 Cadmium

Data on the concentrations of cadmium in maternal blood and placental and fetal blood indicate that cadmium accumulates in the placenta, reaching the human fetus in detectable amounts (Lauwerys *et al*., 1978; Roels *et al*., 1978; Scanlon, 1972). Babies from smoking mothers, whose cadmium body burden is higher than in nonsmokers, have reduced weight at birth. It is difficult, however, to attribute the decreased birth weight exclusively to cadmium. Among nonsmoking women, cadmium levels in urine were higher in those with infants of below-normal birth weight. Data were not confirmed after adjusting urine cadmium levels by creatinine (Cresta *et al*., 1989). However, Nishijo *et al*. (2002) found an inverse correlation between maternal urinary cadmium excretion and gestational age after adjustment for maternal age.

Several studies in rats and mice indicate that cadmium may be fetotoxic from oral exposures before and during gestation. This fetotoxicity is most often manifested as reduced fetal weight and neurobehavioral toxicity (Baranski, 1987; Gupta *et al*.; 1993, Kostial *et al*., 1993; Sorell and Graziano, 1990; Whelton *et al*., 1988).

Nagymajtenyi *et al*. (1997) reported behavioral and neurotoxicological changes in rats treated with cadmium chloride during pregnancy, lactation, and 8 weeks after weaning (at doses ranging from 3, 5, to 14 mg/kg). Open-field behavior and spontaneous and evoked cortical activity were investigated at the age of 12 weeks. Vertical exploration and open-field center exploration were increased; spontaneous and evoked electrophysiological variables showed dose-dependent and generation-dependent changes, thus indicating that low-level exposure of rats to inorganic cadmium can affect some nervous system functions. Changes in some electrophysiological parameters and higher order nervous functions were confirmed by Desi *et al*. (1998) in rats at low-level prenatal and postnatal inorganic cadmium exposure during pregnancy.

4.4 Chromium

Little is known about the developmental effects of chromium in humans or animals. A descriptive geographical study on congenital malformations in communities around a site heavily polluted by chromium waste was carried out by Eizaguiree *et al*. (2000). A 10-km circle centered on the polluted site was subdivided into one circle of 2-km radius and eight 1-km wide rings. The relative risk of congenital malformations for the closest circle seemed to be markedly lower than that for the rest; the relative risk peaked in the rings 2–4 km away from the polluted site. The authors excluded a possible teratogenic effect of the chromium coming from waste with different distribution of the two forms of chromium. Chromium (VI), which accumulates in erythrocytes, was transferred readily to the fetal mouse on day 13 of gestation, whereas there was little transfer of chromium (III), which is bound mainly to plasma proteins. Later in gestation, both chromium (VI) and chromium (III) accumulated in calcified areas of fetal skeleton (Danielsson *et al*., 1982).

Chromium trioxide (at a dose that produced maternal toxicity) caused fetal death and increased the incidence of cleft palate and skeletal anomalies in hamsters on day 7, 8, or 9 of pregnancy; administration on day 10 or 11 had little effect (Gale, 1978; Gale and Bunch, 1979). Different rates of malformations were also observed in different strains of hamsters after chromium treatment (Gale, 1982). Similar results of fetal lethality and malformations in survivors were produced in mice after intraperitoneal injection of approximately 20 mg Cr(III)/kg (Matsumoto *et al*., 1976). However, no effects were noted in pregnant mice receiving chromium (VI) during organogenesis (Junaid *et al*., 1996b). Danielsson and coworkers (1982) investigated the relative toxic actions of chromium (III) and chromium (VI). Cartilage formation in differentiating chick fibroblast cultures was sensitive to damage by chromium (VI) but unaltered by chromium (III). These data suggest that the developmental effects of chromium (VI), which are greater than those of chromium (III), may result from increased uptake and greater direct toxicity.

4.5 Nickel

Nickel has not been shown to affect development in humans, and available data are based on animal experimental studies. In term infants, cord blood and maternal concentrations of nickel are nearly equal. However, detectable amounts of nickel have been identified in human fetal tissues and are somewhat higher than concentrations in full-term infants (Casey and Robinson, 1978).

In experimental animals, nickel was localized within embryos on day 8 of gestation after intramuscular or intraperitoneal injections of nickel (Sunderman *et al*., 1978) and in the visceral yolk sac, gastrointestinal tract, and kidney of the fetus on day 16 in the mouse (Olsen and Jonsen, 1979). Fetal tissue concentrations of nickel are generally higher than maternal levels in late gestation (Jacobsen *et al*., 1978).

The embryotoxicity and teratogenic effects of nickel have been reviewed by Sunderman *et al*. (1983). Malformations after the administration of soluble nickel salts have been reported in hamsters (Ferm, 1972; Sunderman *et al*., 1980), mice (Lu *et al*., 1979; Storeng and Jonsen, 1981), and rats (Sunderman *et al*., 1979); anomalies included ocular, skeletal, and neural defects and were generally observed after single parenteral administration. Nickel carbonyl seems to be the most teratogenic of the nickel salts even after exposure by inhalation (Sunderman *et al*., 1979; 1980). Neither $NiCl_2$ nor $Ni3S_2$ produced malformations in rats when injected on day 6 or 8 of gestation, although fetal weights were reduced (Sunderman *et al.,* 1978). Chronic and/or oral treatment is more likely to produce diminished growth without increased frequency of malformations (Ambrose *et al.,* 1976). Available animal data suggest that the developing fetus and neonates are sensitive targets of nickel toxicity, although effects were often reported at maternally toxic doses (Nieboer, 1988).

4.6 Arsenic

Human data about congenital malformations or developmental effects potentially caused by arsenic derive mainly from investigations of populations living near smelting factories or arsenic-processing plants (Nordstrom *et al*., 1978a,b; 1979a,b). In these studies, however, the analyses gave limited consideration to potential confounders (e.g., smoking), and there were no data specifically relating the apparent effects to arsenic exposure alone. After evaluating 13 studies of human populations and 43 laboratory animal or *in vitro* studies, DeSesso *et al*. (1998) concluded that neither the human nor the animal studies were of sufficient robustness to be conclusive.

Structural malformations in experimental animals were induced only when maternal arsenic blood concentrations were very high and depended on the route of administration under conditions not usual or relevant for human exposure (Holson *et al*., 2000b).

The inhalation of high amounts of arsenic, as As_2O_3, produced developmental effects in laboratory animals. Exposures lower than 2.2 mg As/m^3 caused modest decreases in fetal body weight, and those lower than 0.20 mg As/m^3 had no effects. However, it is unclear whether these effects occur only at doses that cause maternal toxicity (Nagymajtenyi *et al*., 1985).

In other experiments, no increases in fetal resorptions, fetal mortality, or malformations, and no decreases in fetal birth weight occurred when rats were exposed to 0.2–7 mg As/m^3 (as As_2O_3) before mating and during gestation. Around the 8 mg/m^3 exposure level, toxicity was observed in the dams (Holson *et al*., 1999).

4.7 Vanadium

Decreased fertility, embryolethality, fetotoxicity, and teratogenicity have been demonstrated in rats, mice, and hamsters after vanadium exposure, but it is not certainly established whether vanadate (V^{+5}) and vanadyl (V^{+4}) compounds are reproductive and developmental toxicants (Domingo, 1996). The same author (Domingo, 2002) reported reproductive, developmental, and behavioral toxicity, as well as mitogenic activity affecting the distribution of chromosomes during mitosis, inducing aneuploidy-related endpoints.

4.8 Uranium

Little is known about the potential toxic effects of uranium on reproduction and development in animals and humans.

A study of more than 13,329 Navajos born at the Indian Public Health Service Hospital was carried out to address the association between birth defects, stillbirths, and other adverse outcomes of pregnancy and exposure to uranium from mining and milling operations (Shields *et al*., 1992). The only statistically significant association between uranium pollution and unfavorable birth outcomes was identified with the mother living near tailings or mine dumps; these associations were weak and attributed to radiation, not to the chemical toxicity of uranium.

In vitro tests indicate that alloys with depleted uranium may be both genotoxic and mutagenic (Bosque *et al.*, 1992; 1993a,b). Exposure of pregnant rodent dams to uranium produced fetotoxicity and developmental effects. Various effects include reduced fetal weight, cleft palate, and dorsal and facial hematomas in animals dosed with uranyl acetate on gestational days 6–15 (Domingo *et al*., 1989); decreased fetal weight and incomplete bone (Bosque *et al*., 1993a); increased number of dead and reabsorbed fetuses, reduced fetal weight, and a higher incidence of skeletal variations occurred among surviving offspring (Bosque *et al.*, 1992); decreased fertility, teratogenicity, and reduced growth of the offspring was also observed (Domingo 2001).

4.9 Aluminum

As assessed by Domingo (1995), there is relatively little information regarding the embryotoxic and teratogenic activity of aluminum. The developmental toxicity of aluminum is dependent on the route of administration (Cranmer *et al*., 1986; Golub *et al*., 1987; Gomez *et al*., 1990; Paternain *et al*., 1988) and the solubility of aluminum compounds (Colomina *et al*., 1992; Gomez *et al*., 1991; Partridge *et al.*, 1989). Although there was no evidence of teratogenicity when high doses of aluminum hydroxide were administered to pregnant mice (Domingo, 1989) or rats (Gomez *et al*., 1990) during organogenesis, developmental effects were seen in rats and mice fed with aluminum chloride, lactate, or nitrate (Bernuzzi *et al*., 1989; Domingo *et al.*, 1987; Paternain *et al*., 1988). Neurotoxicity and behavioral changes have been reported in offspring at doses of aluminum that did not produce maternal toxicity in rabbits (Yokel, 1987), rats (Bernuzzi *et al*., 1989; Muller *et al*., 1990), and mice (Donald *et al*., 1989; Golub *et al*., 1987).

4.10 Lithium

Human data indicated that lithium, at doses resulting in serum levels typical of the therapeutic range, might cause developmental toxicity and an increased risk of major malformations (particularly cardiac). Because other information on teratogenic effects is contradictory, it is prudent to exercise caution in treating pregnant women with lithium (Leonard *et al.,* 1995). Effects seen in animals, such as nephrotoxicity or behavioral alterations in offspring, have not been confirmed in children of lithium-treated women (Moore *et al*., 1995)

5 DEVELOPMENTAL EFFECTS FROM NEONATAL EXPOSURE

Neonatal and early postnatal periods are lifespan segments during which sensitivity to toxic agents is high. The postnatal period is characterized by rapid growth and development, with higher caloric and nutritional requirements and with the activation of specific metabolic pathways.

Neonates differ from adults in metal absorption, distribution, metabolism, and excretion. For example, absorption from the gastrointestinal tract is influenced by higher neonatal gastric pH and the immaturity of the intestinal mucus; gastrointestinal absorption has been shown for certain metals to be higher in the developing organism than in adults. Higher respiratory rates may also put the developing child at increased risk for exposure to and absorption of airborne metals. Metal distribution may also differ, because of the different percentage of total body water (higher in infants and decreasing till 10–12 years), and the low level of plasma proteins, resulting in a higher amount of the diffusible fraction of metals. The weight and body ratio for each organ must be also considered. The brain/whole body ratio, for instance, is higher at delivery and decreases in subsequent years. Many enzymes such as those active in kidney for excretive function of metals are present at birth in lower concentrations and begin to increase during the first year of life.

Another factor of higher susceptibility during perineonatal periods is the relative immaturity of the different organs or tissues. For example, the blood–brain barrier is not fully developed until 6–9 months, and the toxic elements may be transferred to the brain more readily during the first months of life. High absorption of toxic elements may also be related to "normal" infant behaviors such as mouthing of objects or ingestion of nonfood materials that may be contaminated. Last, food intake may not only determine the amount of toxic metals but may also influence their absorption,

bioavailability, and toxicity. Differences in exposure and metabolic responses to metals have been reviewed by Sanstead *et al*. (1983) and Mahaffey (1983).

For breast-fed children, the main source of metals during the neonatal period is maternal milk. The nutritional habits of the mother and her current or past environmental or occupational exposure strongly influence the kind and level of xenobiotics excreted by the milk, caused, in part, by the redistribution of cumulative maternal bone stores. Ettinger *et al*. (2004) studied the relationship between maternal breast milk lead and infant blood lead levels among 255 mother–infant pairs exclusively or partially breast-feeding in Mexico City. Although the levels of lead in breast milk were low, they clearly influenced infant blood lead levels. These findings are balanced by the fact that human milk is an excellent source of nutrition for young infants, and environmental sources of lead contribute more to neonatal lead intake than do dietary sources (Lin-Fu, 1980) despite the more recent significant lowering of environmental lead pollution in many industrialized countries.

5.1 Lead

Lead is the most intensively studied metal to which neonates and infants have been exposed. Numerous studies in children and animal models have confirmed the adverse effects of lead exposure on cognition and other neurological functions, which constitute a serious public health problem in a global perspective.

The evidence for lowered cognitive ability in children exposed to lead has come largely from prospective epidemiological studies. In the past decade, children's blood lead levels have fallen significantly in a number of countries, and current mean levels in developed countries are typically below 50 μg/L. Despite this reduction, childhood lead poisoning continues to be a public health problem mainly for certain high-risk groups. The current World Health Organization (WHO)/Centers for Disease Control and Prevention (CDC) blood level of concern stands at 100 μg/L. However, recent studies have extended the association of blood lead and intellectual impairment to lower levels of lead exposure (Lanphear, 2005), and the 100 μg/L level should, therefore, not be interpreted as a toxicological threshold or no-effect value The recent studies cast doubt on whether a toxicological threshold and no-effect value exists for lead-related developmental toxicity as will be discussed in greater detail later.

The neonatal period is particularly sensitive to the effects of lead, because there is a higher amount of absorption from the gastrointestinal tract, a greater transfer of metals to the brain, and a higher vulnerability of the developing central nervous system (Cory-Slechta and Schaumburg, 2000; Leggett, 1993). The prevalent opinion is that there is an inverse relationship between IQ and blood lead level, but the magnitude of this effect has been the subject of some debate (Dietrich *et al.,* 1993; Tong *et al*., 1996; Winneke and Kramer, 1997). In children, severe, clinically overt neurobehavioral effects are usually associated with blood lead levels higher than 700 μg/L, but the current concern is focused on more subtle adverse effects caused by significantly lower levels. Great efforts have been directed to ascertain safe levels of blood lead (Adams and Victor, 1993; Cory-Slechta and Schaumburg, 2000; Moreira and Moreira, 2004). From a public health perspective, however, current lead exposure accounts for a small amount of variance in cognitive ability (1–4%), whereas social and parenting factors account for 40% or more.

Long-term follow-up studies carried out during the 1990s indicated that neuropsychological deficits persisted into adulthood and also involved social/emotional development (White *et al.,* 1993). Sciarillo *et al*. (1992) and Stokes *et al*. (1998) observed an increased incidence of depression, somatic complaints, and aggressive behavior in 4–5-year-old lead-exposed children.

To improve dose-response information on the neurobehavioral effects of environmental lead exposure in children, the WHO in collaboration with the Commission of the European Communities executed an international study between 1984 and 1989 in eight European countries (Winneke *et al*., 1990). Blood lead concentration (PbB) was the main marker of exposure. A number of tests evaluated psychometric intelligence, visual-motor integration, reaction performance, and behavior ratings; 1879 school-age children with PbB ranging from less than 50 to about 600 μg/L were examined. The strongest and most consistent effects occurred for the Bender Gestalt test (visual motor integration) and serial choice reaction performance. Psychometric intelligence was also negatively affected, although the association with PbB was only borderline.

The NHANES III, conducted from 1988 to 1994, assessed the relationship between blood lead concentration and performance among 4853 children in the United States aged 6–16 years (Lanphear *et al*., 2000). The mean blood lead concentration was 19 μg/L; 172 children (2.1%) had blood lead concentrations >100 μg/L. After adjustment for many covariates and confounders, the data showed an inverse relationship between blood lead concentration and scores on four measures of cognitive functioning. For every 10 μg/L increase in blood lead concentration, the following decrements in mean scores were noted: 0.7-point in

arithmetic, approximately 1-point in reading, 0.1-point in nonverbal reasoning, and 0.5-point in short-term memory. An inverse relationship between blood lead concentration and arithmetic and reading scores was observed for children with blood lead concentrations lower than 50 μg/L.

Other recent studies of neurobehavioral effects and cognitive function of children exposed postnatally to lead include one by Tong *et al*. (1996), who examined 375 children born near the lead smelting town of Port Pirie, Australia, for an association between environmental lead exposure and children's intelligence at age 11–13 years. Verbal, performance, and full-scale IQ were inversely related to PbB, with no apparent threshold. The expected mean full scale IQ declined by 3.0 points (95% CI, 0.07–5.93) for an increase in lifetime average blood lead concentration from 100–200 μg/L.

Walkowiak *et al*. (1998) investigated the effects of low-level lead exposure (mean, 43 μg/L; 95th percentile, 89 μg/L) on cognitive functioning in a cohort of 6–7-year-old German children. Impaired attention was demonstrated, whereas visual perception, visual memory, fingertapping, and reaction time were unaffected.

Wasserman *et al*. (1998) studied 379 3-year-old children born from mothers living near a lead smelter in Yugoslavia (geometric mean PbB of 409 μg/L in the exposed subjects versus 98 μg/L in controls). At age 3, small but significant decrements were found in destructive and withdrawn scores, somatic and sleep problems, and anxious-depressed subscale, after checking for confounders. Wasserman *et al*. (2000) conducted a subsequent investigation of 442 children from the same Yugoslavian lead-polluted area, comparing the relative contribution of prenatal blood lead with that of relative increases in PbB in either the early (0–2 years) or the later (from 2 years on) postnatal period to child intelligence measured at ages 3 and 4. This study confirmed that elevations in both prenatal and postnatal PbB were associated with small decrements in the children's intelligence.

Canfield *et al*. (2003) measured blood lead concentration in 172 children at 6, 12, 18, 24, 36, 48, and 60 months of age and compared it with the Stanford–Binet Intelligence Scale at the ages of 3 and 5 years. Blood lead concentration was inversely associated with IQ. Estimated by a linear model, each increase of 100 μg/L in the lifetime average blood lead concentration was associated with a 4.6-point decrease in IQ ($P = 0.004$). When estimated in a nonlinear model, IQ declined by 7.4 points as lifetime average blood lead concentrations increased from 10 to 100 μg/L.

Carta *et al*. (2003) assessed 64 adolescents living in a polluted area in Italy. By applying a linear model, it was found that the blood lead concentration was inversely associated with IQ, with an extrapolated decline of 1.29 points in total IQ for 10 μg/L increase in blood concentration.

Coscia *et al*. (2003) examined the effect of lead exposure on cognitive growth patterns in 196 children at ages 6.5, 11, and 15 years. Children with higher lead levels, measured at 15 years of age, showed lower verbal comprehension scores over time and greater decline in their rate of vocabulary development. Lead exposure was not significantly correlated with growth in perceptual organization test scores. Moreover, socioeconomic status and maternal intelligence were statistically significantly associated with growth patterns for both test scores, independently from the effects of lead.

Counter *et al*. (2005) assessed the effects of PbB concentration on nonverbal intelligence in Andean children working in glass manufacturing and chronically exposed to lead. A significant negative association ($r = -0.331$; $P < 0.0001$) was noted between PbB level and a non-language-based test of cognitive function—an approximate two-point decrease in IQ for each 100 μg/L increase in PbB.

Koller *et al*. (2004) reviewed the studies of Lanphear *et al*. (2000) and of Canfield *et al*. (2004) along with others in the field of low-level lead effects on children's cognitive development (Al-Saleh *et al*., 2001; Calderon *et al*., 2001; Garcia Vargas *et al*., 2001; Prpic-Majic *et al*., 2000; Rahbar *et al*., 2002; Wang *et al*., 2002) and concluded that these findings are scientifically important and that efforts to reduce childhood exposure to lead should continue.

Lanphear *et al*. (2005) carried out pooled analysis to examine the association of intelligence test scores and blood lead concentration, especially for children who had maximal measured blood lead levels <100 μg/L. They collected data from 1333 subjects from seven population-based longitudinal cohort studies, followed from birth or infancy until 5–10 years of age. The full-scale IQ score was the primary outcome measure. The geometric mean PbB peaked at 178 μg/L and declined to 94 μg/dL by 5–7 years of age. After adjustment for covariates, the estimated IQ point decrements associated with an increase in blood lead from 24–100 μg/L, 100–200 μg/L, and 200–300 μg/L were 3.9, 1.9, and 1.1, respectively. This suggests that the dose-response curve is steeper at the lower levels compared with the higher ones.

As alluded to previously, no clear and generally accepted threshold value has been identified. The current WHO/CDC blood level of concern stands at 100 μg/L but is being challenged by the recent studies showing adverse effects below this level. Meta-analysis on both cross-sectional and prospective studies

in lead-exposed children have concluded that a typical doubling of PbB from 100–200 μg/L is associated with an average IQ loss of 1–3 points. Proportional regular decrements of IQ (around 5–10 points) may be observed at levels of exposure up to 300 μg/L.

Lead toxicity and nutritional status may also be associated. Kordas *et al*. (2004) examined whether nutritional status variables account for part of the association between cognitive performance and lead exposure in 724 first-grade children aged 6–8 years. The mean blood lead concentration was 115 + 61 μg/L, and 50% of the children had concentrations >100 μg/L. Lead was negatively associated with four cognitive tests and was also inversely correlated with iron status, height-for-age Z scores, and head circumference. In multivariate models, the association between lead and cognitive performance was not strongly affected by nutritional variables, suggesting that the relation of lead to cognition was not explained by iron deficiency or growth retardation.

Chelation therapy has been considered as a possible means to decrease lead body burden and related effects, but it is unclear whether it can prevent or reverse the neurodevelopmental sequelae of lead toxicity. Liu *et al*. (2002) analyzed 741 children who had blood lead levels between 200 and 440 μg/L and were 13–33 months old at random assignment to chelation or placebo. By 6 months after randomization, blood lead levels had fallen by similar amounts in both chelated and placebo children, despite the immediate drops in the chelated group; there was no association between change in blood lead level and change in cognitive test score, and chelation therapy may not be indicated at this blood lead range. The improvement in scores in the placebo group implies that factors other than declining blood lead levels alone are responsible for cognitive effects. Chelation therapy lowered average blood lead levels for approximately 6 months but resulted in no benefit in cognitive or behavioral endpoints (Dietrich *et al*., 2004).

Several correlates to the observations made in humans and proposed mechanisms of action have been produced in experimental studies. In animals (rats and monkeys), observations on the basis of a broad spectrum of learning and retention models support the hypothesis that lead-induced neurobehavioral deficit extends long into adulthood, primarily after preweaning exposure; the evidence is less clear after postweaning lead exposure.

Lead administered to lactating experimental animals appears in their milk. This has been exploited as a model for postnatal exposure to lead. A significant fraction of maternally administered lead is transferred to rodent pups in milk (Keller and Doherty, 1980a,b; Momcilovic and Kostial, 1974). Although lead rapidly enters the brain from blood, its removal is slow (Goldstein and Diamond, 1974). The effect of several constituents of milk on lead absorption has been investigated in rodents. The addition of iron did not suppress uptake of lead in rats (Kostial, 1983); however, calcium and phosphorus reduced lead absorption (Mahaffey, 1981). Data from Barltrop and Khoo (1976) also suggest that the fat content of milk is responsible for increasing lead absorption. Neurological damage and neuropathology have been produced in rats and mice by exposing neonates to lead through milk (Bornschein *et al*., 1980). Effects include altered dendrite development and synapse formation (Alfano and Petit, 1982; Alfano *et al*., 1982; McCauley *et al*., 1982); changes in hippocampus structure and function (Alfano and Petit, 1981; Campbell *et al*., 1982); neurochemical alterations (Silbergeld and Hruska, 1980); and disruption of calcium homeostasis in the immature neonates (Winneke *et al*., 1996).

5.2 Mercury

Neonates may be exposed to methylmercury exposure through breast milk. In the Iraqi outbreak in 1971/72, mercury levels in milk were found to correlate with maternal blood levels (Bakir *et al*., 1973), with milk mercury approximately 5–8% of maternal blood levels (Amin-Zaki *et al.,* 1976). Infants born before the outbreak accumulated significant amounts of mercury through milk from mothers who ate contaminated bread (Amin-Zaki *et al.,* 1974b). Delays in motor development, speech development, and toilet training, as well as abnormal reflexes were noted in these children (Amin-Zaki *et al*., 1980). The half-life of mercury in blood was shorter in lactating women (about 42 days) compared with nonlactating women or men (about 75 days), but the difference could not be accounted for by secretion of mercury in milk (Greenwood *et al*., 1978).

In more recent studies in the Faroe Islands, children were examined during the first year of life for three developmental milestones (sitting, creeping, and standing). In this case, children who reached milestones early had significantly higher levels of mercury at 12 months of age. This could be due to the duration and advantages of breastfeeding, although this resulted in higher mercury levels in the children (Grandjean *et al.,* 1995).

Most animal studies of methylmercury have focused on prenatal exposure (see previous section of this chapter). Methylmercury was secreted into the milk of experimental animals (Mansour *et al.,* 1973; Yang *et al*., 1973) and was readily absorbed from the gut of neonates (Zepp *et al*., 1974). Neonatal rodents are reported to excrete only a small fraction of their body burden of methylmercury until weaning (Doherty and Gates, 1973; Thomas *et al*., 1982) with an abrupt change

in excretion rates taking place at weaning. Little is known about excretion patterns of methylmercury in human infants.

A number of effects have been noted in animals exposed postnatally to methylmercury. One group of nonhuman primates exposed to low levels of methylmercury (50 µg Hg/kg/day) from birth to 7 years, was followed for various types of behavioral changes more than 20 years. Mercury-related deficits were found in auditory, somatosensory, and visual function (Rice and Gilbert, 1982; 1990; 1992; 1995). Auditory impairment was found to increase with age between 11 and 19 years (Rice, 1998a). However, tests for cognitive impairment performed at age 20 years were negative (Rice, 1998b). This is consistent with other studies in which macaques were exposed to methylmercury *in utero* and postnatally; there was no reported mercury related impairment of discrimination reversal tasks when animals were tested as infants or juveniles (Rice, 1992). However, evidence of delayed toxicity has been suggested. Animals at 13 years of age (6 years after exposure stopped) began showing signs of clumsiness and impaired fine motor performance. Clinical neurological assessments revealed insensitivity to touch (Rice, 1989). Similar delayed signs of neurotoxicity were reported in animals exposed to methylmercury *in utero* and up to 4 years of age (Rice, 1996).

In rodent models, behavioral/locomotor alterations (passive avoidance response and Rotorod performance) were noted in rats exposed *in utero* and postnatally to moderate doses of methylmercury. Histopathology of the brains included focal cerebellar dysphasia (Sakamoto *et al.,* 2002). Exposure of rats exclusively during the postnatal period to methylmercury (5 mg/kg/day for 30 days) resulted in severe paralysis of the hind limbs and widespread neuronal degeneration in many areas of the brain (Sakamoto *et al.,* 1998). Earlier studies had documented changes in cerebellar structure and cell division (Choi *et al.,* 1981; Rodier *et al*., 1984; Sager, 1988; Sager *et al*., 1982; 1984). Other groups have noted neurochemical effects after postnatal exposure. Movement and postural disorders in rats correlated with altered metabolism of brain catecholamines and indoleamines (O'Kusky *et al*., 1988) and altered biogenic amines (Taylor and Di Stefano, 1976).

Recent attention has focused on another form of organic mercury, thimerosal (ethylmercury thiosalicylate, also known as thimerosal). Although thimerosal has been used as a preservative in vaccines and other medicinal products since the 1930s, there is relatively little information about the potential reproductive or developmental toxicity of the compound. Reports have suggested that the increased number of infant vaccines containing thimerosal correlated with the increasing rate of autism (reviewed by IOM, 2001). Many countries in Europe removed thimerosal from infant vaccines several years ago. In 1999, the United States took steps to remove thimerosal from routine vaccines for children under the age of 6. However, exposure of infants and children to thimerosal-containing vaccines still occurs in countries where multidose vials of vaccine are used (Knezdvic *et al*., 2002).

Mercury levels after exposure to routine vaccinations containing thimerosal have been evaluated in several studies. Stajich *et al*. (2000) measured total mercury levels in blood before and after administration of the birth dose of hepatitis B vaccine (12.5 µg mercury). Blood mercury levels in 15 preterm and 5 term neonates, measured at 48–72 hours after immunization, showed elevated levels of mercury. Postvaccination levels were about three times higher in preterm infants than term infants (mean, 7.36 µg/mL vs. 2.24 µg/mL). Pichichero *et al*. (2002) examined levels of mercury in blood, urine, and stool 3–28 days after 2- and 6-month-old infants were exposed to thimerosal through vaccination. They found that blood levels of mercury were lower than expected (means, 8.2 nmol/L in 2-month- olds; 5.15 nmol/L in 6-month-olds), on the basis of extrapolation from methylmercury. Significant amounts of mercury were excreted in stool (81.8 ng/g dry weight in 2-month-olds; 58.3 ng/g dry weight in 6-month-olds). In addition, the half-time in blood of mercury derived from thimerosal seemed to be shorter than that for methylmercury (about 7 days vs. 45 days). A follow-up study in neonates, 2-month, and 6- month-olds in Argentina, who received routine vaccinations containing thimerosal, confirms the earlier study (Pichichero *et al*., 2002).

An outbreak of ethyl mercury poisoning occurred in Iraq in 1960, resulting from the consumption of seed grain treated with a fungicide containing ethyl mercury toluene sulfonanilide (Damluji, 1962; Jalili and Abbasi, 1961). Although five children (age 4–8) were included in the Damluji study, there did not seem to be any differential toxicity. The children represented mild to moderately severe cases. In addition, elevated mercury levels in blood and tissue were reported in five infants with omphaloceles who were treated with thimerosal (Fagan *et al*., 1977). Other cases of mercury-related adverse events were also reported in infants and adults receiving this treatment (Stanley-Brown and Frank, 1971; reviewed by Magos, 2001).

Several studies have examined the correlation of thimerosal containing vaccines and autism. Controlled epidemiological studies in Denmark, Sweden, the United Kingdom, and the United States provide no evidence for an association between thimerosal exposure through vaccination and autism (Andrews *et al.,*

2004; Heron *et al*., 2004; Hviid *et al*., 2003; Parker *et al*., 2004; Verstrachen *et al.*, 2003). In these studies, rates of autism continued to rise despite removal of thimerosal from infant and childhood vaccines. Two uncontrolled studies led to the same conclusions (Madsen *et al.*, 2003; Stehr-Green *et al*., 2003). Although other studies reported correlations, these studies were cited by the IOM report as having serious methodological flaws or nontransparent analytic methods. Thus, the IOM concluded that the evidence favors rejection of a causal relationship between thimerosal-containing vaccine and autism (IOM, 2004).

Studies have been conducted in animal models to assess the distribution of mercury to tissues after exposure to thimerosal and/or ethylmercury compared with methylmercury. In studies in infant macaques exposed to injected thimerosal (with appropriate infant vaccines) or oral methylmercury, initial distribution of mercury from both exposures was similar (Burbacher *et al.*, 2005). However, total mercury was cleared from blood more than twice as fast after thimerosal exposure (T1/2 6.9 days vs. 19.1 days). In addition, the faster clearance from blood resulted in no significant accumulation in blood after repeated thimerosal exposure compared with methylmercury. Total mercury accumulation in brain was three to four times less after thimerosal exposure, although inorganic mercury levels were higher; inorganic mercury represented 71% of total brain mercury after thimerosal versus 10% for methylmercury. Blood to brain partitioning ratio for total mercury was slightly higher in the thimerosal group (3.5 vs. 2.5). This may indicate greater deethylation versus demethylation in brain. An additional study in neonatal mice confirmed that thimerosal exposure resulted in lower blood and brain levels of total mercury compared with methylmercury (Harry *et al*., 2004).

During the first half of the 20th century, mercuric oxide and mercuric chloride were administered to infants, frequently in teething powders. Acrodynia or pink disease, characterized by painful red hands and feet, affected only a small fraction of the children exposed (reviewed by Warkany, 1966). More recently, a few cases of acrodynia were identified in Argentina, where phenyl mercury had been used as a fungicide in diapers (Astolfi and Gotelli, 1981). In addition, transient effects on kidney function were noted for infants whose urinary mercury levels reached a "threshold" level (Gotelli *et al.*, 1985).

Little is known about exposure of infants to mercury vapor. One case of pink disease has been reported in an infant after contamination of the bedroom (Curtis *et al*., 1987). Mercury vapor levels near the floor where children are likely to spend time may be much higher because mercury vapor is heavier than air.

5.3 Cadmium

Few experimental animal studies have shown neurotoxic behavioral effects after neonatal cadmium exposure, and there are no published reports on early postnatal cadmium absorption or related developmental effects in humans.

Infants absorb up to 55% of the ingested dose compared with 10% absorption in adults (Alexander *et al*., 1974). Little maternally administered cadmium is secreted in milk in experimental animals (Kostial, 1983; Lucis *et al*., 1972). Factors affecting cadmium uptake include levels of dietary ascorbic acid, calcium, copper, iron, protein, selenium, vitamin D, and zinc (Fox, 1974).

Postnatal exposure to cadmium at high doses (6 mg/kg) was reported to induce synthesis of metallothionein; it did not, however, protect against the toxic effects of cadmium (Bell, 1979; 1980). Discrimination learning was not affected in rats exposed prenatally and postnatally to cadmium, although spontaneous activity decreased (Hastings *et al*., 1978).

5.4 Nickel

Information is solely based on experimental studies. Perinatal toxicity associated with nickel chloride exposure in drinking water was studied in female Long–Evans rats, which were exposed beginning 11 weeks before mating and through two successive gestation and lactation periods (Smith *et al.*, 1993). The frequency of perinatal death is the most significant toxicological finding of the study. The number of dead pups per litter was increased at each dose during lactation periods. Prolactin levels in pups were unchanged by treatment and were reduced in dams at the high dose.

5.5 Arsenic

Arsenic accidentally incorporated into a milk formula was responsible for the exposure of a large number of infants in Japan many years ago. Follow-up studies of these children revealed that arsenic trioxide, when ingested in amounts sufficient to produce clinical signs in infants, may cause permanent neurobehavioral deficits that are qualitatively different from arsenic poisoning seen in adults (Japanese Pediatrics Society, 1973).

Health risks caused by chronic exposure to arsenic-contaminated groundwater have been recognized in many Asian and Latin American countries. The health effects among adults have been well documented, but those among the younger generations, including children, infants, babies, and fetuses, have been rarely reported.

Calderon *et al*. (2001) examined the effects of chronic exposure to lead, arsenic, and undernutrition on the neuropsychological development of children. After checking for significant potential confounders, verbal IQ ($P < 0.01$) decreased with increasing concentrations of arsenic in urine (AsU). Higher levels of AsU were related to poorer performance on parameters such as long-term memory and linguistic abstraction; lower scores in tests measuring attention were obtained at increasing values of PbB.

Watanabe *et al*. (2003) reviewed data from an arsenic-contaminated area in Bangladesh. The authors concluded that although some human data suggest possible effects on developmental endpoints, the data are not sufficient to determine whether arsenic represents a serious developmental risk.

A cross-sectional study was carried out to investigate intellectual function in 201 children aged 10 in a contaminated area of Bangladesh (Wasserman *et al.*, 2004). Exposure to arsenic was associated with reduced intellectual function after adjustment for sociodemographic covariates. Water arsenic levels were associated with reduced intellectual function in a dose-response manner; children with water As levels >50 µg/L achieved significantly lower performance than did children with water As levels <5.5 µg/L. The association was generally stronger for water arsenic than for urinary arsenic.

Early postnatal administration of arsenic at sublethal doses produced little effect in experimental animals. Postnatal rats fed 1.5 mg/kg/day of arsenic trioxide from day 7–21 postpartum did not seem to differ from controls; 50% mortality occurred at 15 mg/kg/day (Tamura, 1978). Heywood and Sortwell (1979) reported high mortality among infant rhesus monkeys fed 3.75 or 7.5 mg/kg/day arsenic; no growth or developmental effects were noted among the survivors.

5.6 Aluminum

No evidences of human developmental effects of aluminum have been reported, but taking into account that gastrointestinal absorption of aluminum may be enhanced by certain dietary constituents, it may be advisable to avoid the consumption of high doses of aluminum during gestation and lactation (Domingo, 1995).

Although knowledge of aluminum toxicity has improved markedly in recent years, information concerning the developmental toxicity of this element is still limited. When high doses of aluminum were given concurrently with acids (citric or lactic), developmental and behavioral toxicities were shown in mice and rabbits (Domingo, 1995). A recent article by Colomina *et al*. (2005) assessed the potential combined influence of maternal-restraint stress and aluminum exposure on postnatal development and behavior. The only effect noted was an increase in the platform quadrant swim time in treated rats.

5.7 Mixed Metal and Multichemical Exposure

In addition to an article by Calderon *et al*. (2001) on neurodevelopmental effects quoted in the preceding section (5.5 Arsenic), timing of menarche has been investigated in relation to several ubiquitous environmental pollutants to which children are commonly exposed at low levels (i.e., dichlorodiphenyl dichloroethylene [p,p′-DDE], hexachlorobenzene (HCB), polychlorinated biphenyls (PCBs), mirex, lead, and mercury (Denham *et al.*, 2005). The study was conducted on 138 girls, 10–16.9 years of age, and residents in an industrialized area. Metals and organic compounds were measured in blood. Mercury levels were at or below background levels, whereas all lead levels were well below 100 µg/L, the CDC action limit, and PCB levels were consistent with a cumulative, continuing exposure pattern. The median age at menarche for the total sample was 12.2 years compared with a predicted age in a control group of 10.5 months later. The analysis of multichemical exposure among adolescent girls suggests that the attainment of menarche may be sensitive to relatively low levels of lead and of certain PCB cogeners.

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CHAPTER

13

Ecotoxicology of Metals—Sources, Transport, and Effects in the Ecosystem

POUL BJERREGAARD AND OLE ANDERSEN

ABSTRACT

Metal smelting and combustion processes are the major anthropogenic sources for emission of metals into the atmosphere. Metals emitted this way are generally bound in small particles with a potential for atmospheric long-range transport and contamination. Sediments are the ultimate sink for metals emitted to the aquatic environment, most evident in coastal and estuarine sediments. Besides direct emissions, man-made environmental changes such as acidification, oxygen depletion, and draining of water-logged areas may mobilize metals not otherwise available to organisms. The speciation of metals is an important determinant for the bioavailability, uptake, and toxicity of metals in the environment. Organisms have developed a wide range of mechanisms for internal handling and storage of essential and/or toxic metals. No other metal than mercury is consistently biomagnified along food chains. In the environment, the most adverse impacts (on populations) from metals have been caused by organic tin compounds used in antifouling paints and alumina mobilized by acid rain. High concentrations of organic mercury and cadmium in certain organisms may cause concern for human health in populations relying heavily on these types of organisms as food items.

1 SOURCES FOR METAL EMISSION

Human activities may augment the concentrations of metals naturally present in various compartments of the environment, leading to metal contamination or pollution in the environment. These anthropogenic activities may cause direct mobilization and emission of metals into the environment, but in some cases, human activities may indirectly cause changes in the environment that mobilize metals that were otherwise bound in stable forms. Several ways of assessing the anthropogenic impact on metal fluxes have been used (Salomons and Förstner, 1984), and one of them is calculation of the "anthropogenic mobilization factor," which is the ratio between anthropogenic and natural emissions to the atmosphere (Lantzy and Mackenzie, 1979). Because lake, coastal, and oceanic sediments are the ultimate sinks for a considerable fraction of the metals emitted as a result of human activities (see Section 2), the so-called sediment enrichment factor can be used to assess contamination of aquatic environments with metals. The sediment enrichment factor is the ratio between metal concentrations in anthropogenically affected sediments and the natural background concentrations (Salomons and Förstner, 1984).

In certain geological areas, naturally high toxic metal concentrations occur. Examples are acidic tropical Al-Fe soils; ultramafic soils with high concentrations of Ni, Cr, and Mg; and soils covering or close to ores containing one or more of the metals and metalloids Cd, Zn, Fe, Ni, Co, Pb, Cu, Cr, Hg, Mn, Se, and As. In volcanic areas, ground waters may contain high concentrations of As compounds.

1.1 Direct Emissions of Metals into Nature

1.1.1 Emissions to the Atmosphere

Combustion of fossil fuels, incineration of garbage, and metal smelting have been some of the major

sources leading to metal contamination of the atmosphere.

1.1.2 Emissions into Water

Mining activities and a number of industrial processes involving metals (e.g., catalysts in chloroalkali processes, anticorrosive surface treatment) are some of the major point sources from which metals are released into the aquatic environment, but metals from more diffuse sources such as atmospheric deposition, storm water (erosion of gutters, wearing of tires, etc.), and ordinary household waste water may end up in the aquatic environment. Deliberate emission of metals into the aquatic environment has taken place when copper has been used as an herbicide to prevent algal growth or used in fish farms to treat parasite infections in the gills of the fish.

1.1.3 Emissions to Soil

On a general scale, atmospheric deposition has constituted one of the major inputs of metals into soil, especially in areas affected by deposition from metal smelting activities. In certain areas, specific sources for metal contamination may, however, be important. Phosphate fertilizers have been important sources for contamination of agricultural soils with cadmium and likewise sludge from sewage treatment plants has added metals (especially cadmium has caused concern) to farmland when used as soil amendment. The addition of copper to, for example, the feed of pigs may lead to copper concentrations in the pig manure that are high enough to exceed the soil quality criterion for copper if the manure is used as soil amendment over several years.

Some metals and metalloids (e.g., copper and arsenic compounds) have been used as pesticides and thus spread deliberately in the terrestrial environment.

1.2 Indirect Mobilization of Metals

1.2.1 Acid Rain

When acidic precipitation falls on soils in which the buffer capacity has been exceeded, cations bound to the negative charges on the surfaces of soil particles are exchanged with hydrogen ions, and the concentration of metals in the pore water of the soil increases. As rainwater percolates, the metal ions will be transported either toward the groundwater or toward streams and lakes. The acidification of lakes is often associated with increases in metal (i.e., alumina, cadmium, lead, mercury) concentrations (Nelson and Campbell, 1991) in both lake water and biota, and it is especially the elevated concentrations of alumina and mercury that cause concern from an ecotoxicological and toxicological point of view (Scheuhammer, 1991).

1.2.2 Oxygen Depletion

Eutrophication of coastal marine areas has led to an increasing number of incidences of oxygen depletion in the bottom layers of the water column during recent decades. In bottom waters with full oxygen saturation, the upper few millimeters of the sediment are normally oxidized, and manganese and iron are found in their oxidized forms (MnO_2 and Fe^{3+}). If the oxygen tension decreases below approximately 16% of full saturation, MnO_2 is reduced to Mn^{2+} that may leak out of the sediment into the water column. The manganese concentrations in anoxic or oxygen-depleted waters may increase from approximately 1 µg/L to up to 1 mg/L (Kremling, 1983). The reduced manganese in the water column is oxidized fairly slowly (Section 8) on reintroduction of conditions of full oxygen saturation, and benthic organisms may be exposed to increased manganese concentrations for extended periods of time. This leads to accumulation of manganese in benthic organisms, and the use of the manganese content of lobsters has been suggested in monitoring programs for oxygen depletion (Baden and Neil, 2003; Baden *et al.*, 1994; Eriksson and Baden, 1998).

1.2.3 Pyrite Oxidation

If waterlogged, anaerobic terrestrial areas are drained, oxygen typically penetrates deeper into the soil, and this leads to oxidation of pyrite (FeS_2) in the soil, and the oxidized iron compounds (a complex mixture of iron oxides and hydroxides) will leach toward the streams together with the sulfuric acid from the oxidation of the reduced sulfur. The iron contamination in streams of such areas may have severe consequences for both invertebrates and fish in the streams.

2 THE BIOGEOCHEMICAL TRANSPORT OF METALS

2.1 Atmospheric Transport

Metals undergo—as the other elements in the Earth's crust—natural, biogeochemical cycles in which they are transported between lithosphere, hydrosphere, and atmosphere. For a number of metals, human activity has increased the mobilization from the lithosphere to a degree where the addition of metal to the hydrosphere—especially via the atmosphere—from anthropogenic sources far exceeds the addition from natural sources.

Metals are naturally added to the atmosphere with windblown dust, volcanic eruptions, sea spray, forest fires, and directly from the vegetation (Nriagu, 1989). The anthropogenic addition of most metals to the atmosphere takes place primarily by combustion of fossil fuels, metal smelting, garbage incineration, and, in the case of lead, also previously by the combustion of leaded petrol. Except for metals for which important gas phase releases take place (such as mercury), most of the metal emission to the atmosphere is in the form of particle-bound metal. The industrial emissions have exceeded (Nriagu, 1979) and still exceed (Nriagu, 1988; 1989; 1996; Nriagu and Pacyna, 1988) the natural background flux for several metals. In the 1970s, the anthropogenic mobilization factors were as high as 100 for lead, 83 for silver, 45 for molybdenum, 39 for antimony, 23 for zinc, 19 for cadmium, and 13 for copper (Salomons and Förstner, 1984). The anthropogenic sources to pollution of the atmosphere with metals are all caused by combustion processes, and some of the particles created have such a long residence time (days to months) in the atmosphere that the metals discharged may be dispersed over large distances. The particles formed by natural processes (also transporting metals from the lithosphere to the atmosphere) are, on the other hand, typically in the size range of 2–100 µm that precipitate fairly quickly—typically within 10 km of the emission site.

The small particles dispersing globally in the atmosphere are primarily brought back to the sea or earth surface with precipitation, either because the drop or crystal formation takes place with the particles or because the particles are scavenged by the falling precipitation.

2.2 Metal Speciation in Water

Metal ions dissolved in pure water are hydrated by an inner hydration shell, where water is tightly bound by the Coulombic field, and an outer hydrations shell, where water is more loosely and randomly bound. ^{18}O-water exchange studies indicate extensive differences in exchange rate between different ions, Rh(III), Cr(III), and Al(III) ions being particularly slow exchangers, whereas most ions have very rapid, almost instantaneous, hydration water exchange. The half-times of inner shell water exchange reactions of ions vary within about 15 orders of magnitude (Raspor, 2004). This has important for other complexation reactions, such as during complexation of metals with various inorganic and organic ligands; the overall exchange rate is often determined by the very first dehydration reaction (Morgan and Stumm, 1991). Most often the inertness or lability of other complexes of the ion follows the stability of the inner hydration shell. The stability of complexes is generally considered to increase with increasing formation constant, but thermodynamically unstable, but highly inert, complexes because of very slow ligand exchange rate may exist for extended time periods. Also, phase transfer reactions, including sediment binding and biological uptake of metals, are highly dependent on both ΔG and kinetic factors. In general, soft (class B) ions form complexes with monodentate and multidentate ligands that are both more stable and kinetically more inert than hard (class A) ions, with borderline ions in between, both with hard and soft ligands (Andersen, 1999).

In the aquatic environment, metals exist in a chemical equilibrium between free, dissolved ions, organic and inorganic complexes, and metal bound to organic and inorganic particles (see Figure 1). The surface of both inorganic particles (e.g., clay particles, iron, and manganese oxides) and organic particles (e.g., unicellular algae) is dominated by O^-groups. Depending on the affinity of the individual metal for O^--dominated surfaces, the equilibrium between free, dissolved cations and particle-bound metal may be shifted toward particle-bound metal. Metals are present as various aquo- and mixed aquo complexes with amines, humic, OH^-, CO_3^{--}, in seawater Cl^-, or bound to organic and inorganic compounds, which may be dissolved or associated to sediment. In estuarine waters, speciation is changed as salinity increases. For some metals (e.g., cadmium) this may result in solubilization from particulate matter because of formation of chlorine complexes, but often,

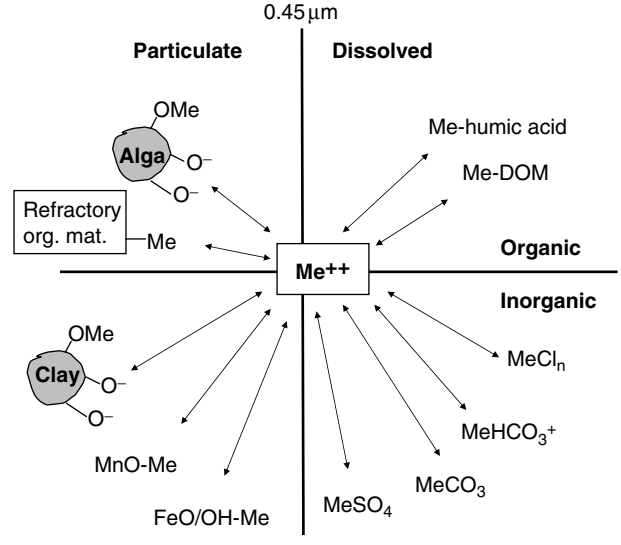

FIGURE 1 Schematic presentation of equilibria between free metal ions and various organic and inorganic chelators (including both particulate and dissolved ligands). Dissolved material is operationally defined as any material that will pass a filter with a pore size of 0.45 µm. Not all potential ligands are included, and stoichiometric adjustment is not made.

most metals transported to estuaries are precipitated because of flocculation of dissolved and suspended organic matter. Also, the fraction of hydrated metal ions, which is often the most bioavailable, is reduced. To ease speciation calculation, computer software have been developed, which on the basis of experimentally determined stability constants and chemical description of natural waters (concentrations of Cl^-, CO_3^{--}, PO_4^{---}, SO_4^{--}, Na^+, Ca^{++}, etc.), calculate metal speciation. Examples are CHESS (Santore and Driscoll, 1995), MINEQL+ (Schecher and McAvoy, 1995), and MINTEQA2 (Brown and Allison, 1987). Also, interactions between metals and natural organic matter (NOM) have been integrated in speciation modeling programs as WHAM (Tipping, 1994) and NICA (Benedetti *et al*., 1995), the latter model incorporating multiple binding sites and competition between cations. Because of the extensive heterogeneity of NOM, this kind of modeling represents an extensive task. Also, all such programs assume equilibrium and cannot predict speciation in dynamic systems.

2.3 Metal Transport in the Ocean

When metal-carrying particles hit the surface of the oceans, the metals may—depending on the character of their binding to the particles and the chemical properties of the individual metals—stay bound to the particles or dissolve in the seawater.

The upper hundreds of meters of the oceans are the habitat of filter-feeding zooplankton organisms that obtain their food by filtering particles (mainly unicellular algae) from the water. Because only a minor fraction of the metal content of the food is taken up into the organisms during the passage of the gut (for many metals considerably less than 10%), the main fraction of the original metal content of the unicellular algae is excreted in the fecal pellets of the zooplankton organisms. A considerable part of the pelagic filter feeders are crustaceans (copepods, euphausiids, etc.), and the fecal pellets of these organisms are lined by a thin chitin membrane. Whereas unicellular algae and fine clay particles sink very slowly down through the water column (~2 m per day), crustacean fecal pellets have much higher sinking rates, approximately 400 m/day (Fowler and Small, 1972; Komar *et al*., 1981; Small *et al*., 1979). This means that the activity of the filter feeders transfers the particle-bound metal in the upper layers of the water column to a form in which it is transported downward in the ocean. On their way down through the water column, the fecal pellets may be eaten several times, and the original content of organic material, nutrients, and metals is gradually liberated to the water masses, but some of the content reaches the ocean bed.

For a number of surface-active metals, these processes result in low concentrations in the upper part of the water column, because the metals are being bound to particle surfaces and transported downward. It is characteristic for these metals (e.g., cadmium, copper, nickel, zinc) that their depth profile is similar to the depth profile of the major nutrients (Bruland, 1980; Bruland *et al*., 1978).

The metals are removed from the upper layers of the water column by a biologically mediated, downwards transport process (Fowler *et al*., 1987). The higher the affinity of a specific metal for binding to biological material is (e.g., reflected in concentration factors for the metals in zooplankton fecal pellets), the faster the metal is removed from the water column, leading to short residence time for the metal in the ocean (Cherry *et al*., 1978; Higgo *et al*., 1977). Concentrations in the seawater and residence times for metals (and also nonmetallic elements) in the ocean show a positive correlation (Whitfield, 1981).

The partitioning coefficient (K_y) for a metal (Y) is defined as the concentration of the metal in seawater and the average concentration in the crust of the earth ($K_y = [Y]_{seawater}/[Y]_{crust}$). Metals with a high K_y do thus occur at a high concentration in the seawater relative to the average concentration in the Earth's crust. For elements, an electronegativity function, Q_{yo}, can be defined, such that $Q_{oy} = (x_y - x_o)^2$ and x_o and x_y are the electronegativities of oxygen and the element Y, respectively. For elements whose concentration in the seawater is controlled by solid-phase chemistry, there is a negative correlation between the partitioning coefficient (K_y) and the electronegativity function, Q_{yo}, illustrating that metals with a high affinity for binding to O^--dominated surfaces are removed faster from the ocean water than metals with a low affinity for binding (Whitfield, 1982).

2.4 Transport of Metals in Freshwater and Estuaries

Metals are continuously added to freshwater systems because of the erosion caused by rainwater. The erosion in the drainage areas of river systems typically is in the order of 5 cm/1000 years, and the rivers of the world each year transport approximately 24 billion tons of material to the oceans. Human activities such as ploughing, forest destruction, etc. are estimated to have increased the amount of material transported by the rivers by a factor 2–3 (Salomons and Förstner, 1984).

The metal contents and chemical composition of freshwater in different river systems vary strongly with the geological characteristics of the drainage areas. Whereas the chemical composition of seawater (regarding inorganic content) shows little variation,

freshwater is characterized by large differences in pH value, alkalinity, hardness, and ion content. The variability in the content of particles and dissolved organic material is generally also larger in freshwater than in seawater. Also in freshwater, metals exist in equilibrium between free and bound forms (Figure 1), but the variability in the character of freshwater makes it more difficult to generalize concerning the speciation of the metals in freshwater than it is in seawater.

Most trace metals in river systems are transported in a particulate form; this is particularly true for the metals that constitute an important part of the mineral structure of the inorganic particles (Fe, Al). Most of the particle-bound metal is transported in the river water by suspended particles with a diameter less than 100 μm; the highest metal concentrations are generally found in/on the smallest particles, because the surface to volume ratio increases with decreasing diameter.

A fraction of the particle-bound metal sediments in the river system itself, the sedimentation depends on the flow and the velocity of the water and the variation in these (potential resuspension of sedimented material) and on the physical shape of the river system.

The metal remaining in the river water is transported toward the sea. In the zone in which river water is mixed with seawater, the concentration and speciation of the metals are affected—often in a fairly dramatic way. On the metals' way out through the mixing zone or the estuary, the processes taking place are influenced by these changes:

1. The ionic strength increases
2. The velocity of the water decreases
3. The pH value increases
4. The freshwater is "diluted" by seawater
5. The particle content (the *turbidity*) increases

The physical shape of the estuary plays an important role in the changes in the composition of the water. The following description is based on a slightly idealized situation in which the salinity increases gradually from the river mouth to full seawater strength, and the effect of tides and sea currents is ignored.

In estuaries, a maximum in the amount of suspended material is often seen where the river water first meets the seawater (Meade, 1972). Part of the increased particle load is explained by the fact that dissolved organic material in the freshwater flocculates under the influence of the increasing ionic strength and the increasing pH value, leading to particle formation. The particles may also be of marine origin (marine sediments resuspended by wave action, sea currents, etc.). At the same time as particle formation takes place in the estuary, there will also be a tendency for particles to precipitate because of the lower flow velocity.

When considering the fate of the river-borne metals in the passage of the estuary, it is important to note that even if the largest fraction of the metal content of the river water is bound to particles, the concentration of dissolved metal is most often higher in the river water than in the seawater.

If the chemical speciation for a specific metal were totally unchanged during the passage from river water to seawater, the concentration of dissolved metal would merely decrease along the estuary; the metal is then said to behave conservatively. Metals may also, however, under influence from the increasing ionic strength in the estuary, be liberated from particles because of, for example, ion exchange and potential complex binding to, for example, chloride. This may result in an increase (when the dilution is taken into account) in the concentration of dissolved metal out through the estuary. As a third possibility, metals with a sufficiently high affinity for binding to particles may be removed from solution (by a shift in the equilibrium between free and particle-bound metal, see Figure 1), so that the dissolved concentration decreases faster than predicted by the dilution alone. The fate of metals in estuaries may vary tremendously—party depending on the chemical properties of individual metals. Also, a particular metal may behave very differently in different estuaries.

When particles precipitate in estuaries, the metal contained by the particles is removed from the water column. In considerations concerning the overall biogeochemical transport of metals, it is, therefore, interesting to know which fraction of the metal content of the rivers that precipitate during the transport from river systems to seawater. Investigations in the St. Lawrence River and its estuary have shown that more than 90% of the river's iron and manganese precipitate in the estuary, whereas the values for nickel, cadmium, copper, and zinc vary between 25 and 60% (Yeats and Bewers, 1982). It is true for a number of metals that a major proportion of the content transported in the river water precipitates in the estuaries of the rivers. For strongly surface reactive metals like the transuranic elements americium and plutonium, it has been found that up to 99% precipitate in the coastal areas after discharge from the nuclear reprocessing plant for nuclear fuel at the Irish Sea.

The fact that a considerable amount of the metal content of the rivers precipitates in the estuaries has the effect that the anthropogenic pollution of the rivers with metals leads to an increase in the metal concentrations in the sediments of many estuaries and coastal areas.

The downward transport of metals will also take place in lakes and in polluted areas; elevated metal concentrations in lake sediments have been demonstrated.

2.5 Metals in Sediments

Sediments are important sinks for pollutants, including metals. Metals bind both to the organic content of sediment, to clay minerals, and form mixed complexes with available oxyanions. Even at high sediment concentrations of, for example, cadmium, the binding capacity of sediment is not saturated. Compared with organic chemicals, metal transfer between sediment and water depends on highly complex processes. Although organic pollutants, in principle, partition between the water phase, the sediment phase, and biota in aquatic systems mainly on the basis of their lipophilic character (i.e., affinity for the organic fraction in sediment and lipid fractions in biota), the partitioning of metals depends much more on water chemistry and sediment chemistry, mainly relative amounts of sulfur and iron compounds, organic content, and redox status, and also on metal characteristics, mainly hard and soft character. Metals interact with particles by means of physical-chemical sorption (i.e., nonspecific attraction [van der Waals forces]) acting between surface charges on particles and the charges of metals/metal compounds. Chemical processes include precipitation and coprecipitation, ion exchange, isomorphous substitution, and solid-state diffusion.

Sediments are most often already anoxic a few millimeters below surface, and in this reducing environment, alternative terminal electron acceptors include sulfate, nitrate, iron, and, in some cases, other metals, and organic matter (methanogenesis). The redox status of sediments extensively affects the speciation and solubility and thereby toxicity of sediment associated metals. The toxicity of metals associated with sediments depends on the bioavailability of the metals, mainly governed by the water concentration (pore water and overlying water) and speciation (see later). For sediment eating and suspension feeding invertebrates as, for example, polychaetes and mussels, direct exposure to metals during digestion of sediment and suspended matter is an important exposure route.

Four chemical processes are considered to increase the transfer of metals from sediment to water: elevation of salinity, changes in sediment redox status, reduced pH, and the presence of organic complexing compounds. The first three processes release "free" metal in the form of mixed complexes with small inorganic ions and water, the fourth releases organically bound metal with another bioavailability than that of the inorganic metal complexes.

Metal ions form highly insoluble hydroxides and sulfides, and for some metals, also phosphates, whereas chlorides, carbonates, and sulfates are more soluble. Also, metals are bound strongly to various oxygen-containing iron compounds. Iron oxide hydroxide (FeOOH) and other iron oxides form a lattice where phosphate ions can exchange with OH, thereby locally increased pH may occur from liberated OH–, and also, a range of metal ions can bind to FeOOH. The metal sequestering capacity of FeOOH is especially high when some of the hydroxy groups are exchanged by phosphate. For transition metals, the ions with the lowest oxidation step typically form more stable complexes, leading to metal mobilization in case of sediment oxidation. The pool of sulfide available for metal binding (acid volatile sulfide) is an important determinant of the toxicity of sediment-associated metals. This pool is smaller in oxidized sediment, leading to metal release. On the other hand, FeOOH is formed only at oxic conditions. Accordingly, the effect of changing the redox status of sediment on the bioavailability of sediment-associated metals depends on the chemical composition of the sediment.

3 UPTAKE AND ACCUMULATION OF METALS

3.1 Bioavailability, Uptake, Accumulation, and Elimination

The term bioavailable refers to the fraction of total metal that can be taken up into an organism (Sanders and Riedel, 1998).

Terrestrial animals absorb metals in their food over their digestive epithelium, volatile metal compounds through the lungs, and for the smaller invertebrates, over the body surface. Aquatic organisms absorb toxic metals from water over the gill epithelium and over the entire body surface, besides from food and imbibed water over digestive epithelium.

The bioavailability of metals varies extensively among metals and organisms and also depends on the speciation of the metals. Accordingly, changes in the physicochemical characteristics of exposure media, especially water, influence the proportions of metal species that can be absorbed.

In principle, only lipophilic metal complexes (e.g., with carbamate pesticides) or organometals (e.g., mercury and tin compounds) can diffuse over biological membranes, whereas hydrophilic metal complexes including free hydrated ions must pass cell membranes by other mechanisms than free diffusion.

Metals can be taken up by endocytosis (e.g., $FeOH_3$), by diffusion (e.g., $Hg(CH_3)_2$), by facilitated diffusion (mainly soft class B ions with affinity for S- or N- containing ligands in membrane carrier proteins), or by active ATP-dependent uptake via cation pumps

(mainly hard class A ions that do not bind to carriers and are transported against concentration gradients) (Philips and Rainbow, 1993; Simkiss and Taylor, 1989). In biological fluids, ligand exchange to form thermodynamically stable complexes with multidentate biological molecules (chelation) prevents back diffusion. Apparently, the soft class B metals can be transported against a concentration gradient transferring the ion along a series of ligands with increasing affinity along a thermodynamic gradient, the metals finally being intracellularly sequestered by the ligand with the highest affinity. This can lead to storage or subsequent transport, as, for example, seen for metallothionein.

Some metals are absorbed from solution as hydrated ions, explaining the increasing bioavailability and toxicity of, for example, Cu and Cd, at decreasing salinity (Mclusky *et al*., 1986). Besides the uptake of hydrated metal species, inorganic and organic neutral metal complexes with increased lipophilicity (e.g., $CdCl_2$, $HgCl_2$, $Hg(CH_3)_2$) may be absorbed by direct diffusion (Sanders and Riedel, 1998)).

Metals with hydrated ionic diameters similar to those of essential metals can be taken up by the ATP-driven pumps and calcium channels, thus Pb, Cd, Mn, and Co can be absorbed as metabolic analogs by means of the Ca pumps (Markich and Jeffree, 1994). The relevance of this mechanism depends on the organism, for molluscs and malacostracan crustaceans with extensive calcium uptake for shell and exoskeleton formation (Bondgaard and Bjerregaard, 2005; Norum *et al*., 2005), this route may determine total uptake.

3.2 Metal Transport in Aquatic Food Chains

Aquatic organisms may take up metals either directly from solution or from ingested food. Phytoplankton organisms do, of course, accumulate metals directly from solution (Whitfield, 2001), but for most pelagic filter-feeding herbivores (such as, e.g., copepods) metals in the ingested food seem to be the major source (Mason *et al*., 1996; Reinfelder and Fisher, 1991); differences between metals and species do, however, occur (Wang, 2002). It has been shown that metals taken up into the cytosol of the unicellular algae and subsequently becoming associated with proteins (Reinfelder and Fisher, 1991) are assimilated to a higher degree in copepods than metal bound to structural elements in the algae (i.e., cell walls). Recent investigations (Ng *et al*., 2005), however, show that this phenomenon cannot be generalized across all species (phytoplankton as well as herbivores) and metals. In some benthic filter feeders such as the blue mussel *Myrtillus edulis,* uptake from water seems to be the dominant route for accumulation of cadmium for example (Fischer, 1988; Riisgard *et al*., 1987).

For most predators, food seems to be the dominant source for metal uptake (Luoma and Rainbow, 2005), that is, for cadmium in fish (Pentreath, 1977) and crabs (Bjerregaard *et al*., 2005).

Except for mercury (Section 7.10), there is no consistent tendency that metal concentrations increase along aquatic food chains.

4 DEFENSE AGAINST AND STORAGE OF METALS

Various mechanisms protecting against metal toxicity exist among different species, several acting by accumulating metals in inert granules that either are excreted or deposited for the lifetime of the organism. Other strategies involve heat-stable metal chelating proteins like metallothioneins (MTs) and metallothionein-like proteins that occur in eucaryotes (invertebrates, vertebrates, and plants) and have been characterized in a variety of species (Vijver *et al*., 2004).

Speciation of metals, homeostatic strategies for essential metals, and protective strategies for toxic metals at the cellular level in organisms may be as follows:

1. Free or complexed ionic species: Mixed aquo, chloride, phosphate, or carbonate complexes. Complexes with metabolic compounds (amino acids, carbohydrate, metabolites, etc.).
2. Bound in active centers of LMW peptides or functional proteins: Hemoglobin, hemocyanin, zinc finger proteins, cytochromes, carbonic anhydrase, superoxide dismutase.
3. Bound to transport or sequestration proteins: Metallothionein, ferritin, transferrin.
4. Bound in lysosomal vesicles as metal granules: Precipitated in extracellular deposits. Hair, feathers, exoskeleton, mineral deposits, residual bodies.
5. Bound to cellular constituents potentially causing harm: Enzyme poisoning, binding to DNA, or ion channels.

There is high sequence homology between MTs from different species, and they apparently all have similar functions as mammalian metallothioneins (i.e., storage, transport, and compartmentalization of essential metals and detoxification of toxic metals). Also, their metal-binding profiles and gene expression regulation are similar to that of mammalian metallothionein (i.e., binding Zn, Cu, Cd, Hg, and induction by these and

a few other metals). In mammalian species, the regulation of MT gene expression has been explained and shown to involve a nuclear receptor, metal-responsive transcription factor (MTF1) (Selvaraj *et al*., 2005). After binding of metals to the MTF1, it binds to metal response elements (MRE) upstream structural genes for MT (Andrews, 2000). This mechanism has been demonstrated in a few nonmammalian species including *Drosophila melanogaster*. Sequencing of MTF1 genes demonstrates high structural conservation from *Drosophila* to mouse, rat, and humans, strongly indicating that this regulation mechanism is general in all higher eucaryotes.

Some terrestrial invertebrates have adapted to a life in areas polluted by high levels of toxic metals. One such example is the wood louse *Porcellio scaber* (isopod, crustacean), which is capable of concentrating extensive amounts of metals in intestinal cells as various types of precipitates (metal granules). This protective system comprises three types of granules: type A mainly binds Ca, Mg, Zn, and Pb by precipitation as phosphates; type B granules contain Cu and Cd bound to MT; and type C binds excess Fe in the form of hemosiderin. Mixed-type granules can form by precipitation of phosphates around existing type B or C granules, depending on the pattern of metal exposure. All granule types are scavenged by lysosomes forming excretory vacuoles.

The intestinal mucosa is composed of both digestive cells sloughed at regular time intervals so the metal content is disposed of, and storage cells, whose metal content remains in the animal's digestive system (Hames and Hopkin, 1991). *Porcellio scaber* also deposits toxic metal in granules in hepatopancreas, a digestive organ in invertebrates combining hepatic and digestive functions.

Porcellio scaber is predated by the spider *Dysdera crocata*, which can ingest extensive amounts of toxic metals with its prey and has a slightly different protective system than *Porcellio scaber*, also involving digestive epithelium sloughing.

Based on numerous studies, formation of metal granules seems to be a widespread protection mechanism in invertebrate species. In marine prosobranch gastropods (snails), one study of more than 40 different species reported the formation of various types of phosphate granules in digestive gland containing mainly essential metals (Gibbs *et al.,* 1998). Other species with described metal granules include bivalves, collembolans, crustaceans, and oligochaetes (Vijver *et al*., 2004).

4.1 Metal Toxicity and Defense Systems in Plants

Plants have an extensive capability for adapting to metal stress. Two major situations have been described. In numerous situations, plants have during geological time scales adapted to growth on soils with very high metal or metalloid levels by invading areas where ore deposits are close to the soil surface. A gradual selection has created new endemic plant species, metallophytes, with an absolute, genetically determined metal tolerance to even extreme metal concentrations.

In other situations, plants (especially grasses) have rapidly (within few decades) developed tolerance to elevated soil levels of metals in areas polluted with metals because of human mining and other industrial activities. This tolerance is most likely because of selection of certain alleles of preexisting genetic polymorphisms and creates what has been called races, subspecies, ecotypes, and physiotypes, with varying degrees of metal tolerance (Peterson, 1993). In some taxa with a high degree of genetic plasticity, different ecotypes have evolved that are resistant to several metals. Especially in species within the genus *Silene* (*Caryophyllaceae*), a large number of metal-tolerant subspecies have developed. In *Silene vulgaris* (bladder campion), several alleles of a few loci have been identified to encode for resistance to high levels of metals (Schat *et al*., 1996). Cut1 and Cut2 alleles cause tolerance to Cu and possibly Mn; Znt1 and Znt2 alleles cause tolerance to Zn and possibly Cd. In plants carrying a combination of Cut and Znt alleles, Co tolerance has been described. Whether specific (now unknown) genes are needed to confer tolerance for each metal or group of closely related metals is presently not known, but this mechanism is likely as the target of metal toxicity can be various metal transporters in the cell membrane, metalloenzymes involved in metabolic functions, as well as other specific functions (Larcher, 2003). In a more general sense, five different mechanisms have been described to cause increased metal tolerance (Ernst, 1996; Larcher, 2003):

1. Formation of intracellular chelates by metal-binding proteins and polypeptides (phytochelatins).
2. Chelation by molecules from internal metabolism followed by compartmentalization into vacuoles.
3. Active export.
4. Impeded transport over the cell membrane.
5. Immobilization in the cell wall especially by pectins.

Cell vacuole–based protection mechanisms have been observed to dominate in leaves of metallophytes, whereas cell wall–based mechanisms dominate in roots, which makes sense in relation to the kinetics of metals in plants. Metals are taken up in roots and transported in cell sap.

Metallophytes can absorb extensive amounts of metals, especially in hyperaccumulators, who have been reported to concentrate metals by factors of 10 to almost 4000, thereby reaching extreme concentrations of tens of grams per kilogram (various metals in various plant species). This is used for phytoremediation of metal polluted soils or waters. In special cases, hyperaccumulator plants can be harvested for use as fuel and the ashes used for metal refining (phytomining).

5 TOXICITY OF METALS IN ECOSYSTEMS

Despite the fact that some of the first observations on the ecotoxicology of metals came from terrestrial studies on, for example, the toxicity of organomercurials used in farming, today, by far most experimentation, data, and mechanistic understanding in ecotoxicology relates to the aquatic environment, both in general and specifically for metals. Research indicates that the primary site for toxic actions of most metals in aquatic organisms is the gill, especially in acute exposures of freshwater organisms.

The functions of the gill are much more complicated than those of terrestrial lung. Besides gas transport and acid-base regulation, some kidney functions (e.g., N-metabolism, water, salt, and metal homeostasis) are in part regulated by the gill. These functions are executed by an array of negatively charged gill membrane proteins that bind cations. This is likely the reason for the high sensitivity of the gill to toxic metals. During acute exposure to toxic metals, extensive histological damage is rapidly induced in gills, leading to reduced gas exchange and efflux of essential ions and eventually mortality.

At lower level chronic exposures, metals, including Cd, Cu, Ag, Zn, and others, tend to bind to specific gill structures involved in metal homeostasis: Monovalent cations (e.g., Ag^+ and Cu^+) affect Na^+ transport, whereas divalent cations (e.g., Cd^{++} and Zn^{++}) affect Ca^{++} transport. Some metals cross the gill membrane and exert a toxic effect after systemic distribution (e.g., Hg^{++} and Pb^{++}). Although Cu in aquatic environments predominantly exists as the divalent Cu^{++} ion, it is most likely reduced to Cu^+ before or during interaction with the gill.

Early in the development of ecotoxicology as a science providing data for risk assessment and risk regulation, it became clear that in aquatic environments metal toxicity was poorly correlated to total metal concentrations, stimulating intense research in speciation, to identify determinants of toxicity (i.e., relations between water chemistry, dominant metal species, and toxicity). Selective analytic procedures have been developed to try to quantify labile (bioavailable) fractions of total metal. These methods include voltammetry, ligand competition, and resin equilibration. At present, the only technique allowing reliable measurement of free metal concentrations is the use of ion selective electrodes and only at relatively high (toxicologically relevant) concentrations.

Accumulating evidence on disproportionality between concentration and effect led to formulation of the free ion activity model (FIAM) (Campbell, 1995), which has been further developed into the biotic ligand–modeling concept (BLM) (Paquin *et al*., 2002), which incorporates metal speciation in description of toxicity. Like the pure speciation models, various BLMs assume equilibrium.

A BLM is a conceptual framework, describing that the acute toxicity of metals is associated with binding of "active" forms of metals (labile complexes or "free" hydrated metals) to biological structures (biotic ligands) as proteins, organelles, etc. Accordingly, the BLM predicts that water chemistry extensively affects metal toxicity, either through binding the metal (e.g., dissolved organic carbon, complexing compounds) or competing for sites at the biotic ligand (e.g., calcium).

Various authors have developed formal BLM descriptions allowing calculation of metal toxicity from metal speciation data acquired from chemical analysis of water and the speciation models described previously, in combination with conditional stability constants for metal–gill tissue complex formation measured experimentally. In this sense, the BLMs are extensions of the previous speciation models including further compartments, the biotic ligands. The presently final modeling approach to predict metal toxicity is the development of a sediment biotic ligand model based on measurement of sediment concentrations of acid-soluble metals (simultaneously extracted metals, SEM) and acid-volatile sulfide (AVS), modeling metal partition using the WHAM, and toxicity modeling using a BLM for metals (Di Toro *et al*., 2005).

The applicability of the various models for predicting metal toxicity is limited by the fact that a central assumption of all BLMs is that metal uptake is under

thermodynamic, not kinetic, control, meaning that the uptake is slow compared with the rate of binding to gill tissue. This may, however, not always be true, especially at low external metal concentration and for metals with slow reaction kinetics (e.g., Ni^{++}, Al^{+++}, Co^{++}, Cr^{+++}) for which metals, even the reaction with gill tissue, may never approach equilibrium.

6 RISK ASSESSMENT OF METALS

6.1 The Aim of Ecotoxicological Risk Assessment

The aim of ecotoxicological risk assessment is to protect ecosystems from damage because of human activities. Accordingly, the aim of ecotoxicological studies is to predict how natural populations respond to contaminant exposure. During this effort, ecotoxicological risk assessment contributes, however, often to protect human health by generating both exposure data and effects data. Because of the widely differing endpoints in human health risk assessment (individual health with emphasis on the most sensitive individuals) and ecological risk assessment (ecosystem health extrapolated from effects at the population and individual levels), the present use of human risk assessment–based methods in ecotoxicological risk assessment is problematic unless relevant extrapolation methods are used to assess effects at population, community, and ecosystem levels from effects at the individual or suborganismal levels, including molecular effects at the subcellular level.

6.2 Integrated Risk Assessment

Historically, risk assessment and risk management have focused on human health, using mainly human-focused toxicological data obtained in rodent and other mammalian test systems. When ecological risk assessment started to come into use only about four decades ago, methods previously developed in data generation for human risk assessment were adopted in a rapidly developing process; however, the assessment was conducted independently on human health risk assessment. The present trend is to perform integrated risk assessment and let the two processes support each other. The contribution of ecotoxicology to support human health risk assessment is mainly related to generation of exposure data. There are, however, important examples that effects observed in ecotoxicology have a major impact on estimating effects in humans, the best examples are within endocrine disruption. Extensive studies have investigated relationships between ecosystem composition (water, sediment/soil, biota), bioavailability, bioaccumulation, trophic transfer, and toxicodynamics of contaminants, including metals. With regard to metals, those compounds with a strong potential for bioaccumulation are most important for top predators like marine mammals and also in relation to human health (i.e., cadmium and methylmercury).

Scope of human health risk assessment:

- Protecting single individuals, ideally the most sensitive (children, pregnant, elderly, diseased, vulnerable genotypes).
- Thereby also protecting cohorts and populations.
- The critical effect is on the single individual.
- And biomarkers can be valuable risk indicators.

Scope of ecotoxicologic risk assessment:

- Protecting ecosystem health = optimal functioning of a biota assemblage.
- Not protecting single individuals—even massive killings are acceptable if fecundity can reestablish original population size.
- Critical effect is on (a) key species (not necessarily the most sensitive).
- (In many cases we do not know which species is/are key species).

6.3 Methods of Ecotoxicology

Classical dose-response assessment of acute toxicity adopted from human-oriented toxicology has been widely used in ecotoxicology to estimate the potency of ecotoxicologically relevant compounds. This method has, however, shortcomings, which can be illustrated from studies of the toxicity of metals (salts of Cd, Cu, Zn) toward one of the most widely used test organisms, the fresh water Cladoceran *Daphnia magna*, which can reproduce both sexually and asexually, the parthenogenic reproduction mode offering the possibility of generating a large number of genetically differing but stable clones in the laboratory. In early toxicity testing, the calculated acute LC_{50} of Cd^{++} in *D. magna* varied several orders of magnitude between different laboratories. Intensive method development under the auspices of OECD, EEC, and ASTM (ASTM, 1987; EEC, 1992; OECD, 2004) led to development of standard tests reducing extensively the interlaboratory variation. Identified error sources for estimating cadmium toxicity included medium composition, including, among other factors, the presence or not of EDTA and water hardness (Barata *et al*., 1998; Guilhermino *et al*., 1997), test temperature (Lewis and Horning, 1991), the *D. magna* clone used (Barata *et al*., 1998), the endpoint of effect (Suedel *et al*., 1997), and the mode of feeding

before testing. By standardizing all aspects of test conditions including the endpoint (24- and 48-hour immobilization test), the interlaboratory variation has been extensively reduced, thus the 48-hour EC_{50} for immobilization varied a factor 4.2, from 0.033–0.14 mg Cd/L among 13 tests in different laboratories (Mark and Solbe, 1998). This is still a rather large variation, but considerably less than earlier observed.

It is an important principle in human risk management to set exposure standards protecting the most vulnerable individuals, elderly, sick, children, or fetuses, or specific genotypes, thereby protecting the entire exposed cohort. Similarly, ecotoxicological risk management should set standards protecting the most vulnerable species, thereby protecting the entire community exposed. The next step in ecotoxicology was development of specific life-stage tests and full life-cycle chronic tests, because reproduction and larval development are especially important life cycle events. In *D. magna*, a 21-day lethality and reproduction test was standardized and validated. This led to further development of mathematical modeling of population composition with regard to the various life stages in response to exposure, and endpoints were transformed to be NOEC instead of LC_{50}. Recently, benchmark modeling has been introduced to further increase the credibility and reproducibility of test results.

The sensitivity of different species to toxins varies extensively, often by several orders of magnitude. Therefore, to protect the most sensitive species, $LC_{50,}$ NOEC, or (e.g., $BMD_{5\%}$) is determined for a number of species in a community, and the concentration protecting 95% of species calculated by modeling. For acute toxicity, this value is called final acute value (FAV).

Still, the relevance of toxicity data obtained under strict laboratory conditions for effects during *in situ* exposure in ecosystems is not straightforward. Toxicity and ensuing effects are modified by, for example, water composition, in freshwater, hardness and organic compounds as, for example, humic and fulvic acid affect the toxicity of metals. Also genetic variability in populations *in situ* as opposed to the use of genetically homogeneous clones in laboratory work will lead to different toxicities. For cadmium, NOEC will be lower and EC_{95} for a natural heterogenous *D. magna* population containing a wide variety of genotypes with regard to sensitivity compared with the sensitivity of a laboratory clone of average sensitivity.

6.4 Practical Risk Management

In the United States, the Toxic Substances Control Act (TSCA) of 1976 resulted in the 1979 TSCA inventory of approximately 60,000 chemicals then in use; today more than 75,000 compounds are enlisted. According to the TSCA, all producers or importers of chemicals not in the TSCA inventory must submit a premanufacture notice (PMN) to the Office of Pollution Prevention and Toxics (OPPT) 90 days before start. The PMN requires chemical, physical, and technical data for the new compound, as well as existing environmental and toxicity data. On the basis of the PMN, risk assessment is performed and risk management implemented. The risk assessment initially follows two avenues:

- Environmental emission and exposure evaluation, resulting in estimation of predicted environmental concentrations (PECs) in relevant scenarios.
- Hazard profile development preferentially using existing toxicity data. Because such data seldom are available, surrogate data are generated using (quantitative) structure-activity relationship (QSAR) analysis by the "ECOSAR" software (ECOSAR, 1998). In most cases either a predicted LC_{50} or a predicted no effect level (PNEC) is calculated.

Based on these exposure and effects assessments, risk characterization is performed to estimate the nature and likelihood of effects. Thus, a risk ratio can be calculated as RR = PEC/PNEC. If RR is around 1 or larger, there are reasons for concern.

For the risk management process, assessment factors are used to correct for uncertainties associated with the quality of data available. The best type of ecotoxicity data are those from field tests, but in most risk assessment cases, only limited toxicity data are available. The RR is a realistic calculation of risk on the basis of often-limited knowledge and not suited for risk management. To take into consideration the quality of data and thereby the uncertainty associated with the RR, assessment factors are used to generate environmental exposure criteria (EEC):

$$EEC = RR/AF$$

Assessment factors used by OPPT for setting concern levels for new chemicals:

Available toxicity data:	Assessment factor:
Limited (e.g., one acute LC_{50} via (Q)SAR	1000
Base set acute toxicity (algae, fish, and daphnia)	100
Chronic toxicity	10
Field test data	1

6.5 Biomarkers as Hazard Indicators in Ecotoxicological Risk Assessment

Biomarkers are important both in human health and in ecotoxicological risk assessment and would *a priori* be expected to play an important role in integrated

risk assessment. This role is not fully achieved, mainly because of limitations in possibilities of extrapolation from biomarkers to likely effect.

Development and use of biomarkers in ecotoxicology and environmental risk assessment:

- Adoption of clinical chemistry methods from human toxicology.
- Adoption of classical markers from ecophysiology and population ecology.

The value of the use of biomarkers in human health risk assessment is restricted by the fact that many biomarkers are hazard indicators rather than risk indicators. Also, because a strong causal link between bioindicator signal and ensuing risk of disease is not always demonstrated, even for many molecular biomarkers, they often function as exposure markers rather than as effect markers. Accordingly, the research needs are related to establishing quantitative relations between biomarker signal and endpoint (risk of adverse effect). Another promising research area involves development of biomarkers for genetic (polymorphisms) or acquired susceptibility to general or specific exposures or diseases.

The value of biomarkers in ecotoxicology is limited by two factors:

1. Problems with extrapolation from biomarker signal typically measured at the individual level or below.
2. Lack of knowledge of the specific ecological significance of the biomarker. Therefore, development and use of markers at higher organizational level (e.g., life cycle traits, reproduction) have high priority.

Because of lack of solid knowledge on long-term ecological consequences of increased mutation load, biomarkers of mutagenic/carcinogenic environmental exposures including metabolic markers are presently most relevant in relation to human risk assessment.

In the case of endocrine disruption, biomarkers have a potential of becoming developed to a stage of very high relevance in integrated risk assessment. Here, a suite of biomarkers is available at the molecular (function and metabolism), histological, and functional levels. Together, these markers are likely capable of forecasting risks of chemicals to both humans and ecosystems because of impaired reproductive functions.

Molecular techniques as genomics, proteomics, and metabolomics are being rapidly used to develop potential biomarkers in human risk assessment with extensive future perspectives. This development is highly accelerated by the human genome project allowing allocation of differential effects at various molecular levels to specific genes. A similar development in ecological risk assessment is impeded by the low degree of sequencing in many key species.

7 MONITORING METAL POLLUTION—BIOMONITORING

The degree of contamination of an ecosystem with metals (and other contaminants) may, of course, be assessed by determining concentrations of metals in air, water, soil, or sediments in the system, but for several reasons, many monitoring programs around the world use selected organisms—so-called monitoring organisms—for monitoring purposes.

The most important advantages in determining metal concentrations in biomonitoring organisms rather than in the abiotic environment are the following:

1. The organisms concentrate chemicals to measurable concentrations.
2. The organisms reflect the average degree of pollution over time.
3. The concentration in the organisms reflects the *bioavailable* fraction of the polluting metal, or, in other words, the fraction that is available for uptake in organisms.

7.1 Mussel Watch

One of the best-known examples of a monitoring organism is the widespread use of the blue mussel *Mytilus edulis* for monitoring the contamination of coastal marine areas (Farrington *et al*., 1983; Goldberg, 1986; Goldberg *et al*., 1978; 1983). The blue mussel has several advantages for this purpose (Bayne, 1989; Widdows and Donkin, 1992):

1. It is distributed all over the world.
2. It is steadfast.
3. It is easily sampled and handled.
4. It is easy to perform experiments with.
5. Its physiology and way of life are well established.
6. It is a dominating species in many coastal areas.
7. It tolerates fairly high concentrations of contaminants in its tissues.
8. It has a relatively low and noninducible MFO-activity (not relevant for metals).
9. It is consumed by humans, so its content of pollutants is relevant for considerations concerning food safety.
10. Mussels can easily be translocated from clean to polluted locations.
11. Mussel populations are relatively stable and the development of contamination can be followed over time.

For correct interpretation of monitoring data on metals—which occur naturally in seawater—it is necessary to have an adequate knowledge about biotic and abiotic factors influencing metal uptake and accumulation in the mussels (Widdows and Donkin, 1992).

7.2 Other Monitoring Organisms

Atmospheric contamination with metals has been assessed by means of moss analyses in a number of countries (Herpin *et al*., 1996; Hertz *et al*., 1984; Hynninen, 1986; Markert *et al*., 1996; Miljøstyrelsen and Statens Naturvårdsverk, 1983; Wolterbeek, 2002).

Macroalgae such as *Fucus* species have been used to assess metal contamination in coastal areas (i.e., contamination with radioactive elements) (Dahlgaard and Boelskifte, 1992; Holm, 1995; Kershaw *et al*., 1999).

Metal contamination in the freshwater environment has been monitored by means of freshwater mussels (Kraak *et al*., 1991; Mersch and Pihan, 1993) and aquatic mosses (Mersch and Pihan, 1993; Say *et al*., 1981).

For information on further uses of biomonitoring organisms, the reader is referred to Philips and Rainbow (1993).

8 ECOTOXICOLOGY OF INDIVIDUAL METALS

8.1 Alumina

Alumina concentrations in the deeper waters of the oceans typically range between 10 and 20 nmol/L (Measures, 1995), whereas the concentrations in the surface waters are strongly affected by inputs from the terrestrial environment and may range between 0.2 and almost 100 nmol/L.

Alumina exists in soils mainly bound in silicates, hydroxides, and oxides, and on acidification of the soil, ionic alumina is released from the silicates. At pH values above 5 the ionic alumina exists as polymeric hydroxy cations that are strongly bound to the surface of the silicate minerals (Savory and Wills, 1991), and pore water concentrations and leaching from the soil are negligible (Savory and Wills, 1991). In the silicate-buffering region, primary silicates are transformed to secondary silicates, transforming lime feldspar to kaolinite, where the Ca^{++} ion is exchangeable:

$$CaAl_2Si_2O_8 + H_2O - >[Al_2Si_2O_5(OH)_2O_2]^{2-}Ca^{2+}$$

In the aluminium hydroxide–buffering region, aluminium hydroxides close to the surface of clay minerals can be released from the solid particles. Combining weathering with low pH can thus liberate extensive, ecotoxicologically effective Al concentrations to soil and surface waters (Kloppel *et al*., 1997). If soil pH decreases below 4.5, increasing amounts of alumina attains a soluble form, and alumina concentrations in the soil water may reach tens of milligrams per liter.

Alumina mobilized from the soil leaches to groundwater, rivers, and lakes, and alumina concentrations in freshwater generally increase with decreasing pH values, typically from concentrations below 100 µg/L at pH values above 6 to 500–700 µg/L at pH 4 (Dillon *et al*., 1983).

The exact mechanism by which alumina exerts its toxic effect toward fish is not known, but it has been proposed (Poleo, 1995) that negatively charged sites on the gill surface act as nuclei for polymerization of $Al(OH)_2(H_2O)_4^+$, which is the dominating species of alumina at pH values between 5 and 6 where toxicity is highest (Lydersen, 1990; Lydersen *et al*., 1990a; 1990b; 1991; Rosseland *et al*., 1992).

Acidification and subsequent mobilization of alumina have profound impacts in aquatic ecosystems (Dillon *et al*., 1983).

The main cause for fish death in acidified lakes and rivers is believed to be impacts on the functions of the gills imposed by toxic Al species with subsequent loss of salts from the blood and disturbances in the gas exchange.

Exposure to alumina at pH around 5–5.5 results in structural changes in the gills (fusion of the secondary gill lamella, increased thickness of the mucus layer), so the diffusion distance between blood and water increases. The increased distance of diffusion means that the uptake of oxygen and the release of carbon dioxide are rendered less efficient. By acute poisoning in acidic water and alumina, the fish die from disturbances in the gas exchange (Malte and Weber, 1988), whereas the cause of death after chronic exposure is less clear.

The concentration of salts (especially Na^+ and Cl^-) in the blood of freshwater fish is higher than in the surrounding water. This means that salts have a tendency passively to diffuse from the blood out into the water. Freshwater fish lose some salts through the urine, but the largest loss takes place from the gills. The loss of salts is replaced by a continuous active uptake of salts over the gills. By uptake of salts in the gills, Na^+ is exchanged with H^+ or NH_4^+ and Cl^- with HCO_3^- or OH^-. At instances of fish death in acidified freshwater areas, it has been found that the concentrations of salts in the blood of the fish decrease in the most heavily affected areas (Leivestad and Muniz, 1976). When fish are transferred from moderately to strongly acidified surroundings, the salt concentration in the blood decreases likewise.

The resistance of the fish toward the combination of acidic water and alumina increases when the salt content of the water increases. This is a natural consequence of the fact that the difference between the salt concentrations of the water and the blood—and thereby at the loss of salts—decreases if the salt concentration increases in the water. Likewise, the fish are far more resistant in hard (calcium containing) water (Wood *et al*., 1988). It is known that calcium under normal conditions participates in the regulation of the gill's permeability to Na^+ and Cl^-, and a high concentration of calcium in the water reduces the salt loss from the gills of the fish.

In freshwater environments affected by acidification (e.g., Norway, Sweden, Scotland, Canada), fish populations have shown marked declines. In the beginning of the 20th century, declines were observed in fish populations in certain Norwegian lakes and rivers. Thus, the sea trout disappeared from approximately 1500 acidified lakes in southern Norway between 1940 and 1980, and the roach started to disappear from lakes in western Sweden around 1930.

Seawater has a fairly high buffering capacity, and acid precipitation does not generally generate problems with decreases in pH and mobilization of alumina in marine areas.

8.2 Antimony

Antimony exists in seawater mainly (>95%) as antimonate (oxidation state V) and minor amounts of Sb(III) and monomethyl antimony (Cutter and Cutter, 1995; 1998; Cutter *et al*., 2001). Antimony concentrations typically range between 0.5 and 2 nmol/L, and the antimony concentration does not show typical nutrient-type profile because the highest concentrations tend to appear in the surface layers (Cutter *et al*., 2001).

Antimony may be released from metal smelters and from combustion of fossil fuels, and the anthropogenic emission of antimony has been estimated to be 38,000 tons annually (Lantzy and Mackenzie, 1979). The mobilization factor for antimony has been estimated to be 39.

EC_{50} values for the aquatic toxicity of antimony of 8 mg/L for daphnia and approximately 50 mg/L for green algae have been reported (Hammel *et al*., 1998).

8.3 Arsenic

The metalloid arsenic appears in nature in the oxidation states −3, 3, and 5 and in a multitude of different chemical compounds (the structures of some of the forms mentioned here are shown in Figure 2).

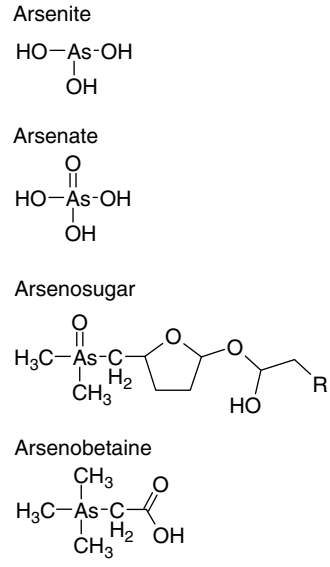

FIGURE 2 Various arsenic forms mentioned in the chapter.

In seawater arsenic is mainly found as arsenate (AsO_4^{3-}; >80%) and arsenite (AsO_3^{3-}; <20%) forms for which the degree of protonation depends on the pH value of the water. Concentrations typically range between 15 and 25 nmol/L (Cutter *et al*., 2001; Santosa *et al*., 1994). In the anoxic pore water of the sediments, the reduced form arsenite dominates. In the photic zone of the oceans, algal methylation of arsenic may lead to concentrations of monomethyl- and dimethyl arsenic of approximately 0.1 and 0.5 nmol/L, respectively (Cutter *et al*., 2001).

Arsenic is emitted into the environment through metal smelting, combustion of fossil fuels, use in pesticides, and from volcanoes. Anthropogenic emissions are far higher than the natural releases of approximately 8000 tons annually (Matschullat, 2000), and a mobilization factor of 3.3 was estimated in the 1970s (Lantzy and Mackenzie, 1979).

Arsenate is taken up from the water by algal cells—possibly via the same route as phosphate. Phytoplankton organisms transform the inorganic arsenic taken up to organic arsenic compounds in the form of arsenosugars (Francesconi and Edmonds, 1997; Francesconi and Kuehnelt, 2004).

In marine animals, most of the arsenic content is likewise present in the form of organic arsenic compounds—mainly in the form of arsenobetain. Whereas the pathways for the algal synthesis of arsenosugars are known, the pathways for the synthesis of the arsenobetain are not explained. The formation of the organic arsenocompounds is considered to be a mechanism for detoxification of the toxic, inorganic arsenic compounds (Francesconi and Edmonds, 1997; Francesconi and Kuehnelt, 2004).

Acute and chronic toxicity of inorganic arsenic to fish and aquatic invertebrates typically occur at concentrations of 1 mg/L (Mance *et al*., 1984c).

In some parts of the world, the soil naturally contains such high concentrations of arsenic that mobilization of inorganic arsenic constitutes a human health problem when groundwater is used to supply drinking water.

8.3 Cadmium

8.3.1 Background Levels and Emissions

Cadmium has been called "the dissipated element," because its uses are most often as minor components (surface treatment, pigments, plastics, solders, fertilizers), and recycling is, therefore, difficult. Coal burning, scrap smelting, and garbage incineration are sources for air pollution with Cd. The anthropogenic mobilization index for cadmium is high. Because of potential toxicological consequences of high anthropogenic emission of cadmium, this element has become substituted from many uses.

Cadmium is a natural component of rocks, soils, and sediments. Average concentrations in the earth's crust and in the upper lithosphere have been estimated to 0.11 mg/kg (Bowen, 1979) and 0.5 mg/kg[1] (Goldsmith, 1958), respectively. Cadmium concentrations in igneous rocks are normally well below 1 mg/kg, whereas sedimentary rocks as bituminous and carbonaceous shales can contain very high Cd concentrations, up to more than 200 mg/kg (Thornton, 1992) and thereby provide extensive exposure of plants and animals because of weathering. A survey of Cd concentrations in surface soils from many parts of the world reported average concentrations between 0.07 and 1.1 mg/kg, and the authors concluded that concentrations >0.5 mg/kg reflect anthropogenic input (Kataba-Pendias and Pendias, 1984).

In the open areas of the oceans, the concentration of total cadmium ranges between 5 and 25 ng/L in the surface water, whereas the concentration in the deep sea typically is approximately 50–100 ng/L (Bruland, 1980; Bruland *et al*., 1978; Yeats *et al*., 1995), somewhat higher in the Pacific and South Atlantic than in the North Atlantic. The relatively low surface concentrations are likely because of scavenging of Cd by microplankton where it may replace zinc (Price and Morel, 1990). In Arctic and Antarctic waters, with much lower organic concentrations, the difference in concentration between surface and deep waters is much smaller (Macdonald and Sprague, 1988). This may explain the very high cadmium body burdens reported in Arctic and Antarctic vertebrates (see later).

Because of the erosion from rainwater in the upper layers of the soil, small amounts of cadmium are constantly transported to freshwater systems. Because of the differences in the cadmium concentration in different types of soils, the natural background concentration of cadmium in freshwater may vary from one place to another; cadmium concentrations in uncontaminated freshwater range between 10 and 100 ng/L. Much higher concentrations may occur in freshwater systems because of emissions with surface runoff, drainage, and sewage (spillover or incomplete treatment) from industrial and municipal areas, especially high concentrations occur near mines with polymetallic sulfide deposits, up to 1 mg/L (Boyle and Jonasson, 1979).

It is estimated that 69% of the cadmium transported in the rivers of the world precipitate in estuaries (Salomons and Förstner, 1984), and an average sediment enrichment factor of 5.3 in coastal areas and lakes has been reported (Salomons and Förstner, 1984).

The biogeochemical cycle of Cd in freshwater follows nutrients like phosphate, silicates, and nitrates. In freshwater, the bioavailability and toxicity Cd has been demonstrated to decrease with lower pH (Andersson and Borg, 1988; Lawrence *et al*., 1989; Yan *et al*., 1990) and with increasing hardness. In general, suspended organic material scavenges Cd and makes Cd less bioavailable and transfers Cd to the sediment. Cadmium is easily bound to clay and organic particles in the freshwater, and the fraction of the total cadmium in freshwater that is found in the particulate form increases with the particle content of the water. The dissolved cadmium will to a smaller or larger degree—depending on the pH value and the hardness of the water—form a $CdCO_3$ complex, and some of the dissolved cadmium will also be bound to organic material dissolved in the water. This means that the free cadmium ions (Cd^{++}) in many instances only will constitute a small percentage of the total amount of cadmium present in the freshwater.

In full-strength seawater, dissolved cadmium is mainly present as chloride complexes ($CdCl_2^0$, $CdCl^+$, $CdCl_3^-$), and Cd^{++} constitutes only between 2 and 3% of the total dissolved cadmium (Mantoura *et al*., 1978; Sunda *et al*., 1978). At reduced salinities in estuaries and coastal areas, Cd^{++} constitutes a higher proportion of the dissolved cadmium.

8.3.2 Uptake in Organisms

The unicellular algae that are the first link in the food chain of the open ocean take up cadmium directly from the seawater so that the concentration in the algae may be up to 1000 times higher than in the surrounding water.

The filter feeders among the zooplankton retain only a minor fraction of the cadmium ingested with the food; the rest is excreted with the feces of the animals. Zooplankton organisms also take up cadmium directly from the seawater—as well as through the gills as on the external surface. The sum of these routes of uptake gives zooplankton organisms (both on 2nd, 3rd, and maybe the 4th level in the food chain) cadmium concentrations that are generally 500–2000 times higher than the cadmium concentrations in the surrounding seawater. The bioaccumulation of cadmium does thus not lead to biomagnification.

Cadmium is taken up by aquatic plants and may reach extensive levels in polluted lakes. In macrophytes from Lago Maggiore (Guillizoni *et al*., 1989) measured 1.6 mg/kg as mean of means, whereas some plants had higher levels: 6.7 mg/kg in *Egeria densa* and 9.6 mg/kg in *Ceratophyllum demersum*, an effective accumulator species for several metals. One of the most efficient Cd accumulators, the water hyacinth *Eichhornia crassipes* that produces MT-like proteins in root tissue has been suggested for remediation of Cd-polluted waste water and freshwater systems because of rapid growth and high biomass production (Nir *et al*., 1990). Several experimental studies with this and other metal hyperaccumulators for remediation of, for example, water ponds in old mining areas are under way with cadmium and other metals.

Both in mammals and birds, cadmium is deposited mainly in the kidneys. In penguins from the Antarctic, an area not polluted by cadmium because of human activities, kidney concentrations between 50 and 80 mg/kg wet weight have been reported in several studies, leading to renal tubular necrotic changes (Elinder, 1992). Also Arctic and Antarctic whales and seals have very high renal cadmium levels; Greenland harbour seals have much higher Cd levels than seals from the North Sea, which receives cadmium discharges because of human activities. The explanation is likely because of extensive uptake of Cd by Arctic and Antarctic amphipods (including krill), oysters, mussels, and squid, which are important food resources for many marine vertebrate species.

It has been found for a number of different marine species that the cadmium concentration in the tissues increases throughout the lifespan of the organism, so that old organisms contain higher cadmium concentrations than young organisms.

8.3.3 Contamination with Cadmium

Cadmium is emitted into the atmosphere by metal smelting and incineration of coal, oil, and different types of garbage. Runoff from gutters and discharge from galvanization industries and other types of chemical industries emit cadmium to the aquatic environment, and cadmium is spread with phosphate fertilizers over agricultural areas. The largest input of anthropogenic cadmium to marine areas comes from atmospheric cadmium. The atmospheric input is, however, distributed over so large an area that it probably only has a minor impact on the cadmium concentrations in the marine organisms.

8.3.4 Cadmium's Toxicity in Water

Cadmium bound in a complex form is apparently not available for uptake in organisms and thereby not toxic. The free, dissolved cadmium ions (Cd^{++}), on the contrary, are toxic. Because a large amount of the cadmium present in seawater forms chloro complexes, cadmium (in the same dissolved concentration) will be far more toxic in freshwater than in seawater, because the concentration of Cd^{++} is higher in the freshwater. Experiments have shown that the lowest concentration of cadmium that has adverse effects on the marine flora and fauna is in the range of 5000 ng Cd/L. There are no well-documented examples that contamination of the sea with cadmium has led to adverse effects in marine ecosystems. On the other hand, contamination of the sea with cadmium can give rise to considerations concerning the use of seafood for human consumption, because blue mussels even from uncontaminated areas contain relatively high amounts of cadmium.

Because calcium reduces the uptake, as well as the toxicity, of dissolved cadmium, the calcium content of the water (hardness) plays an important role for cadmium's toxicity in freshwater. Daphnia are very sensitive to cadmium and in soft water; the reproduction in our most common species of daphnia is affected at 340 ng Cd/L, and the species composition in the zooplankton community is changed at exposure to 200 ng Cd/L (Marshall and Mellinger, 1980).

8.3.5. Cadmium in Agricultural Soil and Uptake of Cadmium into Plants

The uptake of cadmium in a specific plant species is proportional to the cadmium content of the soil. The uptake is, however, somewhat dependent on the pH value of the soil and the content of organic material, clay content, and agricultural practices. The variability in the cadmium uptake between different plant species may be very large. Because most humans have their most important exposure to cadmium from the plant material in our basic food (especially potatoes, cereal products, and rice), the increase in the cadmium concentration of the agricultural soil and thereby in the cadmium content of the crops has caused considerable concern in many countries.

Some species of mushrooms have an extraordinary ability to accumulate cadmium from the soil (Seeger, 1978), also in areas not especially contaminated with the metal. Because some of these mushroom species (e.g., *Agaricus* species) are commonly collected for human consumption, the high cadmium content causes some concern regarding the potential human health aspects.

Cadmium has been added to agricultural soil especially from four sources: atmospheric deposition, phosphate fertilizers, lime, and manure.

In the 1970s, approximately 8000 tons of cadmium were emitted to the atmosphere each year; 7200 tons were the result of human activity, whereas only 800 tons came from natural sources (Nriagu, 1989). Around heavy industry (i.e., metal smelters), the atmospheric deposition of cadmium may be very high—up to 170 g Cd per hectare per year.

The total European emission of cadmium into the atmosphere decreased from around 3000 tons in the 1960s and 1970s to approximately 400 tons in 1999 (EEA, 2003), and as a consequence of this decrease in the emission, the yearly deposition of cadmium in, for example, Denmark declined from approximately 2–3 g Cd per hectare around 1978 to approximately 0.4 g Cd per hectare now.

Phosphate fertilizers contain cadmium. Phosphate fertilizers are manufactured from raw phosphates that are either minerals like apatite (mainly calcium phosphate) with a low cadmium content or former seabed sediments with a high cadmium content. Around 1978, the Danish agricultural areas had approximately 3 g cadmium added per hectare per year with phosphate fertilizers. After introduction of limits for cadmium in phosphate fertilizers, the cadmium addition to the Danish soil from this source has decreased to below 0.1 g hectare per year. The application of lime contributes with approximately 0.4 g Cd per hectare per year; this number has not been changed much since 1978.

In 1978, the total addition of cadmium into the Danish agricultural soil constituted 6 g cadmium per hectare. It is assumed that slightly less than 1 g cadmium disappears from each hectare with draining water, seeping to groundwater and removal of crops. Because the ploughing layer on average contains 510 g Cd per hectare, the cadmium concentration increased by approximately 1% per year in 1978. The increase in the cadmium content of agricultural soil and herbage has been documented in English soil and herbage samples taken between 1860 and 1980 (Jones *et al*., 1987; 1992; Nicholson *et al*., 1994).

The measures taken to reduce the main sources of pollution of the agricultural soil with cadmium should now have lowered the rate of increase in the cadmium concentration in the soil.

8.3.6 Implication for Human Health

Humans in the general population receive most of their exposure to cadmium through basic plant material (rice, potatoes, grain products) in their diet, and the cadmium content in plants generally increases linearly with the cadmium content in the soil in which the plants are grown. Because the margin between the average daily cadmium intake and the intake expected to cause adverse effects in the most sensitive part of the population is very narrow—and in some areas nonexistent—the increase in cadmium concentrations in agricultural soils has caused some concern worldwide.

Mussels and other marine species (e.g., marine mammals) may accumulate cadmium to concentrations that pose a problem to humans whose diet is composed of a large fraction of these organisms.

A detailed description of human exposure to cadmium and the toxicological effects of chronic exposure is given in Chapter 23.

8.4 Cobalt

Dissolved cobalt concentrations in ocean water around 20–40 pmol/L have been reported with no clear vertical profile (Jickells and Burton, 1988; Landing *et al*., 1995). $CoCO_3^0$ dominates in seawater, with Co^{++} as the second most important species (Mantoura *et al*., 1978). Cobalt coprecipitates with other metal hydroxides.

Cobalt is emitted to the aquatic environment mainly in connection with mining activities, and concentrations up to 4.5 mg/L have been recorded (Schrautzer, 1991).

Anthropogenic emissions to the atmosphere are smaller than natural fluxes, and a mobilization factor of 0.71 has been estimated (Lantzy and Mackenzie, 1979). Seventy-nine percent of the cobalt transported by rivers globally is precipitated in the estuaries, and an average sediment enrichment factor of 1.4 in coastal areas and lakes has been found.

8.5 Chromium

Chromium exists in the aquatic environment in an equilibrium between Cr(VI) and Cr(III), which is strongly influenced by redox conditions. In seawater, chromate dominates (3–4 nmol/L reported for Mediterranean water), whereas Cr(III) concentrations were typically around or below 1 nmol/L (Vandenberg *et al*., 1994).

Chromium is emitted to the aquatic environment from galvanizing industries, tanning industries, and

from more diverse sources originating from the use of chromium in dyes, wood impregnation, etc., and an average sediment enrichment factor of 2.2 in coastal areas and lakes has been found. The anthropogenic mobilization factor for chromium is 1.6.

Acute toxicity of chromium toward fish is exerted at concentrations generally above 10 mg/L, whereas sublethal effects (such as reduction in growth) toward various aquatic organisms (e.g., phytoplankton and fish) are exerted from about 10 µg/L (Mance *et al.*, 1984a).

8.6 Copper

Copper is an essential element, and more than 30 copper-containing enzymes are known. In, for example, crustaceans, copper is an integral component of the respiratory pigment, hemocyanin.

Copper concentrations in the oceans show a gradual increase from approximately 1 nmol/L in the surface layers to 3–5 nmol/L in the bottom layers (Bruland, 1980; Yeats *et al.*, 1995). $Cu(OH)_2^0$ is the dominating inorganic Cu species in seawater ~90%), whereas Cu constitutes less than 1% (Mantoura *et al.*, 1978). Uncontaminated freshwater systems show large variability in copper contents (0.2–30 µg/L), with a world median concentration of 3 µg/L (Bowen, 1985). However, copper has a high affinity for binding to organic chelators such as humic and fulvic substances, algal exudates, etc., so the content of dissolved organic material will strongly influence the speciation of copper at a given site. In most aquatic systems, the free copper ions constitute less than 1% of the total amount of copper present.

In the global flux, 19,000 tons of copper are emitted to the atmosphere by natural routes, whereas anthropogenic sources contributed between 56,000 and 260,000 tons during the 1970s (Lantzy and Mackenzie, 1979). The total annual addition of copper to marine areas with rivers is estimated to 2.5 million tons, of which approximately two thirds are retained in the sediments of the estuaries and coastal areas. An average sediment enrichment factor of 2.1 has been reported in lakes and coastal areas. In estuarine areas affected by discharges from mining activities, copper concentrations in the sediments may reach several thousands of µg/g(Salomons and Förstner, 1984). In oxic marine sediments, copper is especially bound to iron oxides and in the organic fraction of the sediments.

Copper has a relatively high toxicity toward aquatic organisms. In the open oceans where the concentrations of organic chelators are low, relatively small increases in the natural background level of copper may inhibit the photosynthetic activity in surface waters (Nielsen and Wium-Andersen, 1970). Copper shows sublethal and lethal effects toward various groups of aquatic invertebrates from approximately 5 µg/L[1] (Mance *et al.*, 1984b). Fish may show mortality at exposure from approximately 30 µg/L (Mance *et al.*, 1984b).

Copper sulfate has been used as an algaecide in aquatic systems, and it is also used in aquaculture systems to treat gill parasites in fish; both of these applications have led to toxic manifestations in the freshwater environment.

Among the mammals, ruminants are relatively sensitive to copper; for example, sheep may develop signs of copper poisoning (liver damage and anaemia) if their food contains 8–10 µg Cu/g. Nonruminants are far less sensitive to the effects of copper, and copper is used as a growth promoter in pig production (up to 250 µg Cu/g). This addition of copper to the pig's feed may lead to elevated copper concentrations in the agricultural soil if the same areas are amended with pig manure for several years, and soil quality criteria may be exceeded.

8.7 Iron

Iron exists in oxic environments as Fe(III) and in anoxic environments as Fe(II). Iron is an essential metal, acting as a cofactor in a number of enzymes and being a constituent of hemoglobin.

Average iron concentrations in surface and deep seawater are 70 and 760 pmol/L, respectively (Johnson *et al.*, 1997). Iron may be rate limiting for phytoplankton growth on the oceans (Whitfield, 2001). In anoxic deep water, iron concentrations may reach 1 µmol/L (Bruland, 1983).

Ninety-nine point eight percent of the iron transported in the rivers of the world is precipitated in the estuaries, and an average sediment enrichment factor of 1.2 in lakes and coastal areas has been found.

Some of the major problems with iron contamination of the aquatic environment are seen in connection with draining of waterlogged areas from which Fe(III) may be released to the streams (Section 1.2.3). For most freshwater invertebrates, sublethal and lethal toxicities are exerted by total iron concentrations in the mg/L range, but larvae of insects such as mayflies show LC_{50} values at a couple of hundred µg/L (Mance and Campbell, 1988).

8.8 Lead

Lead occurs naturally in the earth's crust with an average concentration around 13 mg/kg, and the anthropogenic contamination with lead—mainly because of the use of leaded petrol—has exceeded that of any other metal. During the 1970s, the anthropogenic emissions

of lead to the atmosphere were estimated as between 400,000 and 500,000 tons (Lantzy and Mackenzie, 1979; Nriagu, 1989), a value exceeding the natural cycling of lead by approximately 100 times. An average sediment enrichment factor for lead in lake and coastal sediments of 4.1 has been reported (Salomons and Förstner, 1984).

Tetramethyl and tetraethyl lead are added to petrol as antiknocking agents. During combustion in an engine, the organic lead compounds will be split to methyl and ethyl radicals (that prevent unwanted chain reactions—knocking) and elementary lead. The elementary lead is oxidized to PbO, which will react with HBr and HCl under the formation of PbBrCl and salts of lead, ammonium, chloride, and bromide. Dibromo and dichloroethylene are added to the gasoline with the purpose of removing the lead from the engine (combustion of the ethylene compounds yields HBr and HCl). The lead salts formed are found in the exhaust gas as particles, of which 95% have a diameter less than 0.5 µm. During the decades when leaded petrol was used, lead concentrations in urban air could easily exceed 1 µg Pb/m^{-3}; the effects on human health from this exposure are described in Chapter 31.

The particulate lead will—depending on the size of the particles—be transported for shorter or longer distances before it is deposited again. A minor fraction of the emitted lead (bound in the largest particles) hits the soil quickly and gives rise to lead contamination along the roads. Particles with a diameter <0.5 µm may remain in the atmosphere for long a time, and the lead emitted subsequent to the use of leaded petrol gave rise to global lead contamination.

This emission of lead can be traced in different compartments in the environment, for example, in the Greenland ice cap and in the oceans. The prehistoric lead concentrations in the Greenland ice cap were only a few pg/g. From approximately 1750, lead concentrations increased linearly, reaching approximately 60 pg/g in 1940. After leaded petrol was taken into use in the 1940s, lead concentrations increased very rapidly, reaching levels as high as 400–500 pg/g in the 1970s. When the use of leaded petrol was gradually phased out between the 1970s and the late 1990s in North America and Europe, the lead concentrations in the ice layers decreased correspondingly (Murozumi *et al.*, 1969).

Lead concentrations in the oceans show a peak in the upper 500–1000 m because of the atmospheric deposition. In the Pacific Ocean, peak values of approximately 10 ng Pb/L are found (Schaule and Patterson, 1981), whereas levels in the Northern Atlantic (influenced by emissions from both the North American and the European continents) reach 40 ng Pb/L. Below 2000 m, lead concentrations typically range between 1 and 2 ng Pb/L.

8.8.1 Lead in Ammunition

The use of lead in ammunition has resulted in the dispersal of major quantities of this metal in the environment. In Denmark alone, approximately 800–1000 tons of lead was used for this purpose each year (until around 1990). Approximately 200 tons of these ended up in shallow, coastal areas. Occurrences of up to 10 lead shots/m^2 are not unusual, and in some fiords in Western Jutland, several hundred lead shots/m^2 have been found (Danish EPA, 1989). Extreme values of more than 2 kg/m^2 have been found in shallow waters in the vicinity of shooting ranges. The problem with the dispersal of lead ammunition in the environment through hunting has been attempted by exchanging lead shots with steel shot, and the use of lead shot is now forbidden in many countries. However, the degradation of the lead shots that are already in the environment proceeds fairly slowly, because the surface of the lead shot in the anaerobic zone of the sediments are covered with lead sulfides that have low solubility and prevent further degradation. In terrestrial soil, it is assumed that half of the lead shots will be degraded in 40–70 years, and that all of the lead shots have been degraded in 100–300 years (Danish EPA, 1989).

8.8.2 Effects in Birds and Mammals

Studies of rats (Mouw *et al.*, 1975), pigeons (Hutton, 1980), starlings (Grue *et al.*, 1986), and swallows (Grue *et al.*, 1984) living near trafficked roads while the use of leaded petrol was at its highest showed increased concentrations of lead in the investigated animals and lower ALAD activity than was seen in animals from more rural areas. The reduction in ALAD activity in the swallows did not lead to reductions in the concentrations of hemoglobin, and the breeding success of the animals was not affected (Grue *et al.*, 1984).

Ducks and swans normally ingest sand, gravel, and small stones that in the gizzard participate in grinding the food. If the birds instead ingest lead shot, the lead shots will dissolve in the gizzard and digestive system. The lead taken up causes lead poisoning, and for swans in Western Jutland, lead poisoning used to be the most common cause of death. Lead-poisoned birds initially have paralysis in the esophagus and digestive system, and at later stages in the legs, neck, and wings; concurrently, the sense of equilibrium is lost. One to two lead shots may be a lethal dose.

8.9 Manganese

Under oxidized conditions, manganese exists in a multitude of oxy- and hydroxide forms (e.g., vernadite -MnO_2, hausmannite Mn_3O_4, feitknechtite -MnOOH)

mainly a mixture of oxidation states III and IV (Burdige, 1993); the oxidized form of manganese is often referred to as MnO_2. Mn^{++} dominates under reduced conditions in the environment. Mn^{++} is the dominating species and $MnCl^+$, $MnSO_4^0$, and $MnCO_3^0$ also play a role (Mantoura *et al*., 1978).

Concentrations of dissolved manganese in the oceans are typically higher (~1 nmol/L) in the upper part of the water column than in the deep waters (0.25 nmol/L) (Landing *et al*., 1995), probably because of the impact of atmospheric deposition of manganese transported from the terrestrial environment (Statham *et al*., 1998). Manganese concentrations in coastal waters are generally higher (~one order of magnitude) than concentrations in the surface waters of the oceans (Hydes and Kremling, 1993). Sixty-nine percent of the manganese transported in the rivets of the world are precipitated in the estuaries (Salomons and Förstner, 1984).

Anthropogenic emission of manganese into the atmosphere is lower than the natural flux with a mobilization factor of 0.53 (Lantzy and Mackenzie, 1979).

Manganese plays an important role in oxidation-reduction processes in marine sediments (Burdige, 1993). Under oxic conditions, the manganese present in the upper few millimeters of the sediment is present in the oxidized form, but if the bottom waters (e.g., below thermo- or haloklines) are depleted in oxygen because of eutrophication, manganese is reduced and Mn^{++} leaks out of the sediment to the bottom waters where concentrations approaching 1 mg/L may be reached (Kremling, 1983). Mn^{++} dominates below an oxygen saturation of approximately 16% (Gerringa, 1991), and the reoxidation of Mn^{++} after an incident of oxygen depletion occurs fairly slowly (in the order of weeks) (Dehairs *et al*., 1989).

Increased concentrations of manganese have been found in benthic, marine organisms in areas affected by oxygen depletion (Baden *et al*., 1994; Baden and Neil, 2003; Eriksson and Baden, 1998), but the highest concentrations (approximately 1 mg/L) reached in the seawater are hardly toxic to marine organisms.

8.10 Mercury

8.10.1 Background Concentrations, Uses, and Emissions

Mercury concentrations in the oceans range between 0.7 and 1.1 pmol/L with no apparent concentration gradients between surface and deeper waters (Dalziel, 1995).

In modern times, mercury has been used for many different purposes (chloroalkali industry, seed dressers, antimicrobial agent in the paper industry, etc.), and until the early 1970s, approximately three quarters of the global use of approximately 10,000 tons use was released to nature.

Ecotoxicological and toxicological problems with the uses and releases of mercury were revealed both in the terrestrial and the aquatic environment during the 1960s—mainly in Swedish and Japanese investigations. The use of alkylmercury compounds as seed dressers resulted in elevated levels of mercury in terrestrial wildlife in agricultural areas—especially avian top predators such as hawks, owls, and harriers were affected (Berg *et al*., 1966; Johnels *et al*., 1979). Releases from industrial uses in the chloroalkali and paper industries led to contamination of the aquatic environment, the top predators again showing the highest degree of contamination (Johnels *et al*., 1967). Discharges to the Minamata Bay in Japan caused severe mercury poisoning in the local population after consumption of fish and shellfish from the bay (further described in Chapter 33).

Today, the discharge of mercury from industry has been considerably reduced, but the human mobilization of mercury during the last century has increased the emission of mercury to the atmosphere with approximately a factor of 3 (UNEP, 2002). An average sediment enrichment factor of 8.7 has been reported for lakes and coastal areas (Salomons and Förstner, 1984).

8.10.2 The Transformation of Mercury in Nature

In nature, mercury may be transformed between elemental mercury, divalent mercury, and methylmercury, and these transformations play a vital role both in the global cycling of mercury and for the accumulation and adverse effects of mercury in organisms.

8.10.2.1 Methylation

Inorganic mercury may in nature be transformed to methylmercury by both abiotic and biotic processes, and the biological transformation is believed to be the more important. Most of the knowledge on methylation processes has been obtained from studies in freshwater systems.

Several different types of microorganisms are capable of methylating Hg(II), and the methylation takes place especially at the interface between oxic and anoxic zones in nature—typically in the upper parts of sediments where the oxygen gradient is sharp. Sulfate reducing and methanogenic bacteria are capable of methylating inorganic mercury, and in some environments the quantitatively most important methylation is carried out by these organisms.

The degree of methylation in aquatic ecosystems depends on a number of different factors:

1. Temperature

   The higher temperature, the higher methylation.

2. Dissolved organic material
   a. If the dissolved organic material can be used as an energy source by the microorganisms, the rate of metabolism in these may be increased, which also leads to an increase in the methylation rate.
   b. If the dissolved organic material binds Hg(II)—which is the substrate in the methylation process—the rate of methylation may decrease with increasing concentration of organic material.
3. pH

   The net methylation rate generally increases when the pH value decreases. The rate of both methylation and demethylation may increase with decreasing pH, but because the rate of methylation most often increases more than the rate of demethylation, the net methylation rate will increase.

4. The concentration of bioavailable Hg^{++}:

   The larger the Hg^{++} concentration, the higher rate of methylation.

5. Complex binding ligands (both organic and inorganic, see Figure 1):

The higher amount of ligands, the lower rate of methylation.

Either methylmercury (CH_3–Hg^+) or dimethylmercury (CH_3–Hg–CH_3) is formed in the methylation process. At neutral and low pH, methylmercury (as CH_3–Hg^+Cl^-) is the most stable of the two compounds, whereas dimethylmercury is most stable in an alkaline environment. The pH value in seawater is approximately 8.2 (and thereby higher than in most freshwater systems), and in seawater, the formation of dimethylmercury plays a role, although it is of less importance than the formation of methylmercury in most freshwater systems. Dimethylmercury is fairly volatile, and it may evaporate to the atmosphere. Dimethylmercury is generally found at lower concentrations in the upper layers of the oceans than in the deeper, and this may be because of the evaporation to the atmosphere.

8.10.2.2 Demethylation

Certain types of bacteria are capable of demethylating methylmercury. The demethylation may take place in both aerobic and anaerobic bacteria, but the most quantitatively important demethylation seems to take place in the aerobic environment. The demethylation has been demonstrated both in the sediment and water phase of lakes and it has, likewise, been shown that freshwater algae are capable of degrading methylmercury.

8.10.2.3 Net Methylation

The amount of methylmercury present in a specific ecosystem will, therefore, not only depend on the rate by which methylmercury is synthesized, but rather the equilibrium between methylation and demethylation processes.

The total concentration of mercury in seawater is typically ranging from 1–5 ng/L. The fraction that methylmercury constitutes of the total mercury content in the seawater may vary somewhat, but it is generally in the order of 1%; in freshwater, the fraction of methylmercury is typically somewhat higher.

8.10.2.4 Reduction of Mercury

In the aquatic environment, Hg^{++} may be reduced to elemental mercury that can evaporate to the atmosphere. The reduction of Hg^{++} proceeds both abiotically and biotically—the latter probably predominantly in connection with enzymatic activity on the surfaces of unicellular algae (Poulain *et al*., 2004).

8.10.2.5 Oxidation of Elemental Mercury

Elemental mercury is relatively volatile, and it evaporates from both soil surfaces and the aquatic environment to the atmosphere, where the mercury has a residence time of approximately 1 year. The elemental mercury may be oxidized to Hg^{++} in the atmosphere with the participation of ozone, and the divalent mercury is predominantly bound to particles in the atmosphere (Morel *et al*., 1998).

8.10.3 The Global Mercury Flux

Mercury exists in nature in equilibrium between the elemental mercury, Hg(II) salts, and organic mercury compounds, especially methylmercury, and the overall flux of mercury between soil, atmosphere, and oceans is especially related to mercury's shift in oxidation state between elemental and divalent mercury.

The evaporation of mercury to the atmosphere is larger from soil than from the oceans, and because the fraction of land as well as industrial activity is largest in the northern hemisphere, higher mercury concentrations are generally found in the atmosphere of the northern hemisphere than in the atmosphere of the southern hemisphere (Fitzgerald and Mason, 1997; Mason *et al*., 1994).

The emissions of mercury from industrialized areas are decreasing in Europe and North America but increasing in South East Asia (UNEP, 2002).

Even if the Arctic comprises only 1–2% of the earth's total surface, it is estimated that 6–10% of the total anthropogenic emission of mercury to the atmosphere is deposited there. In the months of spring and summer, the combination of ultraviolet light, ozone, bromine, and low temperature results in a more efficient transformation of the elemental mercury of the atmosphere to reactive mercury species (that are deposited) than at other locations on the earth (AMAP, 2002).

8.10.4 Uptake of Mercury in Organisms and Transport in Food Webs

As a general rule, organisms take up and retain methylmercury far more efficiently than inorganic mercury, and the relatively high content of mercury at the highest trophic levels of aquatic food chains is due to incorporation of organic mercury into the organisms at the lower trophic levels and subsequent transport in the food chains (Kraepiel *et al*., 2003; Morel *et al*., 1998).

The uptake of inorganic mercury in unicellular algae typically takes place by Hg^{++} absorbing to the surface of the algal cell without penetrating the surface. Methylmercury, on the other hand, penetrates the surface of the algae and is thereby taken up into the intracellular parts of the cell (Watras and Bloom, 1992; Watras *et al*., 1998). Mercury concentrations in phytoplankton organisms are typically 10^5–10^6 times higher than the concentrations in the surrounding water, the bioconcentration factor for methylmercury generally is higher than that for inorganic mercury (Watras *et al*., 1998).

Invertebrates generally take up and retain organic mercury more efficiently than inorganic mercury. Shrimps, *Crangon crangon*, exposed to the two mercury species in food for 3 weeks assimilated approximately 75% of the ingested organic mercury and only 4% of the inorganic mercury, and uptake of organic mercury from water was far more efficient than uptake of inorganic mercury (Riisgard and Famme, 1986).

Fish generally assimilate organic mercury efficiently from the food, and retention times for organic mercury may be very long as illustrated in experimental studies with rainbow trout in which half-lives of more than 200 days were found (Giblin and Massaro, 1973; Ruohtula and Miettinen, 1975). In piscivorus fish in the top of food chain, methylmercury typically constitutes more than 90–95% of the total mercury content of the fish.

In aquatic food chains, the methylmercury concentration that the top predators attain will depend on the amount of methylmercury that enters the food chain at the lower levels, because the food (also in predatory fish) is the main source for uptake of mercury. This underlines that the rate by which inorganic mercury in a specific ecosystem is methylated, participates in determining which total mercury concentrations will be attained by the top predators in the system.

The fact that the mercury concentration in fish typically increases with increasing size (and age) of the fish is, of course, partly a result of the efficient uptake and retention of methylmercury, but the fact that the food preferences of an individual fish (and thereby its trophic level in the food chain) changes with size, also play a role. As an example, small perches will feed mainly on plant material (with a low mercury content), whereas larger perches feed on zooplankton with a somewhat higher mercury content. As adults, the perch feed on other fish (with an even higher mercury content), and this means that the mercury dose that the perches ingest with the food increases with the size and age of the fish (Meili, 1997).

It is known from investigations in lakes that the main fraction of the total methylmercury burden in such ecosystems is bound in the biota—especially at the upper levels of the food chains. Generally, the fish in lakes with a low pH and/or alkalinity show the highest concentrations of methylmercury (Gilmour and Henry, 1991; Spry and Wiener, 1991). Contrary to that, the largest pool of inorganic mercury is bound in the sediments of the lakes (Meili, 1997).

Specific problems are associated with metal contamination of the tropical zones, where regulations concerning prevention of environmental pollution are not always followed. Unregulated gold mining has resulted in extensive freshwater mercury pollution, resulting in contamination of fish and birds. Biomagnification has been demonstrated from gastropod molluscs (*Ampullaria* sp) to accipiters (*Rostrhamus sociabilis*) and from invertebrates to fish, birds, and humans (Alho and Vieira, 1997; Pfeiffer *et al*., 1993).

8.10.5 Effects of Mercury in Wildlife

Fish may reach relatively high concentrations of methylmercury in their tissues (easily ~1 μg Hg/g wet weight in low-alkalinity freshwater systems), so high that adverse effects might be predicted in some circumstances. Sublethal adverse effects may be seen in the most sensitive species at body burdens of methylmercury exceeding 1.4 μg Hg/g wet weight, whereas lethal effects are observed in some species at whole body concentrations of 5–10 μg Hg/g wet weight (Spry and Wiener, 1991).

Canadian investigations have shown that the reproduction in fish eating birds—especially the effects on the common loon *Gavia immer*—are affected negatively

in lakes in which the contamination of the fish with methylmercury is high (Chan *et al.*, 2003; Scheuhammer and Blancher, 1994). The adverse effects are related to effects on the nervous system, and the breeding behavior is affected. The effects begin to appear when the mercury concentration in the food of the birds exceeds 0.3 µg Hg/g wet weight (Scheuhammer, 1991); more than this value is widely occurring in Canada, as well as in other countries.

Exposure to methylmercury in the laboratory is capable of reducing shell thickness in the eggs of domestic fowls (Lundholm, 1987; 1995) by affecting the overall calcium metabolism of the bird (a mechanism somewhat different from the one exerted by DDE). Earlier studies, however, showed no such effect of organic mercury in mallards (Haegele *et al.*, 1974) and methylmercury in ring doves and American kestrels (Peakall and Lincer, 1972), and it is questionable whether exposure to methylmercury was a participating agent in the widespread eggshell thinning observed among predatory and fish-eating birds during the 1960s.

8.10.6 Implications for Human Health

Predatory fish—both in the marine and freshwater environment—accumulate methylmercury to fairly high levels and mammals—such as seals, dolphins, and some whales—that feed on predatory fish accumulate methylmercury to even higher levels. Polar bears that feed on seals again attain even higher levels.

Humans who rely on fish or predatory mammals at the top of aquatic food chains as their main food source may be exposed to high levels of methylmercury in their diet—levels that easily exceed the limits recommended by WHO.

It is beyond the scope of this chapter to cover the details of human exposure to and the effects of mercury that are described in Chapter 33.

8.11 Molybdenum

Molybdenum is an essential element with special importance for plants. It exists in seawater in the hexavalent state (as molybdate); surface concentrations of 0.1 µmol/L (Collier, 1985).

Molybdenum is emitted to the environment through combustion of fossil fuels (up to 300 mg/kg in lignite), and fly ash may contain up to 60 mg/kg (Davis, 1991). The mobilization factor for molybdenum is as high as 45 (Lantzy and Mackenzie, 1979).

Ecotoxicological problems with emitted molybdenum have especially been associated with effects on grazing livestock (Davis, 1991).

8.12 Nickel

Nickel concentrations in the ocean vary from 2–3 nmol/L in the surface waters to 5–10 nmol/L in the deeper waters (Bruland, 1980; Yeats *et al.*, 1995). $NiCO_3^0$ is the dominating species in seawater, followed by Ni^{++} and $NiCl^+$ (Mantoura *et al.*, 1978).

Nickel is emitted into the atmosphere, and a mobilization factor of 3.5 has been estimated (Lantzy and Mackenzie, 1979). Ninety-two percent of the nickel transported in the rivers of the world is precipitated in the estuaries, and an average sediment enrichment factor in lakes and coastal areas of 1.3 has been found (Salomons and Förstner, 1984).

8.13 Selenium

Concentrations of total dissolved selenium in ocean water typically vary from 0.5 nmol/L in the surface layers to 1.0–1.5 nmol/L in the deeper layers (Cutter and Cutter, 1995). In the photic zone of the upper few hundred meters, organic selenium compounds (Se(-II) dominate, whereas selenite and selenate exist in equilibrium at depths below approximately 300 m at a ratio of approximately 0.65 (Se(IV)/Se(VI)) (Cutter and Cutter, 1995).

Selenium concentrations in soils show large variability, leading to deficiency symptoms in terrestrial organisms in some parts of the world and toxic symptoms in others.

Selenium is emitted to the environment especially through combustion of fossil fuels, and the anthropogenic emission to the atmosphere was estimated to 14,000 tons (Lantzy and Mackenzie, 1979). The natural emission was estimated to approximately 3400 tons, with only 410 tons bound in particles (Lantzy and Mackenzie, 1979).

Mobilization of naturally occurring selenium as a result of human activity may be seen under special circumstances. In an interaction between fairly complicated geological, chemical, and hydrographical conditions, intensive irrigation in California led to mobilization of soil selenium that ended up in surface waters in the Kesterson Reservoir (Hamilton, 2004); teratogenic effects were seen among the water birds of the reservoir, and reproduction among the fish was affected.

8.14 Silver

In the oceans, the depth profile for silver shows typical nutrient-like characteristics (Flegal *et al.*, 1995; Ndung'u *et al.*, 2001; Zhang *et al.*, 2001), with low concentrations (0.7 pmol/L) in the surface water and

higher concentrations (7 and 20 pmol/L in the Atlantic and Pacific, respectively) in the deeper waters. The concentrations of silver are closely correlated to silicate concentrations (Flegal *et al*., 1995).

Silver is emitted into the aquatic environment from photographic industries and mining activities and into the atmosphere by combustion of fossil fuels, leading to a relatively high mobilization factor of 83 (Lantzy and Mackenzie, 1979).

The accumulation and toxicity of silver has been reviewed by (Ratte, 1999).

8.15 Tin

8.15.1 Inorganic Tin

Tin exists in seawater as Sn(II) and Sn(IV). Surface and deep seawater concentrations of 0.5–5 and 0.2 ng/L, respectively, have been reported (Bruland, 1983).

Tin has been discharged into the environment from tin mining, and elevated concentrations of tin have been registered in sediment dwelling organisms in coastal areas affected by drainage from operating and abandoned tin mines (Bryan *et al*., 1980). The anthropogenic mobilization factor for tin is 8.3 (Lantzy and Mackenzie, 1979).

However, the largest ecotoxicological problems caused by tin compounds are associated with the use of organic tin compounds and especially the use of tributyltin in antifouling paints.

8.15.2 Tributyltin (TBT)

8.15.2.1 TBT as Antifouling Agent

Since man began to use ships as means of transportation, seafarers have had to fight fouling of the water-covered parts of the ships. The fouling with algae and barnacles may affect speed and stability, whereas attacks by shipworm (which is a mussel species) and other boring animals might destroy the woodwork of the ships. As far back as 300 years BC, there were descriptions of ships that had the water-covered parts protected against fouling with plates of lead. In the 17th and 18th century, copper plates were used for the same purpose, and around 1830, painting of ships with copper oxide–containing paints was initiated.

Many different toxic compounds (organic mercury compounds, arsenic compounds, etc.) have been used besides copper oxide, and in the 1960s, the use of organic tin compounds—especially tributyltin oxide—in ship painting was initiated.

Because of their broad versatility and high efficiency, the organic tin compounds have been the most widespread components to protect ships against fouling during the 1970s and 1980s in many countries, and they have, likewise, been used to prevent fouling of equipment in marine aquaculture.

However, tributyltin (TBT) is a highly toxic chemical, and the adverse effects of the chemical reaches far outside the ship hulls in the aquatic ecosystems.

8.15.2.2 Use and Dispersal of TBT

Organic tin compounds are used for many purposes, but of the approximately 35,000 tons that were used globally in the middle of the 1980s, 9% (in the form of TBT) was used as antifouling chemical in paints for ships. Approximately two thirds were used (mainly in the form of dibutyltin) as additive in PVC (Fent, 1996).

The TBT is formulated in the ship's paint in a way such that a gradual liberation to the water phase takes place. In areas with intense leisure time boating, a marked seasonal variation in the TBT concentrations of the water and sediments is observed; the concentration increases during summer when the leisure time boats are in the water (Blanck and Dahl, 1996). The TBT concentrations are highest around harbors and major shipping routes.

8.15.2.3 Uptake and Metabolization of TBT

The chemical character of TBT is dominantly nonpolar (the *n*-octanol/water partitioning coefficient is approximately 5600), and the chemical is accumulated in aquatic organisms to concentrations which for fish typically are approximately 200 times higher than the concentration in the ambient seawater, whereas molluscs such as snails and mussels may concentrate the compound to concentration factors as high as 50,000–100,000. Equilibrium between TBT in the seawater and the tissues is reached after 2–3 months (Bryan *et al*., 1987). Snails and mussels have a low capacity to metabolize TBT, and it is lost from dog whelks *Nucella lapillus* with a half-life of 50–100 days.

Higher organisms may metabolize TBT by hydroxylating and subsequently eliminating the butyl groups one by one such that the TBT is metabolized to dibutyltin and further to monobutyltin and inorganic tin, which are all less toxic than TBT (Lee, 1991).

In seawater, TBT is relatively quickly degraded by bacteria and algae to mono- and dibutyltin (Lee *et al*., 1989). The half-life for TBT in seawater is in the range of days to weeks, whereas the half-life in sediments may be up to 6 months or longer (Seligman *et al*., 1986).

8.15.2.4 Adverse Effects of TBT in the Marine Environment

Of the species investigated so far, molluscs (gastropods, mussels) seem to be the most sensitive toward TBT.

8.15.2.4.1 Effects on Mussels and Oysters During the 1970s, it was observed in French (Alzieu, 1991) and English (Thain *et al*., 1986) oyster aquacultures that oysters with an abnormally thick shell occurred increasingly frequently. Oysters with an abnormal shell development had poor growth, and the soft part portion became so poor that these oysters could not be sold for human consumption. Because the mortality among the oyster spat in the years 1977–1979 was very high and because the frequency of abnormal shell development and larvae mortality was highest in the vicinity of harbors and yachting harbors, TBT was suspected of causing this damage.

Investigations in the laboratory showed that TBT—besides the effect on shell formation in adults—has an extremely adverse effect on the reproduction in oysters and different species of mussels. When the oyster larvae after their free-swimming stage attach to the substratum to develop to oysters, they are very sensitive to TBT. Twenty nanograms TB/L inhibit the growth of the smallest oyster spat (Thain *et al*., 1986), whereas the larger spat are slightly more resistant.

8.15.2.4.2 TBT's Effect on Sexual Development in Snails Snails belonging to the families *Buccinidae* (i.e., the dog whelk *Nucella lapillus*) and *Nassidae* are normally gonochoristic (each individual is either male or female throughout life). In the late 1960s, female dog whelks were found around the Marine Biological Laboratory in Plymouth, which had developed obvious male sexual characters (i.e., penis and vas deferens) (Blaber, 1970). At the same time, an analogous phenomenon was found in the American snail species *Nassa obsoletus* (Smith, 1971; 1980; 1981), and here it was established that the cause for the development of the imposed male sexual characters was exposure to TBT. In these families of snails, the formation of imposed male sexual characters in the females is termed *imposex* (Smith, 1971). Imposex has now been registered in many species of snails worldwide; the number is approaching 200.

The development of imposex in females is divided into seven stages (Gibbs *et al*., 1987): Stage 0, normal female; Stage 1, formation of vas deferens initiated; Stage 2, penis development initiated; Stage 3, vas deferens 50% developed; Stage 4, vas deferens fully formed; Stage 5 vas deferens/prostate tissue begin to block the vaginal opening; Stage 6, the capsule gland contains aborted egg capsules.

Although the development of imposed male sexual characters does not seem to affect the ability to reproduce in *Nassa obsoletus* (Smith, 1980; 1981), the female dog whelks may end up sterile in severe cases, because prostate-like tissue around the vas deferens blocks the vaginal opening of the female. It is assumed that sterile females may die because they abort the eggs (that cannot be liberated via the blocked vaginal opening) in the body cavity (Gibbs *et al*., 1987).

Dog whelks are very sensitive to TBT, and in adult females concentrations as low as 5 ng TBT/L may induce growth of penis. Young and not yet sexually mature females of dog whelks are even more sensitive than the adult individuals and exposure to less than 1 ng TBT/L in nature seems to induce growth of penis and vas deferens tissue in females (Gibbs *et al*., 1987).

Imposex occurs not only in coastal areas but also in open marine areas with major shipping routes (Ten Hallers-Tjabbes *et al*., 1994; 2003) (i.e., in the Danish Belt Area and Kattegat no normal females of the whelk *Neptúnea ántiqua* were found in a recent survey) (Strand and Jacobsen, 2002).

In periwinkles (e.g., *Littorina littorea*) and mud snails (*Hydrobia* sp.) the masculinizing effects of TBT are exerted in a slightly different manner than in the whelks, and in these species the condition is termed *intersex* (Bauer *et al*., 1995). Intersex in the periwinkles is divided into four stages. A penis is developed in the female only in Stage 4 (which is very rarely observed). The periwinkles are far less sensitive to TBT than the whelks.

8.15.2.4.3 Effects in Populations Many of the English populations of dog whelks declined in numbers during the 1970s and 1980s, and it is assumed that population sizes start to be affected when between 20 and 40% of the females have become sterile (Gibbs *et al*., 1991; Langston *et al*., 1990; Spence *et al*., 1990).

8.15.2.4.4 The Mechanism of Action for TBT Several mechanisms of action—that do not mutually exclude each other—have been suggested for TBT's interference with sexual development in gastropods.

It has been proposed that the mechanism of action for TBT is related to the aromatase inhibiting effect of the chemical (Bettin *et al*., 1996). There are also indications that exposure to TBT (albeit at fairly high exposure concentrations) reduces the excretion of testosterone by inhibiting the sulfate conjugation of the testosterone (Ronis and Mason, 1996). Regulation of free testosterone levels may, in certain invertebrates, be associated with the formation of testosterone fatty acid esters (Gooding and LeBlanc, 2001), and TBT affects the equilibrium between free and bound testosterone (Janer *et al*., 2005; Santos *et al*., 2005). TBT accumulates in gastropods in especially high concentrations in the nervous system (Bryan *et al*., 1993), and it has been proposed that TBT exerts its effect through interaction with the formation of neuropeptides involved in

regulation of steroid synthesis (Oberdorster *et al*., 1998; 2005; Oberdorster and Clellan-Green, 2000; 2002).

Besides the effect on hormonal regulation, TBT has other toxicological properties (TBT uncouples the production of ATP in the mitochondria and affects the immune system).

8.16 Vanadium

Vanadium exists in seawater mainly as vanadates. Vanadium is emitted to the environment especially from combustion of fossil fuels, and the mobilization factor is 3.2 (Lantzy and Mackenzie, 1979).

8.17 Zinc

In the oceans, zinc concentrations show the typical nutrient-type profile with concentrations below 0.5 nmol/L in the surface waters and concentrations between 2 and 10 nmol/L in the deeper waters (Bruland, 1980; Landing *et al*., 1995), the Pacific generally showing higher concentrations than the Atlantic. $Zn(OH)_2^0$ and Zn^{++} are the dominating species in seawater (Long and Angino, 1977; Zirino and Yamamoto, 1972).

Zinc is emitted into the atmosphere through combustion processes and metal smelting, with an anthropogenic mobilization factor of 23. Zinc is discharged into the aquatic environment from mining activities and a variety of industrial processes (i.e., galvanizing). Slightly more than half of the zinc transported by the rivers of the world is precipitated in the estuaries (Yeats and Bewers, 1982), and an average sediment enrichment factor of 3.5 in lakes and coastal areas has been reported (Salomons and Förstner, 1984).

8.18 Radioactive Metals

Radioactive metals are produced in various nuclear processes, and they have been released into the environment during nuclear weapons testing, accidents at nuclear power plants, and from nuclear fuel reprocessing plants.

8.18.1 Cesium

^{134}Cs and ^{137}Cs are major fission products in nuclear processes, and with their half-lives of 2.1 and 30 years, respectively, they constitute an important source of contamination of the environment with radioactivity. Major releases have come from nuclear weapons testing, from the nuclear fuel reprocessing plant at Windscale, UK, and from the Chernobyl accident at which 85 and 46×10^{15} Bq of ^{137}Cs and ^{134}Cs, respectively, were emitted (EEA, 2003).

Cesium has a low tendency to adsorb to particles in water, and discharges of ^{137}Cs from Windscale could be traced in seawater in the Irish Sea, north of Scotland, along the Norwegian coast around Svalbard, not reaching "background concentrations" (originating from global fallout from nuclear weapons testing) until the current after 6–8 years and 5000–7000 km reached Northeast Greenland (Hallstadius *et al*., 1986).

Atmospheric transport of ^{137}Cs and fallout may create problems in especially sensitive ecosystems far away from the sites of emission. Oligotrophic freshwater systems are among such sensitive ecosystems. ^{137}Cs is taken up into organisms through the same routes as potassium, and the ability of cells to transport potassium against concentration gradients to high intercellular concentrations also leads to a fairly high bioconcentration of radioactive caesium. After the Chernobyl accident in 1986, a considerable amount of ^{137}Cs was deposited in the northern and central parts of Scandinavia and in a large number of Swedish lakes, the ^{137}Cs content of the fish exceeded the limits (1500 Bq/kg) for human consumption (Hakanson *et al*., 1992).

On deposition from the atmosphere, ^{137}Cs is concentrated in lichens that constitute an important fraction of the food of reindeers during the autumn and winter seasons. Fallout from nuclear weapons testing, and especially the Chernobyl accident, resulted in ^{137}Cs activities in reindeer in certain Scandinavian areas that vastly exceeded the limits for human consumption (Ahman and Ahman, 1994). Fungal mycelia assimilate ^{137}Cs very efficiently from the soil, and mushrooms may be an important source for ^{137}Cs uptake in ruminants such as roe deers (Zibold *et al*., 2001) and goats (Hove *et al*., 1990). Predators with roe deer and reindeer as their main prey, such as lynx, also accumulated ^{137}Cs to high activities after the Chernobyl accident (Ahman *et al*., 2004).

8.18.2 Polonium

Polonium has no stable isotopes, but ^{210}Po is one of the decay products in the decay series of the naturally occurring, radioactive isotope ^{238}U. ^{210}Po has a half-life of 138.4 days, and it is an α-emitter. In the marine environment, some organisms are naturally exposed to very high doses of radiation from ^{210}Po. Polonium is concentrated in phytoplankton to bioconcentration factors of $3–7 \times 10^4$ (Fisher *et al*., 1983c), and the isotope is taken up especially from food in crustaceans; it is accumulated in the midgut gland or hepatopancreas of the animals (Cherry and Heyraud, 1982). In some crustaceans, this results in very high radiation doses. The highest recorded doses have been identified in pelagic

penaeid shrimps receiving yearly doses as high as 3.1 sievert for the entire animal and 26 sievert in the midgut gland (Heyraud *et al*., 1988). These values represent the highest doses of naturally occurring radioactivity received by any organism.

8.18.3 Strontium

^{90}Sr is a major fission product in nuclear processes and with a half-life of 28 years, ^{90}Sr was a constituent of the global radioactive fallout after the intense nuclear weapons testing in the 1960s. Because of its similarity with calcium, ^{90}Sr accumulated in milk and in bone (Bartlett *et al*., 1972).

8.18.4 Transuranic Metals

Transuranic elements such as plutonium, americium, and curium are produced by neutron activation in nuclear processes. Some of these isotopes have long half-lives, and they are α- emitters with a high toxicity.

Emission from nuclear weapons testing and nuclear fuel reprocessing plants has led to contamination of, for example, the marine environment. The aquatic chemistry of plutonium is very complicated, but both plutonium and the trivalent americium are taken up into marine organisms to high bioconcentration factors; this is true for both phytoplankton (Fisher *et al*., 1983a; 1983c), zooplankton (Fisher *et al*., 1983b), and benthic bivalves (Bjerregaard *et al*., 1985).

Contamination with plutonium from the previous emissions from Windscale could be traced in water and seaweeds in the currents north of Scotland and up along the Norwegian coast (Hallstadius *et al*., 1986).

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CHAPTER

14

Risk Assessment*

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ABSTRACT

Risk assessment for metallic substances usually follows the generally accepted framework format for risk assessment for all toxic substances, which involves (1) exposure assessment, (2) hazard identification, (3) assessment of dose-response relationships, and (4) risk characterization. The importance of risk communication is also addressed. Risk assessment/risk communication is of particular relevance for metals and metalloids, because all living organisms are exposed to these elements, and metals such as lead, cadmium, and mercury and the metalloid arsenic have been responsible for many human poisonings and even deaths. It is, hence, imperative that readers of this handbook have a firm perspective on the exposure levels of metallic substances that produce adverse health effects and the various risk assessment approaches that have been used and are evolving to protect the health and well-being of living organisms. Biomonitoring approaches, identification of toxic metallic species for hazard identification, dose–effect relationships, construction of dose-response curves, and the development of benchmark doses for various metallic species are discussed in relation to protecting sensitive subpopulations, because not all individuals within a general population are at equal risk for toxicity. Risk characterization using modern biomarkers that are capable of detecting early cellular effects to low-dose exposures to metallic substances will play an increasingly important role in assessing risk from exposure to this class of toxic substances on an individual or mixture basis. The issue of metal/metalloid-induced carcinogenesis is of ever increasing importance, because many of the elements associated with this cellular outcome produce a number of early cellular effects, including formation of reactive oxygen species (ROS) and apoptosis. Finally, the issue of risk communication/risk management is of great importance, because these issues are critical to addressing the health concerns of exposed populations and the practical, ethical, and financial issues related to reducing hazardous exposures to metallic substances.

1 INTRODUCTION

Risk is a basic and important concept in toxicology and environmental health. The use of scientific information to identify and quantitatively estimate human health risk (i.e., the probability of occurrence of adverse effects that may be caused by exposure to metals) is fundamental to risk assessment in this field.

During the last decades, principles for the assessment of risks to human health from exposure to chemicals have been generally agreed, and four components of a risk assessment are usually included (WHO/IPCS, 1999). The USEPA has also recently published a Framework for Inorganic Metals Risk Assessment (2006a), which addresses a number of cross-cutting issues, such as variability in susceptibility including age, gender, current damage or disease, genetic polymorphisms, and metal mixtures.

The four steps in risk assessment are exposure assessment, hazard identification, assessment of dose-response relationships, and risk characterization. These

*Partly based on the chapter "Risk Assessment" by the late Dr. Lars T. Friberg in the 2nd edition of this Handbook.

four components will be described in this chapter, with a focus on how they can be applied to metals and their compounds. Risk assessment documents for several metals, metalloids, and metal compounds have been published by the World Health Organization in its International Program on Chemical Safety (e.g., WHO/IPCS, 2001). Weight-of-evidence evaluations of carcinogenicity of metals and compounds are published by the International Agency for Research on Cancer (e.g., IARC, 2006a). There are also Union and National criteria documents.

As a result of risk assessment, further steps are often required in terms of risk control and risk management. Risk communication is an increasingly important component of these processes. It comprises the interaction between risk assessors and those concerned (i.e., industrial workers for occupational exposures and the general public for environmentally distributed substances). Risk assessment of widespread substances such as metals often requires careful consideration and judgment of available scientific information to reach widely accepted recommendations.

To perform in-depth risk assessments of a large number of chemical substances is a time-consuming process. It has recently been suggested that simplified approaches may be used, but they are very uncertain and cannot be recommended as a replacement for actual risk assessments. A valuable step for substances with limited quantitative data is to perform the hazard identification step, which may be sufficient for labeling of substances for hazards such as flammability, acute toxicity, irritation, sensitization, carcinogenicity, and reproductive toxicity. Readers of this handbook will see that the information available on the toxicology of metals and their compounds varies widely among metals. Only for a limited number of the metals is there adequate information to make reliable dose-response assessments for humans. In some regions of the world, legislation is being advanced requiring producers and importers of chemical substances, including metals and their compounds, to provide specific data relevant for risk assessment, and we hope that such information provided by this and other mechanisms will give us a basis for improved safety in the use of metals that are presently incompletely investigated.

To perform an adequate risk assessment, there is a need for the responsible scientists, as well as for administrators, to consider certain conceptual and other issues. Some of the major issues of importance for exposure and dose assessment, hazard identification, dose-response estimation, and risk characterization are discussed in the following. The reader is referred to Chapter 9 for a discussion of additional considerations in risk assessment of essential metals.

2 EXPOSURE AND DOSE ASSESSMENT

A description of exposure to the metal compound being considered is a very important component of risk assessment. In combination with results of dose-response analysis, it forms the basis for risk characterization (Section 5).

2.1 Exposure and Dose Terminology

Although efforts have been made to distinguish the terms "exposure" and "dose," there is still no general recognition of such special usage, and the terms usually describe similar conditions as also discussed in Chapter 4. The term "exposure" means that a person or group of persons is exposed to one or more chemical agents such as a metal, and sometimes this term is reserved for the external contact between the human being and the environment. This is the main focus of the WHO/IPCS document 214, where it is said that human exposure assessment is to identify and to define the exposures that occur, or are anticipated to occur, in human populations. This can be a complex endeavor requiring analysis of many different aspects of the contact between people and hazardous substances (WHO/IPCS, 2000). However, in cell biology, the term exposure is also used to describe exposure of cells and subcellular organelles *in vitro* to solutions of metal compounds. The term "dose" originates from pharmacology and means the amount of drug given to a patient. A proportion of the dose is absorbed systemically; this is the absorbed dose, also named internal dose (see also Chapter 3). A fraction of the internal dose is transferred to the organ in the body suffering damage at relatively low exposures (i.e., the critical organ). The concentration of a metal compound in the critical organ is of considerable interest in risk assessment of metal exposures as discussed further in Sections 2.4, 2.5, and 4.1.2. The assessment of metal exposure and dose, thus encompasses primarily the contact between human beings and metals in surrounding media such as air, drinking water, food, and media in contact with the skin but may also include estimation of internal dose and accumulation in critical organs. This section will deal with exposure and dose assessment in this broader scope.

2.2 Exposure, Applied/Inhaled Dose, Daily Intake

Exposure assessment will provide a description of the exposure, often taken as external exposure, by exposure route and its distribution in time and space.

Exposure in terms of concentration of metal/metalloid or its compounds in inhaled air, in drinking water, in food, or in media contacting the skin can be measured or estimated from mathematical models describing concentrations in exposure media from emissions of pollutants into air or water. Such models are often available as computer software for application in risk assessment (e.g., EUSIS, the European Union system for the evaluation of substances) (Vermeire *et al*., 2005). Actual measurements of concentrations of metals and their compounds in air are also performed both in the general environment and particularly in workroom air. Concentrations of metals in drinking water and recreational waters are recorded in environmental monitoring programs.

Emission to air or water can be measured or estimated from the inherent chemical properties of the metal compound in question, patterns of usage, natural occurrence, and waste disposal. Often industrial emissions and other point sources releasing metals into the environment are regularly monitored.

The daily oral exposure dose (or orally applied dose; cf WHO/IPCS, 1999) or daily oral intake of a metal is the amount of metal taken into the gastrointestinal tract. The gastrointestinal intake can be measured by determinations of the amount of metal in portions of food of the same composition as those consumed, the so-called double portion method. It can also be estimated from records of consumption and determinations of metal concentration in various foodstuffs and for metals with limited uptake in the gastrointestinal tract by determinations of the amount of metal in feces, successfully used, for example, for cadmium (cf Chapter 23). When there is exposure through inhalation, the inhaled dose can be estimated from measurements of the metal or its compound in air in combination with estimations of lung ventilation volume based on time of exposure and work load. The use of different types of sampling procedures for estimating air levels of chemical substances such as area sampling and personal sampling should be guided by the purpose of the study to be performed. If source identification is important, stationary sampling may be preferred, whereas personal sampling will be chosen if an estimate of inhaled doses of individuals is desired (WHO/IPCS, 2000). The importance of quality control in sampling and chemical analysis is dealt with in Berglund *et al*. (2001) and in Chapters 2 and 4.

2.3 Absorbed Dose, Internal Dose

As mentioned in the foregoing text, the absorbed dose or internal dose is the amount of metal taken up into the body. When available, data on the internal dose, and particularly the dose in the critical organ, are very useful when describing dose-response relationships (see Section 4). Internal dose can be calculated based on information about absorption. Gastrointestinal absorption of various metal compounds varies widely from almost no absorption to almost 100% absorption as discussed in Chapter 3. In that chapter uptake of metal compounds through inhalation is also described. The great importance of water solubility for uptake of gaseous metals and metal compounds is pointed out, as well as the importance of solubility in tissue fluids and particle size for metals occurring in aerosol form in air.

Internal dose can be estimated from concentrations of metals in blood, plasma, whole blood, or urine based on knowledge about the toxicokinetics of the metal compound considered. For compounds with short biological half-times and excretion through urine, there is a peak concentration in plasma concentration and a subsequent peak in urinary concentration. Other excretion patterns give rise to other relationships between plasma, whole blood, and urine concentrations. Other media used for biological monitoring are saliva, hair, nails, and *in vivo* determinations of metals in bone, kidney, liver, and other body organs as described in more detail in Chapter 4.

2.4 Dose/Concentration in Critical Organ and Critical Target

The best dose estimate is the concentration of metal at the target molecule that determines the expression of adverse effect. It is seldom possible to measure this concentration. Second best is to estimate the concentration in the organ or tissue where an adverse effect is expressed at relatively low exposures (i.e., the critical organ for the critical effect). For certain metals, for example, cadmium, whose critical effect is exerted on the kidney, *in vivo* determinations of levels of cadmium in this organ can be performed by *in vivo* neutron activation analysis (cf Chapter 23). Such measurements provide an extremely good dose estimate for risk assessment. However, because of the high cost and limited precision in such analyses, they are not used routinely in occupational and environmental health. Indeed, risk estimates are in many studies based on other biomarkers as described in the sections to follow.

2.5 Use of Biomarkers in Estimating Concentration in Critical Organ and Critical Target Dose

A possibility to study the accumulation of chemical substances in exposed people is by the use of blood

and urine samples as an indicator of exposure and for risk estimations. Such biological monitoring takes into consideration interindividual and intraindividual variations in uptake because of variations in, for example, metabolism and physical activity. Biological monitoring measures *total* exposure and not only exposure through, for example, inhalation. Much information of importance for occupational health criteria and standards comes, for example, from studies in which humans have been exposed to metals through contaminated food, even though occupational exposure limit values mostly are set for air concentration. Biological monitoring approaches and practices for a number of substances, including metals, have been recently reviewed by the NRC (2006).

For metals, such as lead and cadmium, exposure through food usually constitutes the major exposure route for the general population. For cadmium, smoking is an additional important source, giving rise to considerable increases in blood cadmium. When exposure through food is low, smoking may contribute to approximately half the body burden of cadmium. Exposure within industry may not necessarily be through inhalation only. Secondary contamination of hands, clothing, and of cigarettes, snuff, and pipe tobacco carried in pockets may be an important exposure source (Chen *et al*., 2006; Piscator *et al*., 1976). For a few metals it is possible to determine the concentration in the critical organ by direct *in vivo* measurements. An example is *in vivo* cadmium measurements of the amount of cadmium in the kidney (Chapter 23).

The rationale for the use of biological monitoring to estimate internal dose and critical concentration in critical organ as a proxy to critical target dose is discussed in Chapter 4. Emphasis is given to the importance of adequate use of chemical speciation, quality assurance of sampling, and chemical determinations. A prerequisite for successful use of concentrations in indicator media for estimation of exposure, accumulation of the metal in critical organs, and risks of adverse effects is knowledge of the toxicokinetics and metabolism of the metal, including data on the relationship between external exposure, internal dose, concentration in critical organ, and concentrations in indicator media. For risk estimation, data are also required on mechanism for elicitation of adverse effects and relationships between critical organ concentration (a proxy of critical target dose) and the appearance of adverse effects.

3 HAZARD IDENTIFICATION

On the basis of all available evidence in humans, experimental animals, and *in vitro*, possible adverse effects of the metal or metal compound are evaluated. The weight of evidence is considered whether specific adverse effects can be caused under exposure conditions existing for humans. The primary consideration is what specific chemical species of the metal humans are exposed to. Subsequently, hazard assessment is performed based on information concerning relationships between such exposures and adverse health effects.

3.1 Speciation

The fundamental importance of speciation in risk assessment has recently been highlighted in "Elemental Speciation in Human Health Risk Assessment" (WHO/IPCS, 2007).

The need to consider different metal compounds (species) separately when evaluating metabolism and effects is well recognized for some metals and has recently been reviewed by Yokel *et al*. (2006) (see also Chapters 18, 19, 24, 31, 33, and 38). Mercury is a classic example. Not only is it necessary to separate organic from inorganic mercury compounds, but also metallic mercury has a different metabolism from both its organic and inorganic compounds, and, for example, methylmercury is much more toxic than phenyl mercury. Arsenic occurs as inorganic, as well as organic, compounds. Man is exposed primarily to certain stable organic arsenic compounds in fish and crustaceans. The toxicity of such compounds is considered to be low, although detailed data on possible long-term effects are lacking. Usually, trivalent inorganic arsenic compounds are considered more toxic than pentavalent ones. Evidence for carcinogenicity is mostly available for the trivalent form or a mixture of trivalent and pentavalent forms, because they occur in groundwater used as drinking water (IARC, 2004). Arsenic concentrations in urine are used as an indicator of exposure and risk, but it may be that a small, poorly soluble fraction of arsenic is of importance in the causation of lung cancer in inhalation exposures, and the relationship between lung cancer risk and urinary arsenic may be different than the one for oral exposures.

In all risk assessment, it is fundamentally important to recognize the different kinetics, metabolism, and toxicity of different species of metals. It, therefore, becomes more and more important to analyze not only the metals as such but also the different forms they occur in. Reliable methods are available (e.g., for analysis of different compounds of arsenic and mercury) (see WHO/IPCS, 2007, and Chapters 2, 19, and 33). The issue of speciation has not been a part of hazard and risk assessment of metals except for mercury. The role of elemental speciation and speciation analysis in human health hazard and risk assessment is also

important for several metals and should be considered by risk assessors and regulators in their daily work (WHO/IPCS, 2007).

3.2 Human Data

When performing risk assessment for human exposures, data from humans are of special importance. In addition to general medical knowledge about human diseases, pathology, physiology, and biochemistry, specific knowledge about adverse effects observed in humans as a result of exposure to the toxicant under consideration is of great value. Case reports may be available and detailed studies of a small number of exposed persons with a specific disease who have all been exposed to the same metal compound. Collection of such data is sometimes named "toxicovigilance," a concept encompassing the active detection, validation, and follow-up of clinical adverse events related to toxic exposures in humans (Descotes and Tesud, 2005). Case studies can generate hypotheses for further studies but can seldom establish causality, except when there are unique features such as an association with a rare disease (e.g., Minamata disease) from methylmercury exposures.

Experimental studies in human volunteers can be performed if ethical considerations according to the Helsinki declaration are observed. Kinetics of metal compounds at low doses can be performed, and mild, reversible effects of low doses can be studied. Only few such studies have been reported in the field of metal toxicology.

Studies on the occurrence of diseases in groups of exposed and unexposed persons (i.e., cohort-type epidemiological studies) often provide useful information if well performed. For uncommon diseases, case-control studies may be more feasible (cf Chapter 8). These so-called analytical epidemiological studies can contribute to the establishment of causal connections between exposure and effect; however, the weaknesses inherent in any epidemiological study should be considered (Rothman and Greenland, 1998). Descriptive and correlation studies (also named ecological studies) are mainly hypothesis generating and can seldom establish causality by themselves. They may, however, be valuable as a complement to other observations.

When establishing causality, several of the classical "Hill's criteria" (Hill, 1965) are still valid:

1. Strength of association, meaning that it is more likely that an association with a large relative risk is causal.
2. Consistency, meaning that if association can be found in several studies by different investigations, causality is supported.
3. Temporality, meaning that exposure must precede effects.
4. Biological gradient, meaning that an association for which a dose-response relationship has been demonstrated is likely to be causal unless there is confounding.
5. Specificity, meaning that the effect is specific to the exposure. In metal toxicology, this is rarely the case. Although the presence of specificity implies causality (e.g., for asbestos exposure and mesothelioma), its absence does not exclude it.
6. Biological plausibility. If the mechanism of injury is known from animal experiments, this supports causality. However, if the mechanism is not known, or if there are no animal experiments reproducing the same effect of a specific exposure that, in itself, is not sufficient reason to reject causality.

In the evaluations of causality for cancer published by the International Agency for Research on Cancer–IARC (cf preamble in IARC, 2006) as result of meetings of expert task group discussions, criteria for causality have been selected and developed. To classify an agent as carcinogenic to humans (**IARC Group 1**), it is usually required that there is sufficient evidence of carcinogenicity in humans. Such evidence is based on epidemiological studies of good quality showing a statistically significant association between exposure to the agent and the occurrence of cancer; in addition, the IARC working group should be convinced that bias and confounding could be ruled out with reasonable confidence. During the last decade, classification in Group 1 can also be supported by a combination of limited evidence for carcinogenicity in humans and sufficient evidence of carcinogenicity in experimental animals and strong evidence in exposed humans that the agent acts through a relevant mechanism of carcinogenicity.

The IARC thus uses their own combination of criteria in their evaluation, some of which are similar to those advanced by Hill, for example, consistency and biological plausibility. Similar criteria are used by other organizations classifying chemical substances and other exposures as human carcinogens (see Sections 3.4, 3.5, and 3.6).

3.3 Data from Studies on Acute and Chronic Toxicity in Animals, Cells, and Molecular Systems *In Vitro*

Although human data are available for several metals and their compounds, there is a lack of such data for most metals not yet used extensively in society (see the respective chapters on individual metals

in this handbook). Data on acute and chronic toxicity in experimental animals provide useful information on potential effects in humans. To provide such useful information, animal studies have to be well performed. Test guidelines are available for the various specific animal bioassays for short-term, subchronic, and chronic toxicity, for developmental and reproductive toxicity, immunotoxicity, and carcinogenicity (see *www.oecd*). Available human and animal data for metals on some of these specific effects are provided in separate chapters of this handbook, for immunotoxicity in Chapter 11, for reproductive and developmental effects in Chapter 12, and for carcinogenicity in Chapters 5 and 10. Animal studies published in recent years often fulfil the OECD requirements or similar guidelines, but older data may not. Positive findings may still be useful even if the size of groups do not meet the requirements of the guidelines. The absence of observed effects, however, cannot be assessed from such incomplete data.

In addition to the categories of bioassays mentioned, specific tests may be required for effects like skin and eye irritation. Traditionally, such irritation effects have been assayed in whole animals, but recent achievements concerning alternatives to animal testing have made it possible to test some of these irritation effects in alternative systems (see *http://ecvam.jrc.it*). Particularly for effects like irritation, implying some degree of animal suffering, it is important from an ethical point of view to use nonanimal assays as much as possible if such systems can provide the same degree of safety for humans in the final risk assessment.

When human data are entirely or almost entirely lacking, hazard identification is based on animal and *in vitro* data.

Only when the nonhuman data are of good quality, can a reasonable degree of safety for humans be achieved. Even limited human data complementing good nonhuman data improves the risk assessment considerably both from a qualitative (hazard identification) and a quantitative (dose-response analysis) point of view (see Section 4).

In vitro data on cells and molecular systems provide very useful information when mechanism of action for toxic metals can be described. In situations in which such mechanisms can be shown to be valid at dose levels occurring in human tissues to realistic exposure levels for humans and animals, such *in vitro* data are very valuable for extrapolation of observations in animals to humans (see Chapter 6).

The role of metals in carcinogenesis was discussed in an international meeting in 1980 (Belman and Nordberg, 1981). No firm conclusions in regard to the use of so-called short-term tests were reached at that time, although lifetime studies on animals were considered predictive of probable carcinogenicity in man, especially when the routes of exposure and the sites of the tumors are the same. These are the same evaluation principles as used by IARC in their documents on carcinogenic risks to humans (IARC, 2006a,b). There are several examples of metals/metalloids (arsenic, beryllium, cadmium, chromium, and nickel) that have proven carcinogenic in both humans and animals. At the international meeting referred to previously (Belman and Nordberg, 1981), some chronological observations were made on the carcinogenicity of metallic compounds as judged by case reports, animal experiments, and epidemiological studies. This evaluation has been updated by us, including all metal compounds classified by IARC as human carcinogens or possible human carcinogens (IARC, 2006a,b) and is shown in Figure 1.

The reason that caution should be exercised when extrapolating data from animals to humans is not only because of the possible differences in metabolism related to species differences. Exposure situations will also differ. Often exposure in animal experiments is higher and more acute than exposure in real life. On the other hand, if only single substance exposures occur, this situation excludes several possibilities for interactions. Furthermore, the number of animals exposed is usually small compared with the number of people that may be exposed and does not include the very young or very old and other risk groups, such as people with deficient or suboptimal nutritional intakes and people who already suffer from diseases.

As mentioned (Section 3.2), the IARC classification system for carcinogenicity in humans uses these principles for classification into Group 1. Experimental data in animals and *in vitro* systems are the main basis for classification of exposures into Group 2, although epidemiological data are also required for classification in Group 2A. As stated in the preamble of the IARC monographs (IARC, 2006b).

3.3.1 IARC Group 2

This category includes agents for which, at one extreme, the degree of evidence of carcinogenicity in humans is almost *sufficient*, as well as those for which, at the other extreme, there are no human data but for which there is evidence of carcinogenicity in experimental animals. Agents are assigned to either Group 2A (*probably carcinogenic to humans*) or Group 2B (*possibly carcinogenic to humans*) on the basis of epidemiological and experimental evidence of carcinogenicity and mechanistic and other relevant data. The terms *probably*

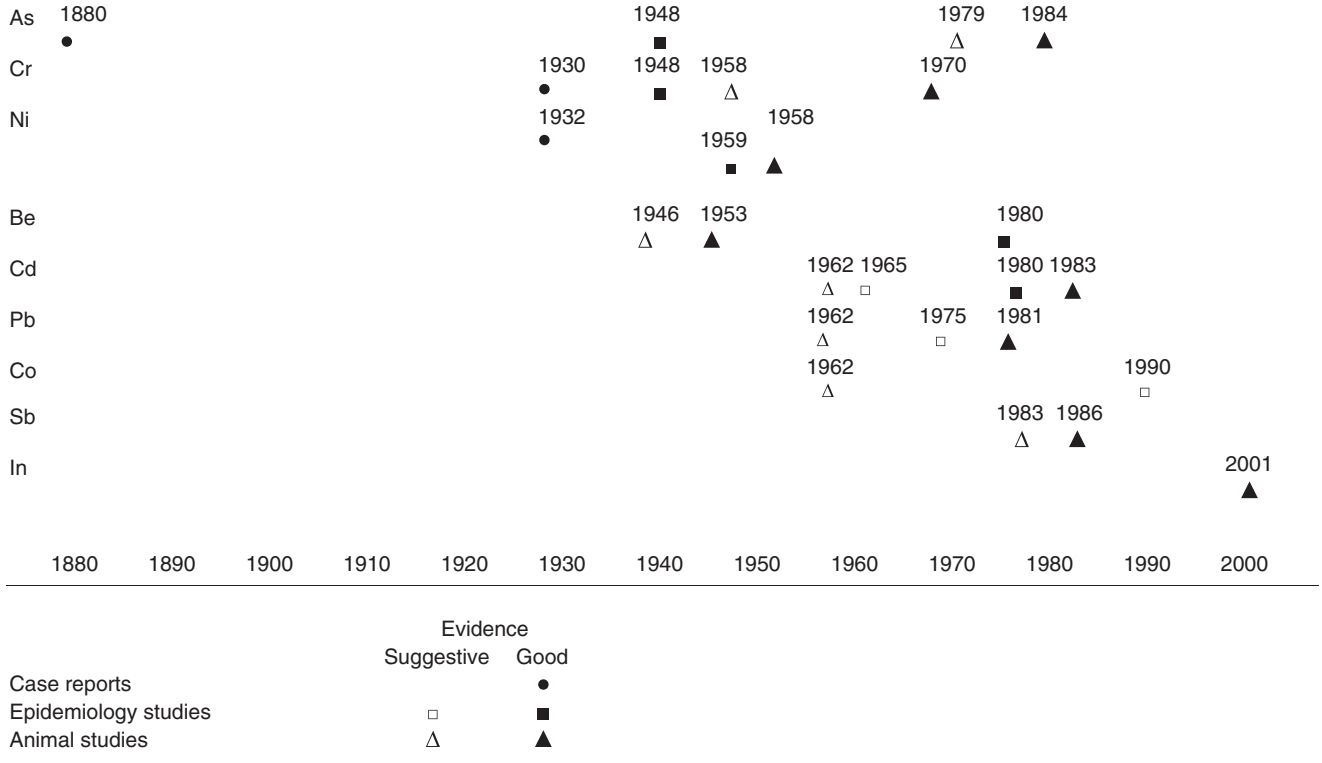

FIGURE 1 Chronology of observation on carcinogenicity of metallic compounds. At an international meeting in 1980 (Belman and Nordberg, 1981), the first five elements listed in the figure were judged as contributing to human cancer. The suspected metallic compounds are not listed in the figure, but as pointed out in the review, specific metallic compounds usually constitute the carcinogenic entity. Data by Takenaka *et al*., (1983), Pershagen *et al*. (1984) have been added as well as data on Pb, Co, Sb as Sb_2O_3, In as InP (review IARC [2006a] IARC [2006b]). The last four metallic compounds are considered by IARC as probable human carcinogens (group 2A). IARC has classified vanadium pentoxide and iron–dextran complex as possible human carcinogens, but they are not listed in the figure.

carcinogenic and *possibly carcinogenic* have no quantitative significance and are used simply as descriptors of different levels of evidence of human carcinogenicity, with *probably carcinogenic* signifying a higher level of evidence than *possibly carcinogenic*.

3.3.2 IARC Group 2A: The Agent is Probably Carcinogenic to Humans

This category is used when there is *limited evidence of carcinogenicity* in humans and *sufficient evidence of carcinogenicity* in experimental animals. In some cases, an agent may be classified in this category when there is *inadequate evidence of carcinogenicity* in humans and *sufficient evidence of carcinogenicity* in experimental animals and strong evidence that the carcinogenesis is mediated by a mechanism that also operates in humans. Exceptionally, an agent may be classified in this category solely on the basis of *limited evidence of carcinogenicity* in humans. An agent may be assigned to this category if it clearly belongs, based on mechanistic considerations, to a class of agents for which one or more members have been classified in Group 1 or Group 2A.

3.3.3 IARC Group 2B: The Agent is Possibly Carcinogenic to Humans

This category is used for agents for which there is *limited evidence of carcinogenicity* in humans and less than *sufficient evidence of carcinogenicity* in experimental animals. It may also be used when there is *inadequate evidence of carcinogenicity* in humans but there is *sufficient evidence of carcinogenicity* in experimental animals. In some instances, an agent for which there is *inadequate evidence of carcinogenicity* in humans and less than *sufficient evidence of carcinogenicity* in experimental animals together with supporting evidence from mechanistic and other relevant data may be placed in this group. An agent may be classified in this category solely on the basis of strong evidence from mechanistic and other relevant data.

Other frequently cited classifications of carcinogenicity are those made by EU, USEPA, and ACGIH, and they will, therefore, be described in the following text.

3.4 Classification According to the European Union (HSE 1999; Nordberg *et al.*, 2004)

For the purpose of classification and labeling and having regard to the current state of knowledge, substances are divided into three categories:

Category 1: Substances Known to be Carcinogenic to Man

There is sufficient evidence to establish a casual association between human exposure to a substance and the development of cancer.

Category 2: Substances that Should be Regarded as if they are Carcinogenic to Man

There is sufficient evidence to provide a strong presumption that human exposure to a substance may result in the development of cancer, generally on the basis of appropriate long-term animal studies and other relevant information.

Category 3: Substances, Which Cause Concern for Man Owing to Possible Carcinogenic Effects but in Respect of Which the Available Information is not Adequate for Making a Satisfactory Assessment.

There is some evidence from appropriate animal studies, but this is insufficient to place the substance in Category 2.

3.5 Classification According to the USEPA (USEPA 1986, 2005)

Group A: "Human Carcinogen"

"This group is used only when there is sufficient evidence from epidemiologic studies to support a causal association between exposure to the agents and cancer."

Group B (1 and 2): "Probable Human Carcinogen"

"This group includes agents for which the weight of evidence of human carcinogenicity based on epidemiologic studies is 'limited' and also includes agents for which the weight of evidence of carcinogenicity based on animal studies is 'sufficient.' The group is divided into two subgroups. Usually, Group B1 is reserved for agents for which there is limited evidence of carcinogenicity from epidemiological studies. It is reasonable, for practical purposes, to regard an agent for which there is 'sufficient evidence of carcinogenicity' in animals as if it presented a carcinogenic risk to humans. Therefore, agents for which there is 'sufficient' evidence from animal studies and for which there is 'inadequate evidence' or 'no data' from epidemiologic studies would usually be categorized under Group B2."

Group C: "Possible Human Carcinogen"

"This group is used for agents with limited evidence of carcinogenicity in animals in the absence of human data. It includes a wide variety of evidence, e.g., (a) a malignant tumor response in a single well-conducted experiment that does not meet conditions for sufficient evidence, (b) tumor responses of marginal statistical significance in studies having inadequate design or reporting, (c) benign but not malignant tumors with an agent showing no response in a variety of short-term tests for mutagenicity, and (d) responses of marginal statistical significance in a tissue known to have a high or variable background rate."

Group D: "Not Classifiable as to Human Carcinogenicity"

"This group is generally used for agents with inadequate human and animal evidence of carcinogenicity or for which no data are available."

Group E: "Evidence of Non-Carcinogenicity for Humans"

"This group is used for agents that show no evidence for carcinogenicity in at least two adequate animal tests in different species or in both adequate epidemiologic and animal studies. The designation of an agent as being in Group E is based on the available evidence and should not be interpreted as a definitive conclusion that the agent will not be a carcinogen under any circumstances."

3.6 Classification According to the American Conference of Governmental Industrial Hygienists, Inc. (ACGIH 2006)

A1: Confirmed human carcinogen
A2: Suspected human carcinogen
A3: Confirmed animal carcinogen with unknown relevance to humans
A4: Not classifiable as human carcinogen. Agents cause concern that they could be carcinogenic for humans, but there is not enough data to classify the agent into any of the other categories.
A5: Not suspected as a human carcinogen.

A number of *in vitro* systems have been suggested as alternatives to animal testing, and extensive research and validation activities are ongoing (see *http://ecvam.jrc.it*). A limited number of tests regarding irritation effects have been found to be useful, and it is hoped that further research and validation will identify useful tests that can be applied in risk assessment for other adverse effects.

Based on so-called structure activity relationships (SAR), rough estimates of the toxicological properties of chemical compounds with similar chemical structure as compounds with known toxicity can sometimes be made. When quantitative estimates are made based on structure similarities, the term "Quantitative Structure-activity relationships"— QSAR has been used. These estimates usually are less precise than estimated based on even limited animal experiments.

At present, epidemiological studies, when available, and animal tests in combination with human data and complemented by information about toxicokinetics and mechanism of action in animals and humans, are the dominant information sources for risk assessment of metal compounds.

4 DOSE-EFFECT AND DOSE-RESPONSE ASSESSMENT

4.1 Concepts in Quantitative Toxicological Analysis

4.1.1 Dose Effect and Dose Response

Terms used when establishing dose-response and dose-effect relationships must be defined and understood. The terms "dose" and "exposure" have been described in Section 1 of this chapter, and their similar meaning has been pointed out. In quantitative toxicological analysis, it is useful to make a distinction between "dose-response" and "dose-effect" relationships (see also Chapter 6). IUPAC (Nordberg *et al.*, 2004) has recommended the following:

Dose-effect relationship: Association between dose and the resulting magnitude of a continuously graded change, either in an individual or in a population.

Dose-response relationship: Association between dose and the incidence of a defined biological effect in an exposed population, usually expressed as percentage.

This terminology has been used for a long time in metal toxicology (see Task Group on Metal Interactions, 1978; Task Group on Metal Toxicity, 1976;) and in previous editions of this handbook. It will be used in this chapter and in other chapters of this edition. There is, however, no general agreement among toxicologists on this usage, because the words "effect" and "response" are synonyms in common language. There may be advantages in replacing the term "dose-response" by more precise terms like "dose-prevalence" and "dose-incidence" (see Chapter 8 for explanation of terms), but up to the present, such terms have not been much used outside of the field of epidemiology; they are seldom used in toxicology. Therefore, the definitions of "dose-effect" and "dose-response," as traditionally used in metal toxicology and listed above according to IUPAC, will be used in this handbook, because they facilitate distinction between these two dimensions of relationships. The dose-effect concept describes the relationship with the severity of effect, and the dose-response concept describes the distribution of a specific effect in the population. Further considerations concerning the relationship between dose-effect, dose-response and variation in sensitivity among individuals in a population has been given in Chapter 6. The use of these concepts supports clear descriptions and discussions on these relationships that are of great importance in risk assessment.

4.1.2 Critical Concentration, Critical Organ, Critical Effect, and No-Observed-Effect Level

In health effects evaluations, one major issue is to identify adverse effects and to establish exposures or doses at which these adverse effects occur. Severe effects, even death of the exposed individual, occur at high doses, and low doses give rise to less severe effects. Sometimes effects can be detected by laboratory methods at exposure levels without evident symptoms or signs of disease. Such subclinical effects, nevertheless, can be adverse effects if they impair the function of organs or other physiological systems. This type of effect is of great importance in occupational and environmental health, because these professions deal with the prevention of adverse effects and promotion of health for the population. If early adverse effects can be prevented, more severe effects, which are pathophysiological consequences of the early effect, will automatically be prevented. A focus on early adverse effects is thus fundamental in the mentioned professions and guides the related risk assessments.

The effect that is used as a basis for setting exposure recommendations and for risk management is named the *critical effect*. This effect is often a subclinical effect.

The related organ, suffering functional impairment, is named the *critical organ*. In some instances, the tissue concentration of a toxicant in an organ can be related to the appearance of effects. The lowest tissue concentration giving rise to an effect in an organ of a particular

individual is the *critical concentration* in that individual. The concept of a critical concentration is only applicable to effects that occur as a result of a specific threshold concentration being reached, so-called deterministic effects or threshold effects.

When a group or population is exposed to a toxic agent and several effects are studied, a set of dose-response curves are achieved as displayed in Figure 2. At high doses, severe and lethal effects occur (curve D in Figure 2). A well-known concept in this context is the median lethal dose LD-50. Experiments in animals determining the dose-response curve for lethality are of very limited value in environmental and occupational health. Such experiments should also be limited based on ethical considerations. At doses lower than those causing lethality, there will be toxic effects leading to disease (curve C, Figure 2). The median toxic dose (TD-50) for a specific toxic effect that may represent a clinical disease not leading to death is shown in Figure 2 (curve C). At slightly lower doses *TD-05, the toxic dose for 5% of the exposed group* may be identified. At even lower doses, adverse functional changes without clinical effects may be identified by biomonitoring (curve B, Figure 2). Such subclinical changes, which can be shown to have a relationship as a precursor to clinical disease, are often taken as critical effect. The part of this curve representing relatively low doses and low response levels (e.g., the level when 5% of the exposed group displays this effect, i.e., the effect dose for 5% (ED-05), is of interest in occupational and environmental health. When a practical threshold is reached, when no adverse (critical) effect can be observed, the *"No-observed-adverse-effect-level" (NOAEL)* is reached (Figure 2). Because

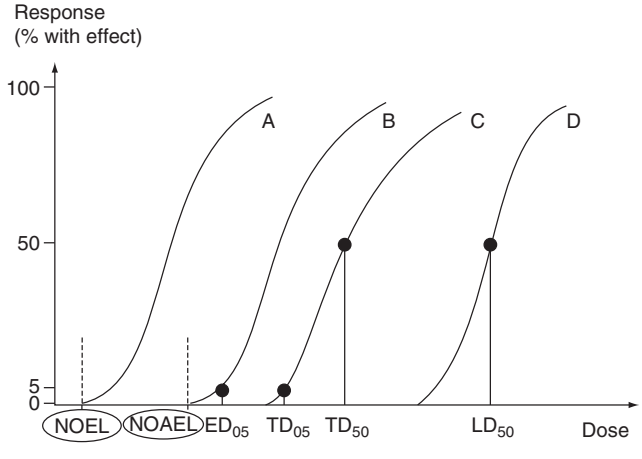

FIGURE 2 Spectrum of dose-response curves for deterministic (threshold-type) effects of a metal compound. No-observed-effect level (NOEL), no-observed-adverse-effect level (NOAEL), effect dose 5% (ED-05), toxic dose 5% (TD-05), toxic dose 50% (TD-50), and lethal dose 50% (LD-50) are indicated.

observations are made in specific dose groups, NOAEL is the highest dose level at which a group of individuals have been exposed without any statistically significant adverse effects being demonstrated. At even lower doses, there may be a dose-response curve describing (curve A in Figure 2) slight, but statistically significant, changes in enzymes for which there is a high reserve capacity, and a small decrease does not imply any decrease in organ function. Such effects are named subcritical effects. Doses below this curve, at which no effects whatsoever occur, represent the *no-observed-effect-level (NOEL)*, which is the highest dose at which no effects at all can be observed (Figure 2).

The S-shaped dose-response curves for threshold-type effects are explained by a Gaussian distribution in sensitivity among persons making up the group studied. This has been further discussed and explained in Chapter 6).

As pointed out in the foregoing text, in preventive medicine, it is of particular importance to identify early effects. If these can be prevented, then later, perhaps more severe, effects can be avoided. Against this background, the terms critical concentration, critical organ, and critical effect have been established for some metals (Nordberg, 1992; Task Group on Metal Accumulation, 1973; Task Group on Metal Interaction, 1978; Task Group on Metal Toxicity, 1976). These concepts are mainly applicable to deterministic/threshold-type effect, but the critical effect concept may also be used when stochastic/nonthreshold effects are considered. Further description and discussion of these concepts and examples of their use can be found in Friberg and Kjellstrom (1981), Kjellstrom *et al*. (1984), Nordberg (1992), WHO/IPCS (1992), and Jarup *et al*. (1998).

Effects that occur as a result of a random interaction of the metal compound with DNA or when they depend on a series of events such as in multistage carcinogenesis are named *stochastic effects or nonthreshold effects*. They are often assumed to display a linear component of the dose-response curve at low doses (curve C in Figure 3). Cancer and some reproductive effects are considered to belong to this category of effects (see also Section 4.2.2).

4.1.3 Benchmark Dose

The concept of benchmark dose (BMD_{Cr}) was introduced into toxicology and risk assessment by Crump (1984) as the statistical lower confidence limit on the dose producing a predetermined level of change in adverse response (the benchmark response, or BMR) compared with the response in untreated animals. There are several advantages in using this concept instead of NOAEL in risk assessment, as discussed in Chapter 6, because the variance in the observation

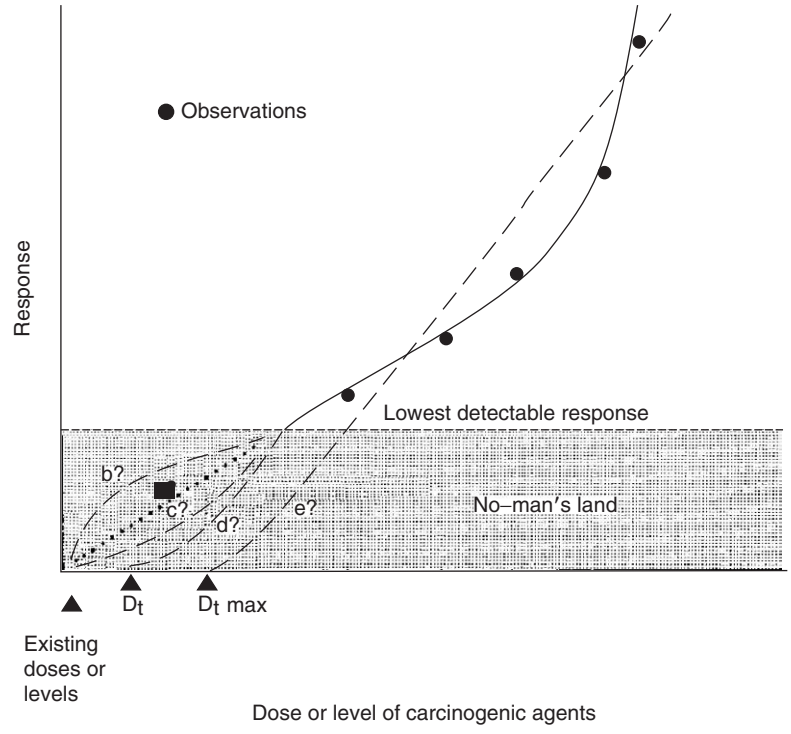

FIGURE 3 Illustration of detection limit for epidemiological and experimental observations of cancer incidence (mortality) as a function of dose (exposure level) of a carcinogenic agent. By response is meant the response caused by the agent, (i.e., the difference between incidence in exposed groups and incidence in the control group). D_t is the threshold dose (Modified from the Swedish Governmental Cancer Committee, 1984).

is considered in a more systematic way. Data with more observations and less variance generate BMD_{Cr} values that are higher, and data with more variance generate lower BMD_{Cr} values for the same BMR. The BMD approach thus uses more of the data and provides more safety. Gaylor *et al*. (1998) redefined the Benchmark dose (BMD_{Ga}) as the point estimate of the dose corresponding to a specified low level or risk, for example, the dose giving rise to a 10% increase in adverse response BMD-10 is the same point in the dose-response curve as ED-10 or TD-10 (see Section 4.1.2) or PCC-10 (Section 4.1.4). Gaylor *et al*. suggested that the lower confidence limit (LBMD) of the BMD_{Ga} be used instead of NOAEL in risk assessments. LBMD as defined by Gaylor is the same concept as BMDL used by USEPA (2006b) and is the same dose as the original BMD_{Cr}. Methods and software for calculating BMD and BMDL are available from USEPA (2006b), and these methods are often used in publications calculating BMD and BMDL; this terminology will be used in this chapter. It is also used in Chapter 8 and in some of the chapters on specific metals, for example, Chapter 23, in this handbook. The software available from USEPA provides various mathematical models, some of which are similar to those used by Crump (1984). These models are in some details different from those used by Gaylor *et al*. (1998). When reporting BMD and BMDL values, the mathematical models and methods used should always be specified, because they do influence the values obtained. The BMD concept was initially developed for use with dose-response data in experimental animals. One important consideration when calculating BMD/BMDL is the background response and how well it is studied. A small "control" group with a large variance will cause difficulties in calculating LBMD regardless of whether experimental data in animals or epidemiological data are considered. In animal experiments, the issue of causality and confounding (see Section 3 and Chapter 8) are not as important considerations as in epidemiology. When calculating BMD and BMDL based on epidemiological data, these considerations must be addressed. Confounding and effect-modifying factors can have considerable influence on BMD values. It was shown, for example, for renal dysfunction in population groups exposed to cadmium in China, that BMDL for Cd was considerably lower in the presence of concomitant exposure to arsenic than in the absence of such exposure (Jin *et al*. [2004]; Hong *et al*. [2004]; Nordberg *et al*. [2005]).

The use of probabilistic risk assessment approaches to take into account variability within exposed populations is a further refinement to these statistical methods.

The BMD, as discussed in the foregoing text, is a point on the dose scale in a dose-response relationship (using so-called quantal data), and the concept has been most frequently used for such data. The advantages of converting dose-effect data (continuous data) to dose-response data have been discussed in Chapter 6. The advantage can be that the occurrence of values considered "abnormal" from a clinical chemistry point of view can be used—usually deviating by more than two times the standard deviation from the mean among healthy persons. When such specific values are known to be related to adverse long-term health outcomes, as for low mol proteinuria in population groups exposed to cadmium (see Chapter 23), the BMD and BMDL are meaningful points in the dose scale that can be used for risk assessment.

The benchmark approach has been extended so that it can be used with dose-effect data (continuous data), also those obtained in epidemiological studies, where confounder adjustment is needed (Budtz-Jorgensen *et al*., 2001; Crump, 1995; Kalliomaa *et al*., 1998). Such benchmark dose calculations may be useful in risk assessments, but their implications in terms of health risk usually have not been defined or discussed. The influence of statistical variance in such calculations has been considered by Sand *et al*. (2003).

When using BMD values in risk assessments, it is important to specify whether dose-response (quantal or dichotomous) data or dose-effect data (continuous data) are used. The BMR should always be specified for dose-response data. It is also important to describe what methods are used to define the magnitude of effect that is under consideration when using dose-effect data. In line with the terminology of this book, the term BME (benchmark effect) is recommended for dose-effect data but has not yet been used as far as known by us.

Measurement imprecision in the dose scale (e.g., in epidemiological studies) will also affect the BMD and should be dealt with as discussed in Chapter 8.

If the considerations discussed in this section are taken into account, BMD/LBMD calculations provide very useful numbers for risk assessment and are recommended when adequate data allowing such calculations are available.

4.1.4 The Critical Concentration on a Population Basis

The critical concentration is established on an individual basis and varies among individuals (Task Group on Metal Toxicity, 1976). The dose-response relationship expressing the response of a particular effect as a function of metal concentration in the critical organ thus displays the frequency distribution of individual critical concentrations.

It is not possible to speak of one single critical concentration even for a specified effect. Because this had sometimes been done, Friberg and Kjellstrom (1981) suggested the introduction of a "population critical concentration" (PCC) instead of a "critical concentration" when assessing data based on a population. To the PCC should be appended a number indicating the response rate expected at this PCC. PCC-50 would be the concentration in the critical organ at which 50% of a population has the critical effect, PCC-20 the concentration at which 20% has the critical effect, and thus has exceeded their individual critical concentrations. The PCC would bring together a number of previously used terms, such as "earliest effect level," "no-observed-adverse effect level" (NOAEL), "critical concentration," without further specification. The use of the PCC concept would be similar to the use of LD50 and ED50 in toxicology and is similar to the benchmark dose concept (see Section 4.1.3).

In risk assessment and risk control of chemicals with specific responses, it is a separate decision to decide on a maximum acceptable response rate for a given effect based on a risk estimation. If 5% is the maximum, then the PCC-5 has to be established. If 0.1% is the maximum, then a PCC-0.l must be established. Standards refer to exposure, however, and it is, therefore, necessary to define the exposure that gives rise to 5% response. To arrive at this exposure, interindividual parameters have to be taken into consideration. Variations in metabolism and sensitivity to a toxic substance will be parameters of importance for the individual. For example, it should be established whether high absorption, unfavorable interactions with other substances, high retention, and low excretion together with high tissue sensitivity within the critical organ may favor an effect even at low exposure levels.

To predict the proportion of a population that has concentrations in the critical organ above the individuals' critical concentrations, knowledge of the distribution of the concentrations of the substance in the critical organ on a population basis is needed together with data on the PCCs. This will give, on the one hand, data on the combined effect of the exposure and metabolism on tissue concentrations, and, on the other hand, information on PCCs. The proportion of a population with concentrations above the critical concentration is then equal to the probability of any individual in the population having an actual concentration in the critical organ above his or her critical con-

centration and the response rate. This proportion has been estimated for cadmium by Kjellstrom (1985) and Jarup (1998); see also Chapter 23. The PCC in terms of urinary cadmium—UCd (biomarker of Cd level in kidney cortex) related to adverse kidney effects has been reported as, for example, PCCUCd-05 (Population critical concentration of urinary cadmium for 5% of the studied group—Jin *et al*. [2004]). These authors also reported the lower confidence limit of this value (i.e., LPCCUCd-05), which is identical to LBMD-05 in the "benchmark dose" terminology (see Section 4.1.3).

Different aspects of the combined effect of distributions of exposure, toxicokinetics, metabolism, and effects have been discussed in Chapter 3 and in papers by Nordberg and Strangert (1978, 1985), Kjellstrom (1977, 1985), Sakurai *et al*. (1982), Jarup *et al*. (1998), Diamond *et al*. (2003), and Chapters 23 and 33.

4.2 Based on Short-Term and Long-Term Studies in Animals

There are relatively good data from epidemiological studies in humans for a limited number of metals and their compounds (see Section 4.3). For other metals and their compounds, mainly data from studies in animals and *in vitro* systems are available. This section will describe how data from animal experiments and combined studies in humans and animals can be used in quantitative risk analysis to arrive at recommended safe exposures for humans.

4.2.1. Threshold-Type Critical Effects

The following considerations are applicable to chemical substances giving rise to threshold-type (deterministic) critical effects but not to genotoxic-type carcinogenic or other nonthreshold effects.

In food toxicology, animal data have been used for a long time as a basis for recommended acceptable daily human intakes (ADIs) of food additives. Traditionally, these recommendations by FAO/WHO have been based on long-term and chronic toxicity studies in animals from which NOAEL has been estimated. To arrive at an ADI, the NOAEL in animals was divided by a safety factor of 100. This method has been widely used in the past but has recently been developed to take into account additional data, when available (see later). Although the method should have been applicable also for metals and their compounds, there are only few metals that have been used as food additives, the most common one is iron. Because human data have been available for iron, an ADI or RDA (recommended daily allowance) entirely based on animal data has not been recommended. Instead, human data have been an important basis for such recommendations (see Chapter 9). For metals and metal compounds occurring as natural components of the environment or as contaminants in food, WHO/FAO sets a provisional tolerable weekly intake (PTWI); this can also be per day PTDI. The same evaluation principles are used as for ADI. The method, dividing NOAEL in animals with 100, to arrive at an ADI (PTWI) is only applicable for substances that are not carcinogenic. For carcinogenesis and other nonthreshold (or stochastic) effects, linear extrapolation is recommended (see later).

The safety factor (or uncertainty factor) of 100 has been explained by a factor of 10 to account for extrapolation from animals to man and another factor of 10 for variability in the human population. The disadvantage with these safety factors is that they do not allow the incorporation of actual data on toxicokinetics and toxicodynamics even if such data are available in animals and in humans. Based on suggestions by Renwick (1993), WHO/IPCS (1999) suggested that (in the absence of actual data) the safety factor (uncertainty factor) of 10 for interspecies differences can be subdivided into two factors: one for interspecies differences in toxicokinetics of 4 and another factor of 2.5 representing interspecies differences in toxicodynamics. The factor of 10 for variability in the human population can be subdivided in a factor of 3.2 for variability in toxicokinetics and another factor of 3.2 for variability in toxicokinetics (Table 1).

The default factors recommended are based on the experience that animals may react quite differently to toxic metals than humans, partly because of a different sensitivity to a particular substance (i.e., a toxicodynamic difference) and partly because of differences in toxicokinetics and metabolism. It may also be difficult to detect certain types of toxic effects in animals, such as neuropsychiatric effects, unless specific tests are performed. It is, therefore, important to look for adequate animal models. The models may differ for different substances and exposure routes. A few examples may illustrate this.

Methylmercury is a very toxic form of mercury to humans. It is highly stable and is accumulated in the body as methylmercury. In rats, this compound is much more readily transformed into inorganic mercury, and the effects are less severe (Chapter 33). In man, as well as in most animal species, arsenic is methylated to a considerable degree as a possible detoxification mechanism, but recent studies have implicated the trivalent monomethyl arsenic acid (MMA-III) as a highly toxic metabolite species that may play a role in arsenic-induced carcinogenesis. In the marmoset monkey, however, there seems to be no methylation at all (Chapter 19). Another monkey, the rhesus monkey, seems to be more resistant to cadmium-induced kidney dysfunc-

TABLE 1 Default Factors in Risk Assessment

Interspecies = 10	Interindividual = 10
TK = 4 TD = 2.5	TK = 3.2 TD = 3.2

TK, Toxicokinetics; TD, toxicodynamics.

tion than man and several other animal species (Tertiary Monkey Experiment Team, 1981; Friberg *et al*., 1985).

When differences between humans and animals have been identified and an adequate animal model for extrapolation to humans can be used, it is, of course, important to use such a model to obtain data relevant for humans. When extrapolating data from animals to humans, the specific observed differences in toxicokinetics could be used in the extrapolation. However, in most instances, such differences are not known, and extrapolation has to be done relying on the default factors listed in Table 1.

It can be seen from the various chapters on individual metals in this handbook that the method that uses the factors in Table 1 in deriving recommended safe levels of exposure for humans has not yet been much used for metals. It may, however, be a feasible option for the future. On the basis of metals on which human epidemiological data are available, the adequacy of the method can be evaluated against actual human dose-response data.

An alternative to the estimation of safety factors (uncertainty factors) are actual estimates of variability in toxicokinetics and toxicodynamics in humans. Probabilistic approaches, calculating risks for adverse effects based on such actual data and estimates of variability in toxicokinetic and toxicodynamic parameters are described in Section 4.3.

4.2.2 Carcinogenesis and Other Nonthreshold Effects

In the evaluations by IARC (Section 3, IARC [2006a,b]), only the weight of evidence for carcinogenicity is considered, and no quantitative estimation of risk is presented. However, quantitative estimations of cancer risks to humans based on combined animal and (limited) human data have been presented by other scientific bodies. The longest history of successful estimations of importance for health protection are those in the field of radiation protection.

In developing rules for protection against carcinogenic effects of ionizing radiation, the assumption has been made that thresholds do not exist and that any exposure involves an increased risk. This philosophy has also been adopted in many quarters for carcinogenic effects of chemical substances, including metals. The form of the dose-response curve for carcinogenic substances has been discussed at an international symposium (Task Group on Air Pollution and Cancer, 1978), and it has been concluded that "in considering protection of human populations, and in the absence of firm evidence to the contrary, it is not justified to assume a 'threshold', i.e. that there is a dose below which no response is obtained." It has also been concluded that "in the absence of relevant dose-response data the most appropriate way to estimate the risk of lung cancer is to assume that it will be directly proportional to the increase in dose.

For small added doses, a simple linear dose-response curve, as used in radiation carcinogenesis, is appropriate." These conclusions imply that basically the same philosophy was adopted as that long used in radiation protection, namely that thresholds do not exist and that any exposure may involve some risk. Similarities with ionizing radiation were considered as best founded in regard to (ultimate) carcinogens (not requiring metabolic activation) acting on directly exposed tissues.

A number of mathematical models have been proposed for extrapolation from high to low doses. They are commented on in Chapter 6 and have also been discussed by an Interdisciplinary Panel on Carcinogenicity (1984) and by WHO/IPCS (1999). It has been pointed out that the dose-response models are quantitatively similar to one another in statistically detectable response rates of 10–100%, but yield substantially different estimates at lower response rates, where estimates may differ by orders of magnitude.

Evidence from epidemiological and experimental data, including certain genetic data, leads to the conclusion that—although knowledge is still incomplete—a linear extrapolation to the low doses in "no-man's land" will be closest to the knowledge concerning mechanistic dose-response relationships for initiators.

Experimental observations, as well as epidemiological data, are almost always based on doses that are considerably higher than those that can be considered as an acceptable exposure. A rationale for extrapolation to low-dose areas and low-exposure levels is, therefore, needed. The levels are within an area where statistically valid conclusions concerning dose-response relationships cannot be drawn from epidemiological or experimental studies. The situation is illustrated in Figure 3. A linear extrapolation according to curve "a" would lead to an underestimation or overestimation of the risk if curve "b" or "c," respectively, was true. Exposures within the dose interval that occur in practice would wrongly be considered "dangerous" if the true relationship showed a threshold dose (D_t) according to curve "d" or "e."

Promoters act basically through nongenetic (nonstochastic) mechanisms, and there is reason to believe that usually a certain exposure (dose in target organs) is necessary for an effect to develop at all. Under such

circumstances, a linear extrapolation from high to low doses may lead to overestimation of risks. Various models have been developed on the basis of initiation, promotion, and, when available, also toxicokinetics (WHO/IPCS, 1999). Such models are used to a variable extent by regulatory agencies in countries around the world for extrapolation of observed relationships to specific response levels like the 10% or 1% level. For further extrapolation into the low-dose range to establish regulations or recommendations of acceptable exposures, linear dose-response relationship is often used.

The linear dose-response relationship means that one may apply the concept of collective dose in risk assessment relative to carcinogenic substances that are persistent in the environment and that—as may be the case for metals—are ultimate carcinogens. The collective dose can be defined as the product of the average dose given to an individual in a defined exposed group and the number of individuals in that group (ICRP, 1977).

The unit risk concept developed by the USEPA (USEPA/IRIS, 2006b,c) is defined as the upper bound lifetime cancer risk estimated to result from an agent at a concentration of 1 μg/L in water or 1 μg/m^3 in air. It is based on a linear extrapolation of observational data (see also Section 6).

4.3 Probabilistic Estimation of Dose-Response Relationships by Toxicokinetic (TK) and Toxicodynamic (TD) Modeling

Probabilistic approaches for characterization of uncertainty have been advanced as an alternative to the "safety factor" approach (Price *et al*., 1997; WHO/IPCS, 1999). Methods using estimated variability in toxicokinetics and toxicodynamics as a basis for risk estimation and estimation of uncertainty in the risk estimates was advanced by Nordberg and Strangert (1978) and has later been developed by inclusion of Monte Carlo simulation as will be described in the following text.

Toxicokinetics (metabolism) describes the movements of a toxicant through the body. It describes to what extent the toxicant is taken up from air, skin, diet, and drinking water. It describes transformation at the site of uptake; transport by blood to various organs; transformation, accumulation, and biological half-life in organs and its excretion in urine, feces, and through skin, hair, and sweat. A detailed description of general principles and mechanism of these events is given in Chapter 3, and this description will not be repeated here except for a few salient points. On the basis of knowledge about particle size and solubility of metals or metal compounds that occur as air pollutants in particle form, their deposition in the lungs can be predicted and their uptake modeled. For metals taken up through the gastrointestinal tract general knowledge about mechanisms are not as much developed, and observational data from animals or humans are required to estimate uptake into blood. The same is true for transport and distribution to various organs in the body and for excretion. Specific transporters and binding proteins are often of great importance for uptake and distribution. Good examples are the DMT1 (divalent metal transporter) carrying Cd and other metals through the gastrointestinal mucosa and metallothionein (MT), a small protein binding Cd in the gastrointestinal mucosa, blood plasma, and in tissues like liver and kidney (cf Chapters 3 and 23). Transformation of metal ions into a bound state (e.g., DMT 1, MT for Cd) or oxidation/reduction reactions (e.g., Cr, Hg) influences distribution and toxicity and needs to be considered in toxicokinetics.

On the basis of an understanding of the toxicokinetics of a metal compound (species) and quantitative measurements in humans and animals, a quantitative toxicokinetic model can be established. Concentrations in critical organs and tissues can be calculated for various exposure scenarios as described in Chapter 3. For example, the accumulation of Cd in the kidney cortex can be calculated as an input into a TK/TD model.

Toxicodynamics (TD) describe mechanisms of action of toxicants, how they can cause tissue damage, and under what conditions in terms of tissue concentrations and time of tissue exposure/dose that adverse effects on tissue structure and function will occur.

4.3.1 Deterministic or Threshold-Type Effects

Some metal compounds (species) elicit tissue damage/adverse changes in organ function when a sufficiently high concentration is reached in the cells of the critical organ. Such effects occurring at a critical concentration that is determined by the sensitivity of the target molecules and the status of defense systems in a particular individual are *deterministic effects* also named *threshold effects*. Organ sensitivity varies among individuals in a population. For example, adverse effects caused by cadmium in the kidney cortex is determined by sensitivity and binding of Cd to target molecules in the renal cell membranes on one hand and the binding of Cd to intracellular MT and other antioxidant defense systems (cf Chapter 23). A relationship can be established by simultaneous measurements of metal concentration in a critical organ directly or indirectly through biomarkers (cf Chapter 4) on one hand and on the other hand measurements of the occurrence of the adverse effects in a large number of individuals in a population.

When there are limited data on humans, but an understanding of threshold-type relationships from animal data, rough estimates of relationships between tissue concentrations and occurrence of effects can be estimated. In such situations, data are often limited to a small number of persons (e.g., workers), who have suffered the adverse effect as a result of high exposure. Experience from such calculations, performed more than 10 years ago for cadmium (cf Chapters 3 and 23) can now be evaluated in relation to epidemiological data from exposed groups in the general population (Chapter 23). Variance of critical concentration in the kidney cortex, estimated from studies carried out in the 1970s and 1980s based on data from small groups of exposed workers has turned out to be somewhat smaller than the variation observed later in epidemiological studies of the general population, because workers are a more homogenous group than the general population. Estimated population critical concentration (PCC) (e.g., PCC_{10}) was adequate for workers but somewhat higher than the actual value for the general population.

In recent calculations using the same basic model, but complemented by Monte-Carlo simulated variation (Diamond *et al*., 2003) adequate estimations of dose response for renal dysfunction in human general populations exposed to background levels of cadmium have been presented.

4.3.2 Stochastic or Nonthreshold Effects

TK/TD modeling is also useful for modeling of dose-response relationships in carcinogenesis. Such models should be combined with an understanding of the mechanisms and quantitative relationships between tissue levels of carcinogenic metals and appearance of tumors. For metals there are not good examples where such calculations have been used in risk assessment, but they have been used for other classes of chemical compounds (WHO/IPCS, 1999).

4.4 Based on Epidemiological Studies

As a result of long-term interest and gradually increased recognition of adverse effects of lead, cadmium, mercury, and their compounds, a considerable amount of epidemiological data are available on human health effects. The considerations of causality in epidemiological studies have been discussed in Section 3.2. It is always important to be reasonably sure that the relationships are causal before a quantitative assessment is made. Often epidemiological data are based on a limited number of exposed persons, for example, in some occupational studies, and it may not be possible to make a calculation of benchmark dose. In such cases, NOAEL can be estimated and used in risk assessment. Sometimes limited data in humans can be combined with TK/TD modeling in calculating risk levels, and NOAEL can be estimated. Such calculations were done in the past to estimate acceptable exposures before epidemiological data were available (Berglund *et al.,* 1971; see also Chapter 33). In recent epidemiological studies, several authors have presented benchmark dose calculations for exposures to cadmium and methylmercury (see Chapters 23 and 33). When using the reported numbers, consideration of possible confounding is important. When confounding and effect modification by other factors can be ruled out, such benchmark doses and their lower confidence levels are of great value in risk assessment and risk management.

4.4.1 Sensitive Groups

The dose-response analysis should focus on the critical effect in the most sensitive subsection of the population (e.g., the fetus of the pregnant mother when adverse effects on the fetus are the critical effect).

4.4.2 Carcinogenic Effects

It was pointed out in Section 4.2.2, that estimation of a safe level of exposure to a carcinogenic metal compound usually involves extrapolation to lower exposures because it is not possible in epidemiological studies to demonstrate low response levels considered acceptable in general environmental exposures (i.e., 1:100 000 in a lifetime). Epidemiological observations on cancer outcome in exposed groups still are of great value in quantitative risk assessment as a starting point for such extrapolations. The best example of a metal/metalloid is inorganic arsenic occurring in drinking water (see Chapter 19; IARC [2004]). There is an increasing body of epidemiological observations on skin, lung, bladder, kidney, and other cancers among population groups in a number of countries exposed to elevated levels of arsenic in drinking water, and dose-response relationships for these effects are also available. Because of the widespread arsenic exposures in drinking water in some parts of the world and number of people exposed, it may be difficult on a practical basis to achieve full population protection at the levels considered desirable for carcinogens in general (see earlier), but this is an ongoing and important discussion.

4.5 Simplified Approach as an Alternative to Risk Assessment

It has recently been suggested that a simplified approach may be used, the so-called threshold of

toxicological concern (TTC) assessment (Kroes *et al.*, 2005). This method is based on classifying chemicals according to their chemical structure and estimating their toxicity, when unknown, from the toxicity of other chemicals with similar chemical structure. Obviously, such a procedure is very uncertain and cannot be used as a replacement for actual risk assessments. It will not be considered further in this text.

5 RISK CHARACTERIZATION

There are both qualitative and quantitative dimensions of risks, and the risk characterization should address both aspects. Some critical effects are severe, like cancer, and a defined numerical risk of such an effect needs to be considered in a different way than the same risk for a critical effect implying an increased urinary excretion of an enzyme or protein without immediate consequences for development of disease. As mentioned in Section 4.1.2, it is desirable to focus dose-response analysis on the critical effect (i.e., the earliest, often subclinical, adverse effect). However, in some cases, particularly when carcinogenesis is involved, such early biomarkers may not be available, and the cancer risks in themselves are the basis for the risk characterization.

The combination of exposure assessment and dose-response analysis generates quantitative estimates of how many persons exceed safe levels derived from NOAEL or benchmark doses and thus are at risk of displaying the critical effect. A concept frequently used in evaluations by expert committees convened by EU is the "margin of safety" (MOS). This is the ratio between NOAEL in animals and the human exposure levels in exposure assessment. Usually MOS of 100 is considered acceptable for noncarcinogenic effects. The considerations as to whether safe levels are exceeded should focus on defined sections of the population (i.e., those most exposed and those most sensitive). Particular attention should be given to estimates of exposure in sensitive groups like pregnant women and children. These sections of the population are particularly sensitive to neurotoxic metals like methylmercury and lead. Risks calculated for such groups often are of crucial importance for risk management.

The risk characterization should also provide information to the risk manager about the quality of the data that has been used in the risk assessment for the manager to be able to judge whether it is reasonably good for decisions about regulatory action. If there are obvious data gaps in the scientific evidence, these problems must be pointed out for the risk managers to be prepared to answer possible questions relating to the risk assessment and risk control measures (for further discussion see WHO/IPCS, 1999).

6 RISK MANAGEMENT AND RISK COMMUNICATION

The scientific database available for the various steps in risk assessment varies considerably among metals as is evident when reading the 31 chapters on individual metals in this handbook. For many of the metals, WHO/IPCS Environmental health criteria documents are available, as well as national documents from various countries (e.g., ATSDR Tox profiles) cited it the various chapters of this book. However, there are a substantial number of metals for which documentation is limited, making it difficult to make a reliable hazard identification and quantitative dose-response analysis. This consideration that is included in the risk characterization is important in risk management.

6.1 Managing Human Exposures by Emission Control, Substitution, Labeling, or Restrictions in Use

Regardless of the risk assessment, exposures are sometimes limited by control of emissions. These considerations are often taken when constructing new manufacturing plants with new production technology and increased automation and encapsulation of productions processes, resulting in considerable reduction in exposure to workers (see also Chapter 16). The "best-practical-means" or "best-available-technology" approach is also used for emissions to the general environment and implies that an industry must use the best available technology to reduce emission of hazardous substances, taking into consideration, however, economic aspects.

The best-practical-means approach, if properly used, carries with it the advantage that resulting concentrations in different media (e.g., in ambient air and soil) around a point source will be as low as possible using the available cleaning techniques. Under certain circumstances, this might mean that, for example, concentrations in the environment may be lower than the levels permissible from the toxicological point of view. Large safety margins may thus sometimes be achieved. The resulting ambient concentrations should, however, be checked against health criteria and other general considerations relating to ecology. The "best-available-technology approach" has been implemented in Swedish environmental legislation since the 1970s and has been effective in limiting exposures of population groups living in the vicinity of point sources of

industrial metal emissions. However, this is not always sufficient to control human exposures that are related to global or regional spread of metals. Despite the successful control of point sources of mercury, high levels still occur in Swedish lake fish as a result of global mercury cycling and the acidification and sulfur pollution of Swedish lakes from European regional sources. Restrictions in intake of certain species of Swedish lake fish, particularly in pregnant women, thus still are recommended by the Swedish Food Agency.

One way to limit human exposures to chemical substances is by labeling. In the EU, as a result of systematic hazard identification efforts, a large number of substances have been classified for hazardous properties. Classification includes acute toxicity (aquatic and terrestrial), irritation, sensitization, carcinogenicity, and reproductive toxicity, and related labeling is implemented (cf European Chemicals Bureau: www.ecb.jrc.it).

If the risk characterization indicates that there are substantial problems with certain uses of a metal, authorities may suggest voluntary substitution of such uses for less toxic substances. If control cannot be achieved by voluntary measures, a ban of such uses of highly toxic metal compounds may be considered. For most metals and uses, this is not possible, but it may be feasible to restrict the use of the most toxic metals. Metals are indispensable in a technological society. All metals are elements that, owing to geological conditions, will be present in varying concentrations in the earth's crust even in nonindustrialized environments. Highly toxic metals often occur in nature together with less toxic metals. Cadmium, for example, is found in ores together with zinc.

In certain instances, the use of some metal compounds has been successfully prohibited. Several governments have, for example, forbidden the use of methylmercury as a fungicide and the use of tetraethyllead as an additive to motor gasoline. Cadmium and lead compounds have been widely used as color pigments. Severe restrictions on this practice are now being enforced in the EU, where many uses of mercury are also being discouraged. For example, many chlor alkali plants are being converted to nonmercury-based technology.

6.2 Controlling Human Exposures by Guidelines and Legislated Permissible Exposure Levels

Exposure guidelines and standards are necessary for toxic metals, often more so than for other substances. Metals are not disintegrated in nature and may be transported over great distances. Furthermore, they have a tendency toward accumulation in nature, as well as in the human organism. We have to live with a certain amount of exposure to metals both in industry and in the general environment. A major problem is to determine what levels of exposure can be accepted with only a minor, defined risk. This discussion focuses on the effects on humans only. Basically, the same principles apply when evaluating effects on the environment. For certain metals, such risks may be the dominating ones, implying that they should receive priority from the standpoint of prevention and control.

Standards for chemicals may be formulated either in terms of concentrations in environmental media (examples are air quality standards, threshold limit values, and drinking water standards) or in terms of amounts of substances that may be ingested into the body within a given unit of time (e.g., acceptable daily intake). These concentrations and amounts should be sufficiently low so that the threshold dose (if one exists and can be determined) will not be reached, or so that the population of concern will not be subjected to "unacceptable risk" even after a lifetime or a working lifetime of exposure.

If a significant exposure takes place via different media, which is often the case for metals, a standard for one media cannot be considered separately (i.e., without looking into possible exposure by means of other media). Take lead as an example. In some places, even the exposure by ambient air, food, and/or water may give rise to concentrations in blood close to or above effect levels. In such situations, occupational exposures must be limited to a very minimum, whereas at other places where ambient levels are low, a higher occupational exposure can be permitted without causing adverse health effects.

Depending on whether the risk characterization indicates that some persons in the population or the work force (for occupational exposures) are at risk of adverse health effects, risk reduction measures are recommended or legislated. Recommendations are issued by international organizations, and various countries or unions may issue legislation about permissible exposure values.

Health-based exposure guidelines are issued by international organizations such as World Health Organization (WHO) in collaboration with Food and Agricultural Organization (FAO). These organizations issue acceptable daily intakes (ADIs) for food additives, tolerable daily intakes (TDIs) or provisional tolerable weekly intakes (PTWIs) for food contaminants. Air pollution guidelines and drinking water guidelines are also recommended by WHO. Recommendations for essential metals have been discussed in Chapter 9.

These recommendations and guidelines are set based on risk assessments using predetermined safety (or uncertainty) factors, and they are thus considered health based, not considering other factors that may be of importance when setting standards in national legislation (see later).

Sometimes the precautionary principle is used when issuing exposure recommendations (cf Hayes, 2005). This principle may imply recommendation of voluntary limiting action against exposures to levels lower that those otherwise recommended based on generally agreed principles of risk assessment. Such lowering of exposures is performed when it can be achieved without any major costs. The precautionary principle is a part of environmental legislation in Sweden and several other European countries. This principle is related to ethical considerations like the paragon of virtue practiced by the ancient authorities in medicine like Hippocrates.

Nongovernmental organizations like the International Commission on Occupational Health (ICOH) sometimes issue policy documents of a precautionary nature. For example, the ICOH Scientific Committee on the Toxicology of Metals in collaboration with the Scientific Committee on Neurotoxicology recently launched the "Declaration of Brescia," recommending lowering of limit values of lead manganese and mercury and other preventive measures (Landrigan *et al*., 2006 and Chapter 16).

Standards (national legislation) for occupational exposures in the United States of America are set by the Occupational Safety and Health Administration (OSHA) and the National Institute for Occupational Safety and Health (NIOSH). There are also drinking water standards and air pollution standards in the United States, which are set by the U.S. Environmental Protection Agency (USEPA) for a number of metals such as lead and metalloids such as arsenic.

For cancer, the USEPA (USEPA/IRIS, 2006a,b) has developed the unit risk concept, which is defined as the upper bound lifetime cancer risk estimated to result from an agent at a concentration of 1 μg/L in water or 1 μg/m^3 in air. The upper bound confidence limit (UCL) is defined as the upper bound of a confidence interval around any calculated statistic that is most commonly an average. The interpretation of unit risk would be as follows: unit risk $= 2 \times 10^{-6}$/μg/L would indicate two excess cancer cases (upper bound estimate) to be expected per 1 million persons if exposed daily over a lifetime to 1 μg of the agent per liter of water or cubic meter of air. For cadmium, which the USEPA considers to be a class B1 carcinogen, the inhalation unit risk estimate is $1.8 \times 10^{-3}(\mu g/m^3)^{-1}$. The USEPA uses mathematical models, based on animal studies, to estimate the probability of a person developing cancer from such exposures.

The EU has issued a limited number of permissible occupational limits (PEL) that are either indicative or binding for member states. They are based on criteria documents issued by the Scientific Committee on Occupational Exposure Limits (SCOEL). Member states like Germany and Sweden have issued a larger number of such limits that are implemented on a national level in harmony with the PELs issued by EU. Other countries like Japan, Russia, and China have their own lists of permissible occupational exposure limits. In the past, there were considerable differences in such limits among countries like the Soviet Union and China on the one hand and the United States and western European countries on the other hand. As a result of the International Program on Chemical Safety, and its predecessors, starting in the 1970s, these differences have gradually decreased (see also discussion by L Friberg in Chapter 1 of this handbook).

The European Union has regulated maximum levels of contaminants, including lead, cadmium, and mercury in foodstuffs (EG466/2001). The EU (1998) has (98/83/EC) adopted drinking water standards for a number of substances including some metals, and these standards are implemented in member states that report values to the European commission.

Biological exposure levels for metals/metalloids in media such as blood for metals such as lead and urine for arsenic are used in both occupational and environmental exposure situations to assess the degree of actual uptake into exposed individuals or populations. These data have been used as screening levels for determining the need for medical interventions or clean up activities. Blood lead measures for occupational and childhood exposures would be the most well-documented and extensively used example. In the United States, the CDC has recommended a blood lead level of concern for lead of 100 μg/L; this level should be used for screening of children.

In Sweden, biological exposure levels in terms of blood concentrations allowed among industrial workers are issued by the Swedish Work Environment Authority (concerning levels for lead see Chapter 31 and for cadmium see Chapter 23).

When national or union authorities issue recommendations and limits, a number of factors are taken into consideration in addition to the risk characterization such as socioeconomic, political, risk/benefit, and cost/benefit factors (cf IPCS/WHO, 1999).

Readers of this handbook will see that the information available on the toxicology of metals and their compounds vary widely among metals. Only for a limited number of the metals is there adequate information to

make a reliable dose-response assessment for humans. An ongoing discussion in Europe is about the new component of chemicals control REACH (Registration and Evaluation of Chemicals; see *http://ecb.jrc.*it), requiring manufacturers and importers of chemical substances, including metals, to furnish a specified amount of toxicological data for the substance to be used in the EU. Some consider that the requirements for toxicological information are too limited (Rudén and Hansson, 2006). It is hoped that this new legislation will provide additional data relevant for risk assessment for some of the metals and metal compounds that are presently incompletely investigated.

6.3 Risk Communication

Risk communication is the process of communication between professionals performing risk assessments and risk managing on the one hand and on the other hand workers in the case of occupational exposures or the general public for general environmental exposures.

In a democratic society, risk communication is of great importance. In the case of occupational risks communication, it may be direct between risk assessors/risk managers in central agencies and workers by means of workers' unions and safety organizations in companies. Such risk communication may sometimes involve considerations like those used when communication involves factors of outrage (see later), but in other cases, such communications are characterized by mutual understanding and acceptance of risks and risk reduction measures suggested by risk assessors.

When communicating with the public, the behavior of the risk assessor is crucial for acceptance.

The following principles are listed by US ATSDR concerning risk communication:

1. Accept and involve the public as a partner. Your goal is to produce an informed public, not to defuse public concerns or replace actions.
2. Plan carefully and evaluate your efforts. Different goals, audiences, and media require different actions.
3. Listen to the public's specific concerns. People often care more about trust, credibility, competence, fairness, and empathy than about statistics and details.
4. Be honest, frank, and open. Trust and credibility are difficult to obtain; once lost, they are almost impossible to regain.
5. Work with other credible sources. Conflicts and disagreements among organizations make communication with the public much more difficult.
6. Meet the needs of the media. The media are usually more interested in politics than risk, simplicity than complexity, danger than safety.
7. Speak clearly and with compassion. Never let your efforts prevent your acknowledging the tragedy of an illness, injury, or death. People can understand risk information, but they may still not agree with you; some people will not be satisfied.
8. Communicate important information to societal decision makers such as legislators, who must frequently make decisions about the need to use public resources for regulatory actions or clean up activities. One mechanism is to use the World Wide Web or Internet for this purpose, because this is a common source of information *(*cf *www.atsdr.cdc.gov/cabs).*

Depending on the situation and the risk under consideration, risk perception by the public may be different than that perceived by the risk assessor. A number of factors, for example, how familiar, widespread, and dreaded the risks are and whether risks are controllable or not determine the degree of outrage by the public (WHO/IPCS, 1999). Increased interaction with the public may be useful in such situations. Communication with the public sometimes is described as a converging process. On the basis of the risk characterization, information about the risk in question is communicated to the group of the public with the highest exposure at a public meeting, and feedback is requested. It is important for the risk assessor, presenting risks to the public, to find out about reasons for possible discrepancies in risk perception. Depending on comments received, answers to questions and new information adapted to the needs of the public are prepared and presented at a new meeting, for example, comparisons between the risks from exposures to the agent under consideration and other risks in society. This procedure may be repeated. Often, a convergence of opinions will occur between risk assessors and the public.

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CHAPTER

15

Diagnosis and Treatment of Metal Poisoning—General Aspects

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ABSTRACT

Metal poisoning may or may not be apparent from the clinical features induced. The exposure pattern in terms of time, concentration, and route of exposure is a determinant of clinical effect. Short-term high- and long-term low-level exposure by ingestion is seen more often in the domestic environment and by inhalation more often occupational in origin. Acute and chronic clinical effects of metal toxicity may involve the gastrointestinal, respiratory, cardiovascular, renal, hemopoietic, and central nervous systems.

In the diagnosis of metal poisoning, the presenting features may be nonspecific, the clinical examination giving no lead on the cause of illness. A full occupational history is required, with a knowledge of the work processes involved and a detailed history where poisoning may occur in the domestic environment, in particular where poisoning results from the long-term absorption of cumulative metals. However, metal accumulation and poisoning may occur in the absence of environmental exposure, as, for example, transfusional siderosis in the thalassanemias, iron overload in idiopathic hemochromatosis, and copper accumulation in Wilson's disease. In addition to history and clinical examination, trace metal analysis in body fluids, tissues, and environmental samples is required, together with biochemical and physiological investigation.

In the treatment of metal poisoning, prevention of further absorption by removal from exposure; minimizing absorption from the gastrointestinal tract; and general supportive therapy that requires maintenance of respiration and circulation, water, and electrolyte balance; and control of nervous system effects is a priority. Elimination of the absorbed poison may be promoted by diuresis, biliary excretion, or by hemodialysis or exchange transfusion. A number of therapeutic agents counteract the effects of an absorbed toxic metal by combining to form a less toxic compound that may be excreted more effectively by displacing it from its receptor site or into a tissue where it cannot exert its toxic effects. Toxic metal antagonists, chelating agents, compete for toxic metals with ligands essential for physiological function by forming a stable complex with the metal in the form of a heterocyclic ring. Chelating agents possess electron donor groups showing a high affinity for the metal to be removed, releasing it from complexes with proteins or other endogenous ligands in a form in which it can be readily excreted. Chelation is indicated in the treatment of metal poisoning, metal storage diseases, blood transfusion in iron overload, and to aid the elimination of metallic nuclides. However, chelation may result in depletion of essential metals or the distribution of toxic metals to other tissues such as the brain. Chelators may also have toxic effects.

Dimercaprol, now a classic chelator with high toxicity, competes with protein sulfhydryl groups for arsenic, forming a stable chelate, and is effective for inorganic mercury, antimony, bismuth, and gold. Unithiol, DMPS, an analog of dimercaprol, is effective in accelerating metal excretion without severe adverse effects in acute and chronic intoxication by inorganic and organic mercury, bismuth, arsenic, and chronic lead poisoning.

Succimer, DMSA, less toxic than DMPS, has been the preferred antidote for lead poisoning, particularly in children, although adverse effects have also been reported. Calcium EDTA, given intravenously, will bind lead with the displacement of calcium. However, it has marked toxic effects and may redistribute lead to the brain after acute or chronic lead poisoning. Penicillamine, an effective chelating agent for copper, iron, lead, mercury, and zinc, is, in particular, effective in the management of Wilson's disease and in which it may be given indefinitely to maintain a negative copper balance. Trien, TETA, is effective in the treatment of Wilson's disease, where intolerance to penicillamine has developed. Desferrioxamine, DFOA, given parenterally, has an affinity for iron and is the treatment of choice in acute iron poisoning, in particular in children. It is indicated in the treatment of transfusional siderosis as in thalassemias and has been effective for the removal of aluminium in dialysis encephalopathy. Deferiprone, L1, is effective orally for the long-term chelation therapy of transfusional siderosis. Lipophilic derivatives of DTPA have been administered as an aerosol to diminish the lung concentration of inhaled insoluble plutonium oxide particles. DEDTC has been effective in the treatment of acute nickel carbonyl poisoning.

A combination of the two chelating agents has been shown to be effective in some cases of poisoning. Combined treatment with EDTA and dimercaprol is effective in acute lead poisoning in children with encephalopathy or blood lead levels greater than 100 μg/L.

Other therapeutic measures in metal poisoning are a modification of tissue response to the poison or an alteration of biochemical or metabolic state of the subject. Thus, the inflammatory process in chronic beryllium disease may be arrested by corticosteroid therapy. In chronic manganese poisoning that closely resembles Parkinson's disease, L-dopa has been effective in reducing symptoms and neurological signs without evidence of a reduced body burden of manganese.

1 CLINICAL EFFECTS

1.1 General Considerations

Metal poisoning may be acute, resulting in death or followed by partial or complete recovery. It may be subacute in onset, or there may be a latent interval between absorption and its development, which may then follow the same course as previously mentioned or give rise to chronic poisoning. However, toxic metals produce other important clinical effects not usually included under this term. These effects are (1) hypersensitivity reactions that may involve the skin, kidney, lung, hemopoietic system, and possibly the nervous system; (2) the induction of cancer after an appropriate latent interval; and (3) teratogenic malformations. Furthermore, an increasing number of metals are being shown to give rise to genetic damage in test systems, and the question arises as to whether mutations may also occur in man, with a consequent increase in abnormal individuals in later generations. These other effects, because of their importance and magnitude, are considered elsewhere.

Adverse effects, which may become manifest by biochemical or physiological tests or by abnormal clinical signs, may occur in the absence of symptoms or in the presence of vague, ill-defined complaints of ill health that do not make up a recognizable syndrome. Common examples are anemia after exposure to lead compounds and renal tubular dysfunction after long-term exposure to cadmium.

The clinical effects described previously imply that an adverse biological change has been produced in the organism with some impairment of cellular function resulting from exposure. The model on which this adverse effect is based assumes that a critical concentration of the toxic metal has been attained in the critical organ to give rise to a critical effect. This critical effect may or may not be of immediate importance to the health of the organism as a whole (Nordberg, 1976). It may be argued that such a critical effect cannot be considered to be synonymous with a clinical effect. Here it is useful to distinguish between critical and subcritical effects. Effects may also occur below the critical concentration in the critical organ that do not impair or seem to impair cellular function (Nordberg, 1976; see also Chapter 12). Although the biological significance of such a subcritical effect may be unknown, it would be reasonable, given the present state of knowledge, to exclude such a subcritical effect from clinical consideration.

1.2 Exposure Pattern and Clinical Effect

Various factors act as determinants of a clinical effect after exposure to a toxic metal. Such factors include dose; route of absorption; the chemical and physical form of the metal concerned; genetic variation manifested through racial, familial, and individual susceptibility; dietary pattern and nutritional status; immunological status; presence of intercurrent disease; and interaction with other chemical compounds in the environment. Some of these determinants are considered in Chapter 7 and in those chapters dealing with the specific metals. The exposure pattern, in terms of concentration, time, and route of exposure, is an important determinant of clinical effects that requires further consideration here.

A short-term exposure may produce a clinical effect very different from that produced by an exposure similar in terms of total dose over a longer period of time. The effects of ingestion of a toxic metal are more often seen in the domestic or general environment than in an industrial setting. Short-term, high-level exposure by ingestion may follow the accidental, suicidal, or homicidal ingestion of a toxic metal compound, giving rise to well-recognized acute syndromes, usually involving the gastrointestinal tract and possibly involving secondarily the renal, cardiovascular, nervous, and hemopoietic systems. Long-term, low-level exposure by ingestion is seen increasingly in the general environment as a result of the contamination of food and drink by metals that have cumulative properties in the organism. Clinical effects may involve any organ system in the body, but the gastrointestinal tract is not primarily involved.

By contrast, short-term, high-level inhalation exposure is most often occupational in origin. It may not only give rise to acute respiratory effects but may also involve the cardiovascular, central nervous, renal, and hemopoietic systems. Again, long-term, low-level inhalation exposure is usually occupational in origin, and its control forms a large part of industrial hygiene practice. However, long-term, low-level inhalation exposure to certain toxic metals may also occur in the general environment and from smoking cigarettes. The effects may involve any organ system in the body and may spare the respiratory system.

Mercury forms a good example of the extreme variation in clinical effect that may be produced, depending on the pattern of exposure and the chemical form of the metal (Kazantzis, 1980). The lung is the critical organ after short-term, high-level inhalation exposure to mercury vapor, resulting in pneumonitis and respiratory failure, whereas the CNS is the critical organ after long-term exposure to mercury vapor. After the ingestion of an inorganic soluble mercuric compound, the kidney becomes the critical organ, manifesting with anuria because of tubular necrosis. As a result of the long-term ingestion of methylmercury as a food contaminant, nervous system effects may develop but with a clinical syndrome different from that seen after the long-term inhalation of inorganic mercury vapor. The variability in clinical effect produced by the toxic metals is further illustrated in the following section. The adverse effects that result after exposure to the individual metals are described in detail in succeeding chapters of this handbook.

1.3 Acute Clinical Effects of Metals

1.3.1 Gastrointestinal Effects

Acute gastroenteritis follows the ingestion of a toxic quantity of most metals in the form of soluble salts. A not uncommon occurrence is the contamination of food or drink, especially if acidic, by dissolution of metal from food containers. Symptoms develop a short time after ingestion, often involve a number of people, and may be labeled as "food poisoning." Vomiting and diarrhea may be followed by circulatory collapse and involvement of other systems, depending on the poison absorbed. Poisoning with soluble compounds of antimony, cadmium, copper, lead, tin, and zinc has occurred in this way. Acute gastroenteritis with collapse may be the predominant feature after the ingestion of rodenticides containing arsenic, barium or thallium, yellow phosphorus, or zinc phosphide. Similar symptoms may follow the ingestion of soluble compounds of bismuth, chromium, iron, silver, and vanadium. The ingestion of a soluble mercuric salt gives rise to gastroenteritis with a bloody diarrhea that may resemble fulminating ulcerative colitis. Lead colic, which may occur as an acute effect after prolonged exposure to lead, has on many occasions simulated an acute surgical emergency.

1.3.2 Respiratory Effects

Acute chemical pneumonitis, which may be accompanied by pulmonary edema, follows the inhalation of a number of freshly formed metal fumes. Particularly toxic in this respect is the inhalation of freshly formed cadmium oxide fume, with acute symptoms developing some hours after an apparently innocuous exposure (Beton *et al*., 1966). The inhalation of antimony pentachloride, arsine, beryllium fume, iron pentacarbonyl, lithium hydride, nickel carbonyl, titanium tetrachloride, selenium dioxide, hydrogen selenide, vanadium pentoxide, or zinc chloride can give rise to a similarly acute picture with pulmonary edema. The inhalation of high concentrations of mercury vapor or dust or inorganic mercury compounds can also give rise to pneumonitis before other symptoms of mercurialism develop. Respiratory symptoms with rigors and fever resembling an acute respiratory infection may follow the inhalation of freshly formed zinc fume, brass fume, or other metallic oxides, giving rise to metal-fume fever (Hunter, 1975). Pneumonic consolidation has followed the inhalation of manganese dust. The inhalation of complex salts of platinum can give rise to acute and severe asthma. Decomposition products or other contaminants of industrial air may be responsible for some of these effects.

1.3.3 Cardiovascular Effects

A number of metallic ions interfere with the normal function of myocardial cells, giving rise to arrhythmias, including ventricular fibrillation. Ventricular

fibrillation may be responsible for a fatal outcome in cases of poisoning by antimony, barium, or lithium salts. Cobalt can give rise to cardiomyopathy (Alexander, 1972). Some metals have been shown to have a hypotensive effect. These include antimony, cadmium, cobalt, copper, iron, and vanadium, a state of shock being a common presenting feature in poisoning with these metals.

1.3.4 Effects on the Central Nervous System

Metal poisoning may present with an acute illness involving the CNS. Most important, because unfortunately still not uncommon in children, are convulsive attacks that may terminate in coma or lead to death as a result of acute lead poisoning. A successful therapeutic outcome in such cases depends on early diagnosis and treatment. Convulsions may also follow the absorption of iron, barium, lithium, thallium, and organic tin compounds. Acute psychosis may also be the presenting feature in metal poisoning. After heavy exposure to tetraethyl lead, a patient may present with delusions, hallucinations, and hyperactivity that may precede coma and death (Beattie *et al*., 1972).

1.3.5 Renal Effects

Renal damage, manifesting as oliguria or anuria caused by acute tubular necrosis, is another way in which metal poisoning may present, although this feature often follows an initial presentation with acute gastrointestinal, circulatory, or respiratory effects. Oliguria and anuria caused by tubular necrosis are common occurrences, especially in children, after the ingestion of soluble mercuric or iron salts. The condition may also follow pneumonitis resulting from cadmium fume inhalation. Renal failure has been a sequel to the absorption of a number of soluble metal compounds, including antimony, arsine, bismuth, copper, uranium, and vanadium salts.

1.3.6 Hemopoietic Effects

Acute hemolytic anemia often accompanied by renal failure may be the presenting feature after the inhalation of arsine or stibine gas (Jenkins *et al*., 1965). Acute hemolysis has also followed the ingestion of large doses of soluble copper salts.

1.4 Chronic Clinical Effects of Metal Toxicity

The term chronic effect is a relative one, signifying that the clinical effect may develop gradually and may persist for a longer interval than an acute effect. Chronic effects are associated with longer-term exposure at lower levels than acute effects. Only examples of chronic effects are given here, the great variety of these being described in the individual metal chapters.

1.4.1 Gastrointestinal Effects

Some chronic gastrointestinal effects may, in fact, be examples of mild acute poisoning after the repeated ingestion of the toxic metal over a period of time. Chronic gastrointestinal symptoms have occurred in people drinking canned juice contaminated with high concentrations of tin or zinc or ingesting drinking water contaminated with copper. However, intestinal colic has been observed in industrial workers and in children with relatively low-level lead exposure, and this can be considered a true example of chronic lead poisoning (Beritic, 1971). Anorexia, nausea, and vomiting in industrial workers may be seen with selenium and tellurium poisoning, when the characteristic garlic odor of the breath may also be elicited. Anorexia, and often digestive disturbances, has also been reported in people living in seleniferous regions and with a high dietary intake of selenium. Anorexia, nausea, vomiting, and diarrhea followed by constipation accompanied by a burning sensation of the tongue and stomatitis have resulted from occupational exposure to thallium compounds.

1.4.2 Hepatic Effects

Several metals are hepatotoxins, giving rise to effects ranging from abnormalities in hepatic enzyme levels to clinical jaundice. Such effects have been reported after exposure to antimony, arsenic, bismuth, copper, chromium, iron, and selenium.

1.4.3 Respiratory Effects

Chronic pulmonary disorders giving rise to dyspnea may result from absorption of metal dust or fume. An inflammatory response consisting of noncaseating granuloma formation followed by pulmonary fibrosis may result from exposure to beryllium after a latent interval of many years (Stoeckle *et al*., 1969). Progressive dyspnea with the clinical, radiological, and functional changes associated with emphysema is seen in workers exposed to cadmium oxide fume or dust. The respiratory abnormality is usually, but not invariably, associated with renal tubular dysfunction. Pulmonary fibrosis has followed occupational exposure to finely powdered aluminium metal dust. Dyspnea, wheeze, and productive cough giving rise to an illness resembling asthmatic bronchitis have followed occupational exposure to vanadium pentoxide dust. Progressive breathlessness associated with pulmonary fibrosis has

occurred in hard-metal workers exposed to the dusts of tungsten and titanium carbides with cobalt used as a binder. Chronic asthma can occur in workers sensitized to chromium after the inhalation of chromate dust or chromic acid fume and also to organic platinum compounds. Once sensitization to platinum has occurred, the reaction can be triggered by exposure to minute quantities of the salts of chloroplatinic acid.

1.4.4 Effects on the Nervous System

Peripheral neuropathy may develop in the recovery stage of acute arsenic intoxication, about 1–3 weeks after exposure. It is a mixed motor and sensory neuropathy, with a "glove and stocking" distribution. Neuropathy develops in those who survive the acute gastrointestinal effects of thallium poisoning, and it may lead to a later fatal termination. With both these metals, skin changes occur at a later stage, with the former metal, arsenical pigmentation results, and with the latter, hair loss. A motor neuropathy involving predominantly the upper limbs with wrist drop and extensor weakness of the fingers is seen in chronic lead poisoning. By contrast, antimony salts of organic acids give rise to a sensory neuropathy that may involve the trigeminal nerve. Bismuth and copper have also given rise to peripheral neuropathy.

Permanent brain damage with cerebral cortical atrophy or hydrocephalus may be the sequel to acute lead encephalopathy. Convulsions may recur over a long period, and idiocy may develop.

The use of lead paints and leaded gasoline has given rise to the widespread occurrence of lead in the environment. Elevated blood lead levels after long-term exposure to house dust with decaying fragments of leaded paint has been shown in epidemiological studies to affect cognitive development in children (Bellinger *et al*., 1992; Needleman *et al*., 1990; Chapter 31).

Degenerative changes in the nerve cells of the basal ganglia giving rise to a parkinsonian syndrome were considered to result from the absorption of manganese after long-term occupational exposure (Mena *et al*., 1970). Parkinson's disease, a not uncommon disorder, is characterized by muscular rigidity, akinesia, tremor, and postural deformities. In a case-control study to determine whether welding-related parkinsonism differs from idiopathic Parkinson's disease in welders exposed to manganese welders had a significantly younger age of onset of parkinsonism, suggesting welding as a risk factor for this condition (Racette *et al*., 2001).

Degenerative changes affecting in particular the granular cells in the cerebellum and neurons in the calcarine, precentral, and postcentral cortex, follow the absorption of alkylmercury compounds and present with a characteristic neurological syndrome whose principal features are paresthesia of extremities and face, ataxia, dysarthria, and concentric constriction of the fields of vision. Pyramidal signs may also occur. Another characteristic neurological disorder consisting principally of intention tremor of the hands, tremor of the eyelids and tongue, and a combination of behavioral and personality changes known as erethism develops after chronic exposure to mercury vapor.

1.4.5 Renal Effects

Certain chronic renal disorders may also follow exposure to toxic metals. Proximal renal tubular dysfunction consisting mainly of tubular proteinuria, glucosuria, aminoaciduria, and phosphaturia may develop after cumulative exposure to cadmium, lead, and uranium compounds. Hypercalciuria has also occurred in chronic cadmium poisoning as a further manifestation of renal tubular dysfunction, and this has led to renal stone formation and to osteomalacia in a few cases after industrial exposure (Kazantzis, 1979).

Osteomalacia has been observed in a population environmentally exposed to cadmium in Japan. Although the skeletal disorder (Itai-Itai disease) only occurred in postmenopausal women, evidence of renal tubular dysfunction was found in the population as a whole (Friberg *et al*., 1974; 1985).

Progressive impairment in renal function terminating in uremia and indistinguishable clinically from chronic nephritis has been observed after childhood lead poisoning. The wide range of abnormal responses of the kidney to absorbed metals can be further illustrated by reference to the nephrotic syndrome. Heavy proteinuria, with hypoproteinemia and edema, has followed exposure to inorganic mercury, organic gold, and bismuth preparations.

1.4.6 Hemopoietic Effects

Chronic arsenic poisoning is associated with an anemia caused by decreased red blood cell formation with increased destruction. The anemia of chronic lead poisoning also results from decreased hemopoiesis combined with increased red cell destruction (Goyer and Rhyne, 1973). By contrast, cobalt has an increased hemopoietic effect and has given rise to polycythemia, but not to increased production of other cellular elements in the blood.

2 DIAGNOSIS OF METAL POISONING

As can be seen from the preceding, metal poisoning may involve any of the organ systems of the body and

give rise to a variety of effects. The presenting features may be entirely nonspecific, the clinical examination giving no lead on the cause of the illness. The principal features that should be considered in arriving at a correct diagnosis are elaborated in the following.

2.1 History of Exposure

In most cases, a history of exposure to a toxic metal will give the necessary clue. In an industrial situation, there may be a clear history of exposure that may be obtained from the patient, a relative, or a coworker. The clinician should not fail to take a full and accurate occupational history when a poisoning of occupational origin is suspected. It may be necessary to seek additional information from the employer or his agent, from an occupational hygienist, or from a health and safety representative. Exposure to a toxic metal may, however, occur without this being suspected by any of the persons questioned, and this can only be inferred by an adequate knowledge of the work processes involved. A good example may be given with exposure to arsine gas, which usually presents as an acute medical emergency. Arsenic may be a contaminant in scrap metals, flue dusts, etc., and arsine may be formed in any reducing situation, or when a metallic dross containing arsenic comes into contact with water. The condition should, therefore, be suspected in a scrap-metal worker presenting with the appropriate clinical features.

In the domestic environment, an appropriate history may give an exposure inferred from medicaments such as iron pills or household chemicals. Unlabeled pills or chemicals should, of course, be kept for analysis. In a homicidal instance, a psychotic factory worker succeeded in poisoning three fellow workers in succession by adding a thallium salt to tea, causing the deaths of two men before the poisoning was suspected (Cavanagh *et al.*, 1974). In the general environment, diagnosis of metal poisoning, especially if caused by long-term absorption of cumulative metals in the body, can be very difficult. Methylmercury poisoning after the eating of contaminated bread in Iraq was diagnosed at the beginning of the outbreak, because the medical officers had previous experience of the condition.

Metal accumulation may also occur in the absence of environmental exposure. The thalassemias are a heterogenous group of genetic disorders of hemoglobin synthesis. Symptomatic treatment of anemia requires regular blood transfusion from childhood. For the prevention and treatment of resulting iron overload, transfusional siderosis, a chelating agent is required, desferrioxamine being the best choice. However, repeated intravenous or subcutaneous injection with possible side effects is stressful to patients, and an orally effective iron chelator, deferiprone, is suited for long-term in-home treatment. This can be combined with desferrioxamine and be effective at lower doses (Kontogiorghes *et al.*, 2000).

Primary or idiopathic hemochromatosis is an autosomal-recessive disorder caused by excessive absorption and storage of iron. Deposition of iron in organs and tissues causes fibrotic changes and functional impairment, in particular arthropathy, cirrhosis with hepatomegaly, diabetic mellitus, hypogonadism, cardiac arrhythmias, heart failure, and skin pigmentation caused by melanin and hemosiderin deposition (Bacon, 2001; Niederau *et al.*, 1994). Repeated phlebotomus has been effective in reducing serum ferritin, and in cases with excessive iron deposits, this has been combined with an iron-chelating agent, in particular desferrioxamine. Because early diagnosis is essential for the successful treatment of this condition, relatives of patients with primary hemochromatosis are candidates for genetic screening (Tavill, 1999).

In Wilson's disease, hepatolenticular degeneration, a recessively inherited metabolic disorder, there is a failure to excrete copper into bile, resulting in copper accumulation first in the liver, then in the brain, cornea, and kidneys (Walshe, 1984). The age of onset may be as early as 5 years up to the fifth decade. Hepatic necrosis releases copper into the circulation, causing damage to erythrocyte membranes leading to hemolytic anaemia. Copper accumulation results in widespread neurological disturbances with tremor, slurred speech, muscular rigidity, mental deterioration, and pigmented rings at the corneal rims of the eye, this being a diagnostic marker. Diagnosis depends on an accurate estimation of copper in an uncontaminated 24-hour urine sample, in Wilson's disease usually more than 100 μg/24 hours. Treatment to produce a negative copper balance is effective with the chelating agents penicillamine, triethylene tetramine, and with zinc, which inhibits copper absorption from food and increases fecal copper excretion. In particular, treatment is more effective in those diagnosed in the presymptomatic phases of the disease (Sarkar, 1999).

2.2 Clinical Features

The presenting clinical features, especially in acute poisoning, may be entirely nonspecific. Lead colic has been mistaken for the acute surgical abdomen, and craniotomy has been performed after encephalopathy caused by lead. Such mistakes would not have been made if a careful history had been taken. Certain clinical sequences should alert the physician to the possibility of metal poisoning. Gastroenteritis followed by

peripheral neuropathy that is, in turn, followed by hair loss is a classical presentation for thallium poisoning. Gastroenteritis followed by oliguria or anuria follows the ingestion of inorganic mercuric and other soluble metal compounds as mentioned in the previous section. Hemorrhagic gastroenteritis, especially if accompanied by collapse and hypotension, should alert the clinician to an acute metal poisoning by ingestion. Similarly, acute pulmonary edema in a subject free of heart disease should raise the suspicion of a toxic inhalation exposure. Acute cadmium fume poisoning should be suspected, especially if soldering or welding had been performed, for iron and steel may be plated with cadmium. Metal poisoning should also be considered in the differential diagnosis of oliguria or anuria of unknown etiology. Several metals give rise to renal tubular dysfunction, in particular cadmium, uranium, and lead. With lead, this condition is seen more often in children, so that lead poisoning should be considered in every case of glycosuria presenting in childhood. The causes of peripheral neuropathy are often obscure, and many cases remain undiagnosed. Metal poisoning should be considered in the differential diagnosis of peripheral neuropathy.

Few of the signs described in this chapter are specific to metal poisoning. There are, however, certain specific signs indicative of metal absorption, although not necessarily of poisoning. Among these, the following should be mentioned: the characteristic garlic smell in the breath of selenium and tellurium workers, the green tongue of the vanadium worker, and the pigmented alveolar margin (the lead line) caused by the deposition of insoluble sulfide in the gums of lead workers. However, the lead line is not seen in edentulous workers, and other metals will deposit sulfide in the same site. A finely mottled brown pigmentation of the skin, with leukodermia, a "rain drop pigmentation" affecting, in particular, the temples, eyelids, and neck is seen in workers in contact with arsenical dusts. Ulceration and perforation of the nasal septum should also alert the clinician to previous long-term exposure to arsenical dusts or to chromates. Chronic ulceration on the fingers is seen after exposure to washing soda or to lime. White striae on the fingernails lines (Mees lines) are indicative of previous exposure to arsenic.

2.3 Toxicological Analysis

A diagnosis of metal poisoning can be confirmed in the acute stage and often in the chronic stage by finding an increased concentration of the suspected metal in the appropriate medium. However, the techniques involved in trace metal analysis in body fluids and tissues present certain difficulties, and such analysis should only be performed in laboratories that are equipped for this purpose and that participate in external quality control. Environmental sampling may need to be performed on air, water, or food. Pills, residues in bottles, may require analysis after an acute poisoning incident, as may blood, urine, feces, vomit, and tissue samples. The examination of blood and urine samples for metal concentration can be considered a routine procedure. However, it should be borne in mind that some metals disappear rapidly from the blood, and others are sparingly excreted in the urine. In thallium poisoning, for example, the diagnosis can be confirmed by taking hair and nail clippings for analysis at a stage when blood and urine concentrations are not helpful. Evidence of methylmercury absorption can be obtained from analysis of hair samples after blood levels have fallen to normal. In the diagnosis of lead poisoning, difficulty may be caused when exposure had ceased some time previously. In such cases, blood lead levels may return to normal by the time diagnosis is attempted. A calcium EDTA provocation test will increase the urinary excretion of stored lead in such a situation.

2.4 Biochemical Investigation

For most metal poisonings, biochemical investigation is required in addition to estimation of metal concentrations in body fluids. The profile of biochemical abnormality is indicated in the chapters on the different metals considered. In general, biochemical tests of renal and hepatic function are always required. Biochemical data are essential for diagnosis with certain toxic metals but are not helpful with others. For example, in the diagnosis of lead poisoning, it is useful to estimate δ-aminolevulinic acid and coproporphyrin in the urine, as well as zinc erythrocyte protoporphyrin and lead concentration in the blood. In contrast, the diagnosis of poisoning by metallic mercury vapor or by alkylmercury compounds is essentially clinical, together with estimation of metal concentrations in blood and for inorganic mercury in urine.

2.5 Physiological Investigation

Electrocardiography, respiratory function tests, electroencephalography, and nerve conduction studies provide examples of possible ancillary investigations for the diagnosis and for the determination of the extent of poisoning by certain metals. The principal disturbances of physiological function in such poisoning are indicated in the chapters on individual metals.

3 TREATMENT

The treatment of metal poisoning includes treatment of the acute and chronic effects and suppression of tissue reaction when sensitization has occurred.

The management of acute metal poisoning requires emergency resuscitative procedures that may need to be initiated in the work or home environment and continued in an acute treatment unit. Those principles on which the management of the acute case of poisoning is based, which are aspects of emergency medicine (see Dreisbach, 1983; Vale and Meredith, 1981), will only be briefly referred to here, whereas therapy related to metal poisoning will be discussed in more detail.

3.1 Prevention of Further Absorption

3.1.1 Removal from Exposure

Absorption of a toxic metal may follow inhalation, ingestion, or skin or mucosal contamination. Occupational exposure most frequently gives rise to absorption after inhalation. After high-level inhalation exposure, the victim should be removed immediately from the contaminated atmosphere. Such an emergency situation may arise with volatile metal compounds such as stibine, arsine, alkylmercury, alkyltin, and alkyl lead compounds. After contamination of the skin with lipid-soluble metal compounds, contaminated clothing should be removed as soon as possible, and the contaminated area must be irrigated and washed, but not rubbed, with copious amounts of cold water. Decontamination may also be required for hair and fingernails.

In chronic, cumulative metal poisoning, it may be sufficient to remove the subject from further exposure. In certain circumstances, for instance, a worker with mild poisoning after exposure to inorganic mercury vapor or to lead fume or dust may require no therapy other than such removal. The normal excretory mechanisms will ensure a gradual recovery from mild toxic effects.

A review of possible routes of absorption in the individual case should include consideration of the possibility of exposure by contamination of food, drink, cigarettes, or clothing. When this possibility exists, food, drink, and cigarettes at the work site should be prohibited, and facilities should be provided for showering and for a complete change of clothing after each shift.

3.1.2 Minimizing Absorption from the Gastrointestinal Tract

3.1.2.1 Removal from the Gastrointestinal Tract

Within about 4 hours after ingestion, some of the poison may be recovered by emptying the stomach. In a fully conscious patient, this may be effected by vomiting. As a first-aid measure in a child immediately after ingestion, this may be induced by pharyngeal stimulation. Ipecacuanha Emetic Mixture 10–15 ml for a child, 30 ml for an adult, may be effective in inducing vomiting.

The stomach may be washed out after the passage of a gastric tube, but this should not be attempted after the ingestion of corrosives or petroleum products or in drowsy or comatose patients because of the danger of inhalation of stomach contents. The procedure of gastric lavage is a skilled one that should only be performed in a properly equipped emergency department, again within about 4 hours of ingestion. If the cough reflex is absent or if consciousness is impaired, a cuffed endotracheal tube should first be passed to protect the lungs from inhalation of stomach contents.

3.1.2.2 Inactivation in the Gastrointestinal Tract

Although gastric lavage can be effectively performed with warm water, some poisons may be inactivated in the stomach by a specific antidote, and after lavage, this may be left in the stomach in a volume not greater than 200–300 ml. However, lavage should not be delayed if the antidote is not immediately available. In an emergency situation, egg white or milk will help to partly inactivate mercury and other heavy metals by precipitation in the stomach. Activated charcoal will effectively absorb many poisons still present in the stomach and will also interrupt the enterohepatic circulation. However, it is ineffective in iron poisoning. Five to ten grams of a thick suspension in water should be given, and this may be repeated at intervals of 20 minutes to a maximum of 50 g. Higher doses may be given, but these may induce vomiting of the suspension. As a first-aid measure, sodium bicarbonate will partly precipitate soluble iron salts in the stomach. Gastric lavage should be performed as soon as possible with desferrioxamine mesylate, 2 g in 1 liter of water. A solution of desferrioxamine, 10 g in 50 mL water, should then be left in the stomach. Effective precipitation of the toxic barium ion can be produced by oral administration or gastric lavage with a 10% solution of sodium or magnesium sulfate. After the ingestion of chromic acid, lavage should be performed with magnesium carbonate. Prussian blue will chelate thallium, and after its ingestion, a colloidal solution should be given by duodenal tube at a rate of 250 mg/kg/day in divided doses.

3.2 General Supportive Therapy

In acute poisoning, resuscitative measures have to take precedence over all others. If the patient can be

kept alive, excretory and detoxicating mechanisms together with the administration of specific antidotes, when these exist, will be able to ensure eventual elimination of the poison. Careful symptomatic medical care is always necessary, with an awareness of delayed effects that may occur.

3.2.1 Maintenance of Respiration and Circulation

Maintenance of respiration and circulation should receive precedence over all other procedures. The patency of the airway must be ensured, especially at the site of the accident and the journey to the treatment center, and artificial respiration may be necessary. At the treatment center, tracheobronchial toilet may be required. The presence of hypoxia should be assessed by determining minute ventilation or preferably arterial oxygen and carbon dioxide tensions. Oxygen may be needed and also mechanical ventilation. Treatment for acute pulmonary edema may be necessary after exposure to beryllium or cadmium fumes, to nickel carbonyl, or to hydrogen selenide.

In an emergency situation, a conscious shocked patient with a blood pressure less than 80–90 mmHg should be reassured, covered with blankets, and kept supine with the legs elevated. Once the patient reaches the treatment center, the circulatory blood volume can be restored with suitable intravenous fluids. Blood transfusion may be required for severe hemolytic anemia after exposure to arsine.

3.2.2 Maintenance of Water and Electrolyte Balance

Imbalance may occur from vomiting, diarrhea, tissue damage, or from measures taken to eliminate the poison. It may be sufficient to give fluids by mouth to prevent dehydration, but an intravenous infusion with appropriate biochemical monitoring is often required. An adequate urinary flow should be ensured, and a catheter may be necessary.

Acute tubular necrosis may give rise to anuria, and dialysis may be necessary. Careful water and electrolyte balance has to be maintained until regeneration of the tubular epithelium leads to recovery.

3.2.3 Control of Nervous System Effects

The general supportive measures just outlined are usually adequate for the management of the patient whose level of consciousness is depressed. There is no indication for the use of analeptic drugs in acute metal poisoning. Convulsions, however, require management with diazepam, 10–30 mg, given parenterally. Cerebral edema may occur, as, for example, in acute lead poisoning. This should be treated with dexamethasone given intravenously. Prolonged sedation with diazepam may be necessary to overcome the hyperactivity and other behavioral disorders that may develop in poisoning with alkyl lead compounds.

3.3 Elimination of Absorbed Poison

3.3.1 Diuresis

The promotion of a diuresis will increase the clearance of many poisons by decreasing their passive reabsorption from the proximal renal tubules. Diuresis can be achieved by giving a fluid load together with osmotic agents like mannitol or by giving a diuretic (e.g., furosemide). Furthermore, the excretion of some poisons is influenced by the pH of the urine, because passive tubular reabsorption is less effective with increased ionization of the solute in the tubular fluid. Although of great value in some common forms of acute poisoning, these methods have limitations in poisoning by metals, although in general, excretion of the toxic metal can be accelerated if a high flow of urine is maintained, as, for example, in acute inorganic mercury and lead poisoning. Forced alkaline diuresis is of limited value in aiding the elimination of lithium salts.

3.3.2 Biliary Excretion

The action of certain toxic metals is prolonged as a result of an enterohepatic circulation where excretion in the bile can occur against a high concentration gradient followed by intestinal reabsorption. The use of activated charcoal in absorbing certain toxic agents from the gut is referred to previously. A promising approach for interrupting the enterohepatic circulation has been described by Clarkson *et al*. (1973). This involves a complexing agent given by mouth that will bind with the metal compound excreted in the bile, prevent reabsorption, and enhance fecal excretion of those heavy metal compounds that undergo an extensive enterohepatic circulation. This synthetic polystyrene resin containing fixed sulfhydryl groups, when added to food in a concentration of 1%, doubled the rate of excretion of methylmercury from mice and lowered blood and tissue levels compared with untreated controls. In man, mercury levels in blood were reduced and the fecal excretion of methylmercury enhanced (Bakir *et al*., 1973).

3.3.3 Dialysis

Hemodialysis will achieve higher clearance rates of toxic metals not irreversibly bound to tissues than can be attained by forced diuresis, although, in general, the yield of metal ion in the dialysate is low. Metals

that may be dialyzable are arsenic, copper, iron, lead, lithium, magnesium, mercury, potassium, sodium, strontium, and zinc. The procedure is indicated when a potentially fatal dose has been absorbed, when the clinical condition is deteriorating despite adequate treatment by other means, and when a complication exists, such as aspiration pneumonia or renal insufficiency. Severe poisoning with lithium salts should be treated with prolonged hemodialysis. Peritoneal dialysis may be used where hemodialysis is not available.

In severe acute inorganic mercury poisoning, dialysis may be performed after giving dimercaprol to remove the mercury complex.

3.3.4 Exchange Transfusion

When there are no facilities for hemodialysis or when the toxic material is poorly dialyzable, exchange transfusion may be lifesaving in severe poisoning by agents that remain in the blood in appreciable concentrations.

3.4 Inactivation of the Absorbed Poison

A limited number of therapeutic agents may be administered to counteract the effects of an absorbed toxic metal. Such a specific antidote may act in different ways. It may combine with the toxic agent to form a less toxic or a nontoxic compound, which may be excreted more effectively in the urine; it may compete with the toxic agent and displace it from its receptor site; or it may displace the poison into a tissue where it cannot exert its toxic effects. The intravenous administration of calcium gluconate will displace lead from its site of action and will temporarily relieve the intense pain of lead colic. An infusion of potassium has been shown to correct the potassium-displacing capacity of absorbed soluble barium salts (Berning, 1975).

Certain antidotes have been designed specifically to compete, for toxic metals, with ligands essential for normal physiological function. These toxic metal antagonists, or chelating agents, form a stable complex with the metal in the form of a heterocyclic ring (Klaassen, 1980). The antagonists and the chelates produced are not themselves without toxic effects, and they should not, therefore, be administered therapeutically in those mild cases of poisoning where removal from further exposure is sufficient to promote recovery. Chelation therapy, because of its value in metal poisoning, is considered in greater detail below.

3.5 Chelation Therapy

Chelation is indicated in the treatment of metal poisoning, in the treatment of metal-storage diseases, in particular in Wilson's disease, in blood transfusion iron overload as in the thalassemias and in hemochromatosis, and to aid the elimination of metallic radionuclides from the body. The pharmacology and therapeutic applications of chelating agents were reviewed by Catsch and Harmuth-Hoene (1976) and updated by Andersen (1999), Andersen and Aaseth (2002), and Andersen (2004). The basic principle underlying chelation is that the therapeutic agent should possess electron donor groups showing a high affinity for the metal to be removed, thus releasing it from complexes with proteins or other endogenous ligands in a form in which it can be readily excreted. The effectiveness of ligand exchange depends on the relative stability constants for the metal–endogenous ligand and the metal–therapeutic ligand complexes, on the rate of ligand exchange, on the effective concentration of the agent in the region of the metal–endogenous ligand complex, and on the stability of the newly formed chelate. The chelating agent should form complexes with the toxic metal that have much greater stability than those formed with physiologically essential metals such as iron, calcium, zinc, or copper. Treatment is most effective if the chelating agent is given while the metal is still in the circulation or in the extracellular fluid compartment, because once intracellular, the metal is less accessible. Many chelating agents are in an ionized form and, therefore, have limited ability to penetrate cell membranes.

Effective chelation forming a less toxic species that may be effectively excreted depends on physical and chemical characteristics of metals and chelators, such as ionic diameter, ring size and deformability, hardness/softness of electron donors and acceptors, administration route, bioavailability, metabolism, organ and intra/extra cellular compartmentalization, and excretion. Account has to be taken that the metal selectivity of chelators may result in depletion of essential metals or to the redistribution of toxic metals to other tissues (e.g., the brain) (Andersen 2004; Andersen and Aaseth 2002).

3.5.1 Dimercaprol

2,3-Dimercaptopropanol (dimercaprol, British antilewisite, BAL), now a classic chelator, was synthesized as a specific antagonist to the vesicant arsenical war gases. It is a dithiol compound that successfully competes with protein sulfhydryl groups for arsenic compounds and for other heavy metals by forming a stable chelate with them. The other metals for which dimercaprol has been shown to be effective are mercury in inorganic form, antimony, bismuth, and gold. In cases with acute lead encephalopathy and increased

intracranial pressure, it has been advised to administer dimercaprol parenterally before calcium EDTA (Griesemer, 2001). The half-life is short, metabolism and excretion being complete within 4 hours.

Dimercaprol is given by deep intramuscular injection as a 5% solution in arachis oil BP (or a 10% solution with benzyl benzoate in vegetable oil, USP). It is usually given in a dose of 2.5–3 mg/kg, every 4 hours, for the first 2 days, every 6 hours on the third day, and thereafter once or twice daily for up to 10 days. In a severe acute case of poisoning, 4–5 mg/kg may be given, every 4 hours for the first 24 hours, but no single dose should exceed 300 mg. There are several variations to this schedule. The drug should be administered at a lower dosage to patients with impaired renal function. It is wise to render the urine alkaline during therapy, because the dimercaprol–metal complex may dissociate in an acidic medium.

Dimercaprol has unpleasant and sometimes alarming side effects when given in full dosage. One of the most consistent is a rise in blood pressure accompanied by tachycardia. Other untoward effects are nausea and vomiting, headache, burning sensations in the mouth and throat, with paresthesia of the hands, a feeling of constriction or pain in the chest, lacrimation, salivation, rhinorrhea, sweating, and abdominal pain accompanied by a feeling of anxiety.

Dimercaprol has been shown in the experimental animal to inhibit the blood pressure–lowering effect of intravenously injected cadmium (Dalhamn and Friberg, 1954) and also of vanadium and cobalt. Although the urinary excretion of cadmium is enhanced by dimercaprol, Tepperman (1947) showed this to be accompanied by a large increase in cadmium concentration in the kidney, and Gilman *et al*. (1946) observed severe renal damage as a sequel. Dimercaprol is, therefore, contraindicated in cadmium poisoning.

The administration of dimercaprol in mercury poisoning effects a redistribution of the body burden of the metal without increasing its excretion. In poisoning with inorganic mercuric salts, dimercaprol decreases the renal concentration and thus protects the kidney. However, with phenylmercury and with alkylmercury compounds, dimercaprol accelerates the uptake of mercury from blood into the tissues and in particular its uptake into the brain (Berlin and Lewander, 1965; Berlin *et al*., 1965). Dimercaprol is, therefore, contraindicated in the treatment of poisoning with both aryl- and alkylmercury compounds. Dimercaprol enhances the toxicity of selenium and tellurium, producing kidney damage, and is contraindicated in poisoning by these metals (Amdur, 1958; Cerwenka and Cooper, 1961).

Because of its high toxicity, dimercaprol is now suited only for brief treatment of acute intoxications (Andersen and Aaseth, 2002). DMPS and DMSA have now superseded dimercaprol in most cases of poisoning.

3.5.1.1 Sodium 2,3-Dimercaptopropane Sulfonate (DMPS; Unithiol)

This water-soluble chemical analog of dimercaprol, with dimercaptosuccinic acid (considered below), is an effective antidote for certain forms of heavy metal poisoning, less toxic than dimercaprol, which has been used extensively in Russia and more recently in the Western world.

Both agents increase the urinary excretion of copper and zinc, but in therapeutic doses, this is not considered to be of clinical significance. Allergic side effects have been reported. The properties and uses of these two chelating agents have been reviewed by Aposhian (1983). DMPS is effective in accelerating metal excretion without causing severe adverse health effects in acute and chronic intoxication by organic and inorganic mercury, bismuth, arsenic, and chronic lead poisoning (Andersen, 1999; Aposhian *et al*., 1995).

DMPS (unithiol) may be given orally or parenterally. It has been given intramuscularly as a 5% solution at the rate of 5 mg/kg three or four times during the first 24 hours, two or three times on the second day, and once or twice on subsequent days.

DMPS has been given successfully in one case of Wilson's disease where intolerance had developed to penicillamine and to triethylene tetramine, resulting in effective cupruresis (Walshe, 1984, 1985). In two other cases reported by the same author, adverse effects were reported. In experimental animals, DMPS has prevented the lethal effects of a number of arsenic compounds, and it has been used in Eastern Europe in the treatment of arsenic poisoning in man. Together with dimercaprol it is, however, contraindicated in arsine poisoning. DMPS has been used successfully in the Soviet Union in the treatment of chronic lead poisoning. It is in the treatment of alkylmercury poisoning that DMPS may have a major role. In a study of the relative effectiveness of DMPS, a thiolated resin, and the penicillamines, in the Iraqi outbreak of alkylmercury poisoning, DMPS was the most effective agent, reducing the mean T 1/2 value for mercury in blood from about 65–10 days (Clarkson *et al*., 1981). Adverse effects of treatment were not seen, and the authors concluded that a reduction in blood mercury level with accelerated excretion would be clinically useful if given before irreversible damage had occurred. DMPS has also been given in inorganic mercury poisoning after the inhalation of mercury vapor and the ingestion of mercuric oxide. It has been shown to be effective in increasing mercury excretion in urine (Mant, 1985).

DMPS has also been given as a diagnostic test for low-level mercury exposure, urinary mercury after DMPS being a better indicator of exposure than unchallenged urinary mercury excretion (Aposhian *et al*., 1995). DMPS has been considered to be the optimal antidote for inorganic mercury poisoning and DMSA as more effective in organic poisoning, but this requires verification (Andersen, 1999).

3.5.1.2 Meso-2,3-Dimercaptosuccinic Acid (DMSA; Succimer)

There are many reports in the Soviet and Chinese literature and more recently in the Western countries testifying to its effectiveness and low toxicity in the treatment of poisoning by arsenic, lead, and mercury (Andersen, 2004). This analog of dimercaprol, among the least toxic of the chelators, may be given parenterally or orally in doses of 30 mg/kg/day for 5–7 days followed by 20 mg/kg/day for 1–3 weeks. DMSA is rapidly excreted in urine and, in particular, decreases the deposition of lead and also of methylmercury in the brain (Cory-Slechka, 1988).

DMSA, being less toxic than DMPS, is usually the preferred antidote for lead poisoning, particularly in children (Angle, 1993). It was registered in the United States in 1991 for the oral treatment of lead poisoning in children, a significant problem in poor population subgroups. Four lead-intoxicated children were treated with DMSA for five courses each lasting 10 days (Chisholm, 1990). After reduction, the blood lead increased again after each course, indicating the need for repeated chelation. A rebound in blood lead concentration occurs after chelation treatment, considered to be predominantly caused by mobilization of bone lead, necessitating the need for repeated chelation.

Five lead-poisoned smelter workers were given DMSA in increasing dosage from the first to the sixth day. A marked fall in blood lead concentration was accompanied by a significant increase in urinary lead and copper excretion but without effect on urinary calcium, iron, magnesium, or zinc excretion (Friedheim *et al*., 1978). The efficacy of DMSA in binding and mobilizing mercury has been shown in a number of animal studies reviewed by Aposhian (1983). The mobilization and removal of methylmercury by extracorporeal complexing hemodialysis with DMSA has given promising results in the dog (Kostyniak, 1982). Although DMSA is effective for the treatment of lead intoxication, adverse effects have been reported. A strong musculocutaneous reaction in one case of chronic lead poisoning (Grandjean *et al*., 1991) and hemolytic anemia was reported in a worker with occupational lead exposure (Gerr *et al*., 1994).

3.5.2 Calcium Disodium Edetate (Calcium EDTA)

Ethylenediaminetetraacetic acid, another classic chelator, and related compounds are able to chelate many divalent and trivalent metals *in vitro*. Infusion of the sodium salt will chelate calcium from the body and may result in hypocalcemic tetany. However, the calcium disodium salt, calcium EDTA, is a valuable therapeutic agent, because it will bind lead with the displacement of calcium from the chelate. However, it has dangerous toxic effects in the chelation of metals other than lead. Calcium EDTA is poorly absorbed from the gastrointestinal tract and so has to be given by intravenous infusion. It is distributed mainly in the extracellular fluid and is excreted rapidly by glomerular filtration, about 50% appearing in the urine within 1 hour. Castellino and Aloj (1965) showed in rats a biphasic pattern of lead excretion, weakly bound extracellular lead being excreted rapidly, with lead complexed within cells being removed very slowly, probably as a direct result of an increased concentration gradient.

The toxic effects of calcium EDTA make it necessary to monitor its administration with care. The most important of these effects is on the kidney. Friberg (1956) showed that cadmium excretion could be increased considerably by giving calcium EDTA to rabbits previously dosed with cadmium. However, severe degenerative and necrotic changes were seen in the tubular epithelium of rabbits with prolonged exposure to the metal. Reversible hydropic degeneration of the proximal tubules was produced in rats by Foreman *et al*. (1956), and proximal tubular damage was also observed in man. It is not known whether this is due to a toxic effect of the chelating agent itself, to the chelated metal, or to breakdown of the chelate in its transfer through the tubule, or even to the binding of a trace metal in an essential enzyme system. Untoward side effects in the treatment of lead poisoning include a febrile reaction with headache, myalgia, nausea, and vomiting. Furthermore, EDTA may redistribute lead to the brain after acute or chronicle lead exposure (Andersen, 2004). Lacrimation, nasal congestion, mucocutaneous lesions, glycosuria, hypotension, and ECG abnormalities have also been reported. Prolonged courses of calcium EDTA give rise to trace-metal depletion, the most marked being due to the excretion of zinc.

Many of the side effects of calcium EDTA have been ascribed to excessive chelation after the administration of too high a dose over a short period of time. The drug is usually administered in adults by the infusion of 1.0 g in 250–500 mL 5% dextrose over a period of 1–2 hours. Two such infusions may be given daily for 3–5 days, but a daily dose of 70 mg/kg body weight should not be exceeded. Not more than 80 mg/kg should be given

daily to a child. After an interval of a few days—during which time redistribution of lead from the intracellular to the extracellular compartments occurs—the course may be repeated. Although a patient is on calcium EDTA treatment, urinalysis should be performed, and blood urea, electrolytes, calcium, phosphorus, and alkaline phosphatase should be monitored regularly. Because of its adverse effects, calcium EDTA is being progressively replaced by DMSA in the treatment of lead poisoning (Aposhian *et al*., 1995).

The local application of calcium EDTA in an ointment is of value in the treatment of chrome ulceration.

3.5.3 Penicillamine (Cuprimine)

b,b′-Dimethylcysteine, or penicillamine, is derived from the hydrolytic degradation of penicillin. The D-isomer is an effective chelating agent for copper, iron, lead, mercury, and zinc, and it will increase the excretion of these metals in the urine. It is probably the chelating agent of choice in the less severe forms of lead poisoning. *N*-Acetyl-DL-penicillamine may be even more effective than D-penicillamine in protecting against the effects of inorganic mercury (Aposhian and Aposhian, 1959), but there have been few valid comparisons. Penicillamine is well absorbed from the gastrointestinal tract and may thus be given by mouth. It is stable once absorbed and is excreted rapidly in the urine.

The most important side effects of penicillamine are acute sensitivity reactions manifested by fever, skin rashes, blood dyscrasias, and occasionally renal tubular damage, proteinuria, and the nephrotic syndrome. There is cross-sensitivity with penicillin, so that people who are allergic to penicillin should not be given penicillamine. Penicillamine antagonizes pyridoxine, although clinical effects after treatment with D-penicillamine are unusual.

Penicillamine is given orally, before meals, in four divided doses at a rate of 0.5–1.5 g daily, although up to 5 g daily has been given without ill effects. The urine should be tested for protein, and a full blood count, including a platelet count, should be performed at weekly intervals, but when treatment continues for longer than 2–3 months, as in the management of Wilson's disease, these tests may then be performed monthly. In Wilson's disease, 500 mg penicillamine is given before meals three times a day. Once stability is attained, this may be reduced to 750 mg/day and continued indefinitely to maintain a negative copper balance. In children, 25 mg/kg is appropriate.

Penicillamine is also effective in the treatment of rheumatoid arthritis and related conditions. Its mode of action is unknown.

3.5.4 Triethylene Tetramine (Trien, TETA)

A new orally active chelating agent, triethylene tetramine 2 HCL (trientine dihydrochloride or Trien), has been used successfully in the treatment of Wilson's disease and is now indicated when intolerance to penicillamine has developed (Walshe, 1983). Initially 600 mg is given three or four times a day, reduced to a maintenance dose of 600 mg twice daily before meals and continued indefinitely. Zinc sulfate or acetate given orally is also helpful in maintaining copper balance by blocking absorption of the metal.

3.5.5 Desferrioxamine (DFOA)

The hydroxylamine desferrioxamine B is a chelating agent with a remarkable affinity for iron. It is poorly absorbed from the gastrointestinal tract, but given parenterally, it complexes with iron in a diffuse chelatable pool and is rapidly excreted as the red ferrioxamine, about two thirds through the kidneys and one third through the bile into feces. Desferrioxamine may be given by the subcutaneous, intramuscular, or preferably intravenous route. Given by mouth, it chelates iron remaining in the lumen of the gut and renders this nonabsorbable and thus nontoxic. It is the treatment of choice in acute iron poisoning, greatly reducing the mortality in children, provided it is given at an early stage. Its effectiveness also depends on an adequate urine output, so that the ferrioxamine complex may be excreted.

If oliguria develops, peritoneal dialysis or hemodialysis may be necessary to aid excretion. Desferrioxamine is also of great value in the treatment of chronic iron overload after multiple blood transfusions. In thalassemia, it is the standard treatment, also given to young children on regular blood transfusion. In this condition it is now given in high-dose subcutaneous infusions. Iron stores can be assessed, by measuring urinary iron excretion in the 6 hours after a test dose of desferrioxamine, 500 mg, given intramuscularly.

Long-term hemodialysis for chronic renal failure has been associated with the accumulation of aluminium in the brain, resulting from high aluminium content in the dialysis water supply causing encephalopathy. Desferrioxamine has been used for the successful removal of aluminum in dialysis encephalopathy (Ackrill *et al*., 1980), resulting in clinical improvement. Desferrioxamine raises serum aluminum to high levels and allows this to be removed by conventional dialysis techniques.

In a severe case of poisoning with shock, desferrioxamine is given by slow intravenous infusion at a rate of not more than 15 mg/kg to a maximum of 80 mg/kg per 24 hours. In the absence of shock, it may be given intra-

muscularly at a rate of 2 g for an adult and 1 g for a child every 12 hours. Gastric lavage may be performed with 5 g desferrioxamine dissolved in 1 liter of water, and 5–10 g desferrioxamine in 50–100 mL water may be left in the stomach to chelate unabsorbed iron from the gut.

While being well tolerated, the rapid intravenous administration of desferrioxamine may cause transient hypotension with histamine-like or anaphylactic reactions. The drug should be used cautiously in patients with impaired renal function.

3.5.6 Deferiprone (L1)

Deferiprone, 1, 2-dimethyl-3-hydroxypyrid-4-one, is effective for long-term iron chelation therapy of transfusional iron overload in thalassemia. Given by mouth, it is suitable for home treatment (Kontoghiorges *et al*., 2000). Deferiprone together with desferrioxamine in lower doses is indicated in cases requiring lifelong chelation (Mourad *et al*., 2003).

3.5.7 Diethylenetriaminepentaacetic Acid (DTPA)

DTPA is another synthetic polyaminopolycarboxylic acid with properties similar to those of EDTA, which also forms stable chelates with calcium. The calcium salt has been used to accelerate the elimination of plutonium and related actinide metals. Because zinc depletion may occur during long-term administration, it has been suggested that the more effective calcium DTPA be replaced by zinc DTPA in the later stages of treatment (Taylor and Volf, 1980). As with all chelators, DTPA is most effective if administered shortly after exposure. Lipophilic derivatives of DTPA are being developed, as, for example, Puchel, which can be administered as an aerosol to decrease the lung concentration of inhaled insoluble plutonium oxide particles (Stradling *et al*., 1981). Small amounts of plutonium and americium have been successfully eliminated with DTPA given in a dose range of about 1 g/day or about 30 mIU mol/kg body weight, in a few cases for long periods without adverse effect (Bair and Thompson, 1974; Taylor, 1982).

3.5.8 Diethyldithiocarbamate (DEDTC)

DEDTC is a chelating agent that has been found to be of value in the treatment of acute nickel carbonyl poisoning, greatly increasing the excretion of nickel in the urine (Sunderman, 1971). DEDTC can be given orally in moderately severe poisoning, initially at a rate of 50 mg/kg in divided doses. At the low pH of gastric juice, DEDTC is degraded to ethylamine and carbon disulfide. This reaction can be minimized by the concomitant administration of 2 g sodium bicarbonate by mouth.

3.5.9 Combinations of Chelating Agents

Combined treatment with EDTA and dimercaprol is most effective in acute lead poisoning in children with encephalopathy or blood lead levels greater than 100 μg/dL (1000 μg/L; 5 μmol/L), showing greater therapeutic effectiveness than EDTA alone. The reason for this is thought to be related to synergistic action of EDTA, which may intensify the inhibitory action of lead on hemosynthesis by a similar action on δ-aminolevulinic acid dehydrogenase. With intravenous EDTA infusion, to prevent a transfer of chelated lead to the brain, intravenous administration of dimercaprol should be given 4 hours before and continued during EDTA treatment (Grisemer, 2001).

The possible therapeutic advantages of using combinations of chelating agents, with particular reference to aiding the elimination of cadmium and plutonium, are considered by Schubert (1983). Experimental observations in mice dosed with cadmium chloride indicated an additive protective effect using the two chelators DTPA and DMPS. Chelator combinations tested for their effectiveness in the elimination of plutonium have included DTPA with desferrioxamine and EDTA together with a number of bidentate ligands. EDTA with bidentate ligands such as *p*-aminosalicylic acid significantly reduced the plutonium content in mouse liver and bone.

The rationale in using two different complexing agents to produce a synergistic effect is that the first agent should be sufficiently lipophilic to mobilize the metal from intracellular binding sites and promote its release into the blood, whereas the second agent will promote ligand exchange to form an ionized chelate that can then be excreted into the urine.

3.6 Modification of Response

Under this heading are included examples of therapeutic measures in metal poisoning that are directed toward a modification of the tissue response to the poison or to an alteration in the biochemical or metabolic state of the subject.

3.6.1 Modification of Tissue Response

Chronic beryllium disease is characterized by an inflammatory reaction that is granulomatous in nature and that seems to result from a hypersensitive response in certain individuals. The inflammatory process may be arrested, although not reversed, by adequate corticosteroid therapy. This treatment has resulted in a change in the clinical course of the disease with a reduction in symptoms and a favorable change in prognosis.

3.6.2 Modification of Biochemical Status

Chronic manganese poisoning has pathological, biochemical, and clinical features that closely resemble those of Parkinson's disease, which, once established, becomes a permanent disabling occupational disorder. The observations of Cotzias *et al*. (1971) on neurochemical similarities between the two conditions have led to the administration of L-dopa in manganese-induced parkinsonism. Modification of the parkinsonian state in chronic manganese poisoning has been obtained with L-dopa (Mena *et al*., 1970). In most patients so treated, hypokinesia and rigidity were markedly reduced, and postural reflexes improved with a restitution of balance. There was no evidence of a decreased body burden of manganese in the parkinsonian ex-miners treated in this way. Beneficial effects can be considered solely as a form of replacement therapy.

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CHAPTER

16

Principles for Prevention of the Toxic Effects of Metals

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ABSTRACT

Prevention of the toxic effects of metals begins with identification of the populations at risk of exposure. Industrial workers, long the principal focus of concern, remain the group at greatest risk. Nonoccupational populations—young children and pregnant women in particular—are also at risk today through exposures in air, food, water, and consumer products. These nonoccupational exposures are the result of wide environmental dissemination of metals in the past century and affect millions of persons. Toxic effects in occupational and nonoccupational populations may be clinically evident or subclinical. Toxic effects may occur in women and children at levels below those that cause effects in workers.

The tools available for prevention of toxic effects include exposure assessment, environmental monitoring, biological monitoring, medical monitoring, and a range of public health interventions. Medical examinations may sometimes be of use and can pinpoint reasons for increased susceptibility that may require additional action. Preventive interventions should seek ways to target the source of the problem and may require action to remove a subject from a dangerous exposure. Treatment may be necessary as a last resort, when prevention fails.

Five principles guide the prevention of toxic effects: (1) *hazard identification*; (2) *reduction of exposure*; (3) *monitoring*; (4) *training*; and (5) *authority. Hazard identification* includes recognition of the sources and routes of exposure and explanation of the range effects on health, including effects on early development. *Reduction of exposure* may involve outright bans of certain uses of toxic metals—the most effective strategy; reduction of workplace or environmental concentrations using the technologies of industrial hygiene and environmental control; and use of personal protective equipment—the least effective strategy. *Monitoring* may involve monitoring of the workplace or general environment or biological monitoring and health examinations of exposed populations. *Training* involves education of managers, workers, health professionals, policy makers, and the general public about the health risks posed by toxic metals and approaches for risk reduction. To be effective, all of these programs must be supported by *authority*, which usually is based in law.

Great success has been achieved in reducing toxic effects of metals in occupational and nonoccupational populations, but much work still remains to be done. Precaution should guide all decisions on whether to permit further dissemination of metals into the environment.

1 INTRODUCTION

The unique properties of metals—their durability, stability, and resistance to chemical breakdown—are simultaneously the basis for the wide and constantly expanding range of industrial applications of metals but also are the basis for the extensive distribution and persistence of metals in the environment and for the hazards they pose to human health.

Metals cause a wide range of toxic effects. Metals are proven and probable causes of human cancer. Metals can cause asthma, renal failure, reproductive impairment, disruption of the immune system, and pulmonary disease. Metals can cause dysfunction of the central and peripheral nervous systems. Metals are especially toxic when exposures occur early in life. Exposures to metals *in utero* and in the first years of postnatal life can cause injury to the developing brain and other organ systems at very low levels that would be considered safe for adults.

Minimizing or eliminating exposure is the single most effective means of preventing the toxic effects of metals. Current approaches to prevention are based on observations about the toxicity of metals that were first made in classical times and that have repeatedly been updated in light of new knowledge. Three trends in recent decades have greatly influenced strategies for preventing the toxic effects of metals:

1. Metals have become widely disseminated in the environment; they have moved far beyond the workplace, the original and traditional focus of concern.
2. Vulnerable segments of the population, such as women of childbearing age and young children, are increasingly exposed to metals and often require special protections against exposure.
3. Recognition has grown that metals can cause harmful effects at low levels of exposure previously considered safe and that there seem to exist no thresholds for subclinical toxic effects.

The quantities of metals used worldwide in the past two centuries exceed their use in all previous eras combined. In consequence of their extensive use in industry, consumer products, and motor fuels, as well as their release to the environment as by-products of industry and fossil fuel combustion, metals now contaminate the environment worldwide (Mage *et al.*, 1996; Patterson, 1970).

Industrial workers—miners, smelter workers, battery workers, workers in metal fabrication and construction workers—remain, as they have for centuries, the groups at greatest risk of exposure to metals and of toxicity (Hunter, 1969).

Because metals have become widely distributed in the environment, the populations at risk of exposure and toxicity have expanded greatly. Elevated levels of metals are now found in the bodies of humans of all ages (Agency for Toxic Substances and Disease Registry, 2002; 2005).

Women of childbearing age and young children are now recognized to be much more extensively exposed than they were in generations past. These groups are exquisitely susceptible to toxicity even at low levels of exposure (National Academy of Sciences, 1992; 1993b). Millions of cases of metal toxicity are reported worldwide each year among these vulnerable groups. The disease and dysfunction that result from exposures to metals early in life can disrupt the development of the brain and other organ systems, reduce intelligence, alter behavior, increase risk for mental retardation, and place major medical and economic burdens on families and societies (Grosse *et al.*, 2002; Landrigan, 2002; Trasande *et al.*, 2005).

Acute poisoning that results from high-dose exposure and produces clinically obvious symptoms was the first form of metal toxicity to be recognized (Hunter, 1969; Ramazzini, 1940). Subsequently, more sophisticated research has documented the presence of less striking, but nonetheless serious, adverse effects at lower levels of exposure and in a wide range of age groups. This sequence of discoveries has led to the recognition that metals can exert a range of adverse effects. Some are clinically obvious, but others are not evident during standard examination and can be discerned only through special testing; hence the term, "subclinical toxicity." The underlying concept is that there exists a dose-dependent continuum of toxicity, in which clinically obvious effects have their subclinical counterparts (Landrigan, 1989).

The concept of subclinical toxicity traces its origins to pioneering studies of lead toxicity undertaken by Landrigan, Needleman, and collaborators (Landrigan *et al.*, 1975; Needleman *et al.*, 1979). These studies showed that exposures to lead in early childhood could cause decreases in intelligence and alteration of behavior in the absence of clinically visible symptoms. The subclinical toxicity of lead in children has subsequently been confirmed in prospective epidemiological studies (Baghurst *et al.*, 1992; Bellinger and Dietrich, 1994) and has been found to extend down to the lowest measurable levels of exposure—below 100 µg/L (10 µg/dl) blood lead (Canfield *et al.*, 2003; Lanphear *et al.*, 2005). Subclinical toxicity has now been found to result additionally from lower dose exposures to arsenic (Wasserman *et al.*, 2004), mercury (Grandjean *et al.*, 1997), and probably manganese (Mergler *et al.*, 1999; Normandine *et al.*, 2004). The environmental impact of these subclinical toxicities on children and pregnant women is discussed further in Section 3.2.

2 GENERAL PRINCIPLES FOR PREVENTION OF THE TOXIC EFFECTS OF METALS

From a public health viewpoint, prevention must target the population groups at greatest risk (i.e., those who have the highest levels of exposure and those who

are the most vulnerable). The tools available for prevention include exposure assessment, environmental monitoring, biological monitoring, medical monitoring, and a range of public health interventions. Medical examinations may sometimes be of use and can pinpoint reasons for increased susceptibility that may require additional action. Preventive interventions should seek ways to target the source of the problem and may require action to remove a subject from a dangerous exposure. Treatment may be necessary as a last resort when prevention has failed.

Five common principles guide all approaches to prevention of toxicity caused by metals, whether in the workplace or the general population: Hazard identification, reduction of exposure, monitoring, training, and authority. The first of these, and general guidelines for the second, will be discussed in Section 2). The next three will be discussed separately for the workplace and general environments in Sections 2 and 3, respectively.

2.1 Hazard Identification

First, the hazard must be recognized and routes of exposure determined. This knowledge may be readily available or may require substantial research. The historical examples of lead, methylmercury, and arsenic show that, as health hazard information becomes more detailed, the range of effects that are uncovered increases in extent and severity. Several examples demonstrate that early warnings of toxicity have frequently been ignored.

2.1.1 Lead

The first description of lead poisoning in young children was presented in a report from Australia published more than 100 years ago. The source of this outbreak was not immediately obvious and was discerned only after several years of investigation. It was traced to ingestion of lead-based paint by children playing on painted verandas (Gibson, 1895). Further reports of childhood lead poisoning from lead paint ingestion in the United States followed over the next several decades and resulted in bans in many nations on household use of lead-based paint. Lead poisoning was, at that time, considered an acute illness from which a child either recovered or died. The realization that lead could cause long-term neurological sequelae was first documented in the 1940s, when 19 of 20 survivors of acute lead poisoning in a clinical follow-up study conducted in Boston were found to have severe and persistent learning and behavioral problems (Byers *et al.,* 1943).

Despite those early warnings, extensive use of lead in petrol, paints, ceramic glazes, and many other products continued through much of the 20th century and caused widespread toxicity. During the 1970s, subclinical neurobehavioral deficits were documented in asymptomatic children with elevated lead concentrations in blood (Landrigan *et al.,* 1975; Needleman *et al.,* 1979). Further studies from many countries confirmed these findings. In response to these demonstrations of low-dose toxicity, health agencies have serially reduced the permissible concentration of lead in children's blood. Current research on the developmental neurotoxicity of lead focuses on the shape of the dose-response curve at very low exposure levels, which seem to cause surprisingly large functional decrements (Canfield *et al.,* 2003).

2.1.2 Methylmercury

Methylmercury was first established as a neurotoxicant in men exposed occupationally (Hunter and Russell, 1954). The toxicity of methylmercury to the developing fetal brain was first recognized in the 1960s in Minamata, Japan, where an epidemic of spasticity, blindness, and profound mental retardation occurred among infants whose mothers had consumed contaminated fish without being affected themselves (Harada *et al.,* 1995). Recent studies have focused on the neurodevelopmental effects of prenatal exposures to methylmercury at doses insufficient to produce clinically obvious symptoms. (Grandjean *et al.,* 1997) (See also Chapter 33 for more detailed discussion.) The US National Academy of Sciences reviewed these studies and concluded that strong evidence exists for the fetal neurotoxicity of methylmercury, even at low-exposure levels (National Academy of Sciences, 2002). These findings have led food safety and public health authorities in nations worldwide to issue dietary advisories warning populations, especially women of child-bearing ages, of the hazards of consuming predatory fish. Substantial reductions have been achieved in mercury use, as well as in its release from various sources, such as hospitals and waste incinerators.

2.1.3 Arsenic

Ingestion of arsenic-contaminated drinking water has long been recognized to cause peripheral neuropathy in adults (Landrigan, 1981). Dramatic documentation of developmental neurotoxicity caused by arsenic was first reported from Japan, where, in 1955, consumption of powdered infant milk contaminated with arsenic led to more than 12,000 poisoning cases and 131 deaths (Yamashita *et al.,* 1972). Arsenic occurs in groundwater worldwide and has been linked to various forms of cancers (National Academy of Sciences, 2001). (See also Chapter 19 for a detailed discussion of the effects of arsenic.) As in the previous examples, continued research revealed

adverse health effects at levels of exposure previously thought to be safe (Wasserman *et al.*, 2004).

2.2 Reduction of Exposure

The second principle of prevention of metal toxicity is to reduce exposure through elimination or abatement of the hazard. It is now widely understood that there exists a hierarchy of procedures for reducing exposure and that the procedures at the top of this list, such as product substitution and engineering controls, are always and inherently more effective than downstream approaches that require personal action by individuals at risk or use of personal protective equipment. This hierarchy of exposure reduction is as follows:

1. Cessation of use or, if not possible, cessation of unnecessary use of the toxic metal.
2. Substitution or replacement of the toxic metal with less toxic agents.
3. Prevention of exposure caused by the spread of metal dust and fumes.
4. Removal of the metal already spread.
5. Reduction of personal contact with the metal.

For further discussion of these approaches, see Burton (1998) and Raterman (1996).

3 PREVENTION OF THE EFFECTS OF METAL TOXICITY IN THE WORK ENVIRONMENT

3.1 General Considerations

Before any action can be taken to control exposure to a toxic metal in the workplace, the hazard must be recognized. This can be a prolonged process, as illustrated by the history of recognition of occupational lead poisoning in the United States in the 20th century (Hamilton, 1914; 1943; Schilling, 1973).

During recent decades, understanding of the hazards of metals has evolved over time and thus contributed to the perspective of prevention. For example, when the International Agency for Research on Cancer first evaluated beryllium carcinogenicity, only one member of the expert committee requested a minority statement that the epidemiological evidence was sufficient. But at a subsequent meeting, after the publication of additional studies, the experts arrived at a consensus that beryllium should be recognized as a human carcinogen (IARC, 1993). Similarly, dose-response relationships have been better defined, and hazards are now recognized at exposure levels that were previously thought to be safe. Perhaps the clearest example is inorganic lead, but other metals also provide examples. The occupational exposure limit for lead was once 5 mg/m^3, but by 1979 the threshold limit value used in the United States had been lowered 100-fold to 0.05 mg/m^3 (OSHA, 2005). Undoubtedly, new studies will generate additional documentation of low-dose toxicity and additional lowering of exposure limits, thus further strengthening prevention efforts (Landfield *et al.*, 2003).

3.2 Reduction of Exposure

In occupational health, several approaches to exposure reduction are applicable and need to be explored:

3.2.1 Elimination of Unnecessary Uses and Substitution of Safer Materials

In some instances, the use of a toxic metal in a process is unnecessary. Examples of cessation of unnecessary uses of metals in the workplace are:

TABLE 1 A Hierarchy of Prevention

Cessation of Use/Substitution—Outright ban or substitution of the toxic metal with a less hazardous material. This may be termed *primary prevention*. The best example is removal of lead from gasoline, which produced massive declines in blood lead levels worldwide (CDC, 1997). Additional reductions in exposure have resulted from banning certain uses of metals, such as the use of mercury in felt manufacturing, plastics plants, and medical devices.

Prevention of Exposure—This may be termed *secondary prevention*. It seeks to reduce exposures both by prevention of spread of metal dusts and fumes and removal of spread that has already occurred. These efforts are important, but generally less effective than outright ban or metal substitution. *Standards-setting* is an important aspect of secondary prevention of exposure. In most countries, standards-setting is a legal, as well as a scientific, process and is often guided by the paradigm of *risk assessment* and *risk management*. Risk assessment/risk management is sometimes modified by application of the *precautionary principle*.

Other examples include:

- Process enclosure in industry
- Exhaust ventilation from industrial plants
- Removal of lead paint from walls to prevent childhood lead poisoning

Reduction of Personal Contact—Through respirators and personal protective clothing. Protects only the person wearing the items. Least effective of the prevention measures, but appropriately used for emergencies and occasional exposures.

Example: Chelation therapy for metals overexposure.

- Removal of mercury-containing thermometers from hospitals and their replacement by electronic devices.
- The use of copper- or nylon-clad bullets with nonlead primers at indoor firing ranges, instead of lead-containing bullets.
- The use of composite materials and ceramics instead of mercury amalgam for dental fillings.
- The introduction of cement with negligible chromate content.

3.2.2 Reduced Use of Toxic Metals in Plant and Manufacturing Design

It is axiomatic that a properly designed new facility that incorporates state-of-the-art control measures will be safer, more efficient, and more economical than an older facility, where ventilation and safety equipment must be retrofitted. Success is usually greatest when technical and medical knowledge can be applied in the planning stages of a new facility to eliminate or limit the use of toxic materials as much as possible. The focus of toxic use reduction is to eliminate or control use of toxic materials in manufacturing at the plant design stage, rather than cleaning up the environment after its use. In 1989, the Commonwealth of Massachusetts (United States) passed a landmark Toxic Use Reduction Act (TURA) (General Laws of Massachusetts, Ch. 21 I @ *www.mass.gov*). Techniques favored under this Act include substitution, product reformulation, redesign of production processes, improved housekeeping, and further recycling and reuse of waste products. For more information on toxics use reduction see Geiser and Commoner (2001).

3.2.3 Other Technical Control Measures

The technical control of metal exposures in the workplace follows the principles of industrial hygiene. Forms of automation can be used to reduce workers' contact with hazardous processes. Whenever possible, all hazardous types of exposure should be concentrated in specially enclosed areas where control processes can be effectively used. Encapsulation and other isolation methods prevent toxic dusts and fumes from being spread about, and special well-ventilated control rooms can be built within the plant to enclose the most dangerous processes. Surfaces should be smooth to prevent dust from attaching to walls and floors. Nooks and corners, where dusts can accumulate, should be avoided. For example, in the case of metallic mercury, the worst problem is evaporation from spilled droplets. Their removal is facilitated by smooth, nonabsorbing surfaces that round up in the corners and slant toward the center of the floor. Secondary lead smelting is such a hazardous process that priority should be given to automation of transportation and oven-filling phases. The preparation of all metal pigments, very hazardous when done by hand, becomes far less dangerous in closed, automated systems. Instead of dust-producing powders, liquid premixes are preferred.

For more detailed information about these approaches, the reader is referred to basic textbooks in industrial hygiene, such as DiNardi (1998), Harris (2000), LaDou (2004), Levy *et al*. (2000), and Plog *et al*. (1996). In addition, Volume III of the *ILO Encyclopedia of Occupational Health and Safety* is particularly useful for metals use and control measures in various mining and metalworking industries (Stellman, 1998).

3.2.4 Local Exhaust Ventilation

One of the best means for controlling dust or fumes in the workplace is a local exhaust system, in which intake is as close as possible to the point of origin of the dust or fume. The system for removal of the polluted air must be constructed, so as not to draw the contaminated air into the breathing zone of the worker on its way to the exhaust. The air taken into this system must be filtered and the metals removed, so that the general environment outside of the factory will not be polluted. All air filters should be inspected frequently and changed on a regular schedule. Self-made "improvements," such as ductwork extensions, may be deleterious to the efficient functioning of the system and may cause deterioration of system equipment. For further details see Burton (1998; 2005), George and DiNardi (1998), and Soule (2000a).

3.2.5 General Room Ventilation

Good general room ventilation is important to the quality of working life within a facility, but it is not a particularly efficient adjuvant to a local exhaust system—indeed, an improperly designed room ventilation system can undermine the efficient operation of a local exhaust ventilation system. Because general systems involve much higher costs, for example, in energy consumption, the emphasis should be put on local controls. It should be stressed that open windows and doors, as well as changes in the working process, may thwart otherwise efficient room and local exhaust ventilation systems. Proper caution and careful maintenance are especially important when recirculated air is used for workroom ventilation, as in heating, ventilation and air-conditioning (HVAC) systems. For further details see AIHA/ANSI Z9 Committee (2004), Burton (1996; 1998), and Harris and Arp (2000).

3.2.6 Housekeeping

Poor housekeeping can render even the best technical control system ineffective. Education and supervision should be detailed and protective measures enforced. Clothing made of synthetic fibers retains less dust than cotton overalls. Pockets should be as few as possible, especially when metallic mercury is present in the workroom. Working clothes should be vacuumed before removal and changed at least twice a week. Metal workers need to be provided with changing and showering facilities, and work and street clothes should be stored in separate lockers to reduce the exposure to the worker himself and to prevent the worker from exposing his family (Baker *et al.,* 1977a).

Because most metals occur in dust form or precipitate as small particles in fumes, exposure can be reduced by keeping the workplace floor permanently moistened and by frequent vacuum cleaning. Dry sweeping should be avoided, because this will re-entrain settled dust into the workroom air. The use of compressed air to blow dust from floors, walls, and ledges should be prohibited. In principle, all cleaning and other operations that may cause settled dust to whirl about should be performed outside of normal working hours by specially trained, well-protected workers.

When moistening the floor, the water should never come in contact with molten metal, because violent explosions can occur. Operations involving the welding, flame-cutting, or burning of metals coated with paint of unknown composition should not be allowed before samples have been analyzed at least for lead, cadmium compounds, and chromates, or until the composition has otherwise been explained. If such metal-based pigments are present, the burning is extremely dangerous and should be performed under powerful local exhaust ventilation and, if possible, outside the normal working hours. Metals coated with metallic cadmium should be treated in the same way. Even less toxic metals, such as zinc, found in galvanized metals and brass can cause metal-fume fever, and welding and burning these demand the same protective measures.

3.2.7 Influence of Personal Hygiene on Metal Absorption and Toxicity

Both the industrial and the general environment present many possibilities for contamination of hands and clothing with metal compounds. Because of the danger of food becoming contaminated from the environment or from dusty hands or clothes, food should never be prepared, dispensed, or eaten in areas where there is metal dust. Dining rooms should be clean. Personal hygiene should be facilitated by providing sufficient washrooms. Good personal hygiene is also important from the point of view of reducing exposure to the worker's family (Baker *et al.,* 1977a). Smoking is another hazard in the industrial environment. Large amounts of metallic dust may accumulate on smoke particles from cigarettes, cigars, and pipes and be transferred to the human body (of the smokers and nonsmokers alike) through respiration or by direct contact with contaminated hands on sandwiches and other foods. Aerosolized metals may also be inhaled through burning tobacco products, causing even more hazardous products of combustion—all the more reason for prohibiting smoking in the workplace.

3.2.8 Reduction of Worker Contact with Toxic Metals and Personal Protective Equipment

When metal toxicity occurs, the ideal solution is immediate cessation in use of the toxic metal. Typically, such drastic action requires a legally mandated ban enforced by governmental authorities, although it may also be achieved by voluntary agreement. A second, less optimal approach is to remove the worker from exposure whenever the concentrations of the metal in the blood or urine exceed the action level, when early symptoms or signs are present, or when reports of exceptional exposure peaks are made. Ideally, the worker should be transferred to a job that is free from exposure. Otherwise, compensated sick leave may be considered, as well as job transfer at the same level of compensation. For lead workers in the United States, the latter approach is legally required under the Medical Removal Protection program established through the OSHA Lead Standard (OSHA, 2005). The worker should not be allowed to return to exposure until his or her blood lead levels are well below the recommended upper limits.

Workers should spend as little time as possible in areas with unusually high concentrations of metal dust or fumes. These areas include the vicinities of smelting furnaces, welding places, and pasting machines in storage battery factories. When engaged in welding or burning outdoors, the worker should be trained to always place himself upwind, and other workers should not be asked to work downwind from the welding or burning operation.

If large-scale engineering methods such as automation and encapsulation fail to ensure adequate safety, personal protective equipment may be used, but only as a last resort. The only situations in which respirators and other forms of personal protective equipment can be considered a primary choice for worker protection are during emergencies and occasional, brief exposures, such as in repair and maintenance operations.

Many types of respirators are on the market, from air-filtering respirators of various types to self-contained breathing apparatus (SCBAs), for which the worker carries the air on a tank on his or her back. Metal fumes require more efficient respirators than are needed for dust; if both are present concomitantly, respirators designed for fumes must be used. Activated charcoal filters do not retain fine aerosol particles; therefore, combined gas and dust filters should be used in spray painting. In certain operations in which exposure is very high, for example when ships covered with thick layers of red lead are scrapped by flame cutting, protection may require either an air-purifying positive-pressure respirator or an SCBA.

If respirators are used in the workplace, an intensive program of test fitting of the respirators to each person and an active program of cleaning and maintaining the respirators and replacing filters regularly is imperative and is mandated by OSHA Standard 1910.134. For details on respirators, their selection, capabilities, and maintenance see Colton *et al*. (1998), Myers (2000), and Nelson (1998).

3.3 Monitoring of the Work Environment

Two complementary approaches exist for monitoring metal exposure in the workplace—environmental and biological monitoring. Measurements of airborne metal concentrations in the work environment are mandatory to:

1. Localize high-exposure areas.
2. Estimate individual exposure (especially when no satisfactory biological monitoring method exists).
3. Detect engineering failures (leakages, areas of insufficient ventilation).
4. Detect the effects of changes in processes or working methods.
5. Document the impact of preventive measures.
6. Ensure that safety standards are being met.

Only a broad outline of monitoring is presented here. For further information about environmental monitoring for vapors and metal fumes see Burgess (2000), Dietrich (1998), Huey (1996), Hahne (1996), Johnson and Swift (1998), and Soule (2000b). Although environmental monitoring provides information about levels of the metals in air, biological sampling of levels of metals in the bodies of workers provides a time-integrated measure of cumulative exposure (Todd *et al.*, 1996). For further information about biological monitoring see Chapter 4 on Biological Monitoring and Biomarkers and Clarkson *et al*. (1988), LaDou (2004), Lauwerys (1998), Levy *et al*. (2000), Que Hee (1998), Rom (1998), Soule (2000c), and Zenz (1994).

3.3.1 Air Sampling Strategy in the Workplace

Metals usually appear in the workroom air as fumes and solid particles and sometimes as vapor (metallic mercury) or liquid aerosols (e.g., chromates above the electroplating bath). Fumes are formed when molten metal evaporates or when metal or metal compounds are burned. The dust particles are either derived from condensed fumes or from metal-compound dust that has whirled up. Although the particle size distribution varies, the respirable dust is composed of small particles that can stay in the air for many hours. Larger particles settle but may become re-entrained as a result of wind currents or nearby activities.

In recent decades, many new and improved direct-reading instruments have been developed. These are especially convenient for the real-time measurement of concentrations of gases and vapors.

The only metal present in many workplaces primarily in vapor form is mercury, and commercial analyzers are widely used for its detection. However, direct-reading instruments are not so helpful in determining concentrations of aerosols (fumes, particulates and/or liquid droplets), the most common form in which metals are present in the workplace, because they cannot differentiate between types of aerosols. They can, however, be used to size these aerosols by means of optical or linked optical and electrical techniques. For further information about the types, capabilities, and drawbacks of direct-reading instruments, see Todd (1998). Because metals in the workplace usually appear as fumes or particulates, we will focus on them in the following discussion along with their associated sampling and laboratory measurement.

Concentrations of metal in air close to a machine or where a process is carried out are typically maximal. They may vary considerably over a distance of a few meters or even centimeters. For most purposes, samples should be taken in regular work areas (area air samples) and also from the worker's breathing zone.

The number of air samples needed depends on the purpose of the measurement. An overall picture of workplace exposure level is obtained from sampling carried out on a long-term basis with samples taken from multiple, different sites. When individual exposure is to be studied, personal air monitoring is the method of choice. The samples should be taken from the breathing zone, no farther than 30 cm from the nose. They may even be taken from within a welding helmet. The sampler is usually attached to the worker's shoulder or waist. No obstruction can be allowed between the air sampler inlet and the worker's face, and the equipment must not disturb the work, for instance, by blocking sight. Workers representative

of each area, operation, and other special conditions should be chosen, and personal breathing zone air samples collected on them. Workers with predicted high exposure should be included, so that the worst case is assessed (Mulhausen and Damiano, 1998). Air sampling may be performed at regular intervals or randomly over time. However, regular-interval sampling must not coincide with regular cycles of events in the workplace that may cause systematic errors in the measured concentrations, such as start-ups of new processes or periodic downtimes.

Short-term air samples (15–60 minutes) are preferred for assessing intermittent exposures as, for example, in welding operations. Long-term samples (7–8 hours) are most relevant when the goal is to ensure that metal concentrations on an average are below the threshold limit value. Because long-term particulate sampling often has to be restricted to about 10 samples or less for practical reasons (e.g., 8 hours per sample), their average should be well below the hygienic standard if a safety margin is to be ensured for variations.

Compared with biological samples, personal monitoring gives a more limited assessment of exposure, because it focuses on the inhalation route of entry, whereas biological monitoring assesses personal exposures on the basis of all routes of entry (inhalation, skin, ingestion, etc.). Furthermore, personal sampling does not allow for differences in physical activity during exposure, personal hygiene, use of respirators, or differences arising from other sources of exposure, such as food and water. If the metal accumulates in the body, exposure assessment during a single day may not be very informative of long-term health risk. Hence, biological monitoring is always preferable whenever reliable methods exist. Such is definitely the case for lead, arsenic, cadmium, and mercury, whereas the experiences with chromium, nickel, and selenium are still debatable (ACGIH, 2003; Meister, 2004; Soule, 2000c).

3.3.2 Sampling Technique

During sampling of fumes and dusts, a measured volume of air is drawn through the sampling train and then captured either by a filter, a cyclone separator followed by a filter, or a multistage cascade impactor (Johnson *et al.*, 1998). The first case (simple filter collection) is called the gravimetric method and measures total particulates (respirable and nonrespirable) with no size separation. The second method uses the cyclone separator as a size-selective presampler to separate the coarse dust from the respirable fraction and then captures the respirable dust on a filter, which is gravimetrically analyzed. Most popular currently is the third case, in which a multistage cascade impactor separates the dust into several fractions, according to the number of stages in the impactor. The volume of the air sample may vary from a few liters to several cubic meters, depending on the concentration of metal, the sampling equipment, and the sensitivity of the laboratory analytical method. Often the concentrations, especially those around or below the hygienic standard, are so low that a large air-volume and high flow rate are needed before enough metal particles can be collected over a reasonable period of time. The sample size should be large enough to allow rate estimations of concentrations within the magnitude of about a tenth of the hygienic standard to be made.

3.3.3 Analysis

The concentration of the particulate matter in the air is calculated from the weight of the dust particles collected, determined by direct weighing and/or chemical analysis, divided by the volume of the air sample. Also, the particles in a known portion of the sample may be counted using microscopy or automatic particle counters. Because particles in the respirable range are most important from the toxicological point of view, the size distribution should also be determined and taken into consideration. The size distribution is most readily determined by use of cascade impactors, or direct reading optical or optical/electrical analyzers, as noted previously (Todd, 1998). Finally, various analytical methods, described in other sections of this book, may be used to determine the concentrations of a particular metal vapor or fume in the ambient environment. This is especially important for analyses in which more than one metal is known or suspected to be present.

In evaluating exposure measurements, a main consideration is whether the samples analyzed are representative of the exposure situation. Results obtained during one day, or from a few sites only, cannot be given too much weight in the estimation of workers' exposure beyond the actual sampling period. Monitoring must be regular if reliability is to be ensured. Plotting the results of successive measurements on a control chart is recommended. Needless to say, the analytical methods used should be strictly standardized, and quality-control programs should be used both within a single laboratory and between laboratories.

3.3.4 Biological Monitoring

In industry, biological monitoring has been carried out for decades, because the absorbed dose was considered a useful tool to protect the individual. The choice of indicators and biological samples varies from one metal to another. In general, the parameter of dose or internal exposure (i.e., the concentration of the metal itself in some biological medium) is most useful.

At present, lead is more suitable for biological monitoring than other metals, because the dose-effect and dose-response relationships are fairly well known. The concentration of lead in blood represents current exposure to inorganic lead compounds better than any other test. Measurement of lead in blood should be preferred to that of, for example, lead in urine. When a sufficiently low-action level is selected, enough safety can be ensured if blood lead alone is used for health monitoring. If the chosen action level is exceeded, exposure cessation is required, and measures should be initiated to better control the exposures.

In the case of occupational exposures to mercury, exposure tests are less reliable. Mercury in urine has been the most widely used exposure test in industry, when inorganic mercury or mercury vapor were the main exposures. However, current ability to conduct real-time measurements of mercury levels in the workplace air has obviated some of the need for its biological monitoring. Cadmium can be measured in both blood and urine. It is believed that cadmium in blood reflects current exposure, whereas cadmium in urine reflects body burden. After exposure has ceased, blood cadmium also reflects body burden of cadmium (Nordberg and Nordberg, 1988). Hence, both blood and urine tests should be used concurrently.

In principle, the frequency of monitoring is determined by the degree of risk (WHO, 1980). For further information about biological monitoring, as noted previously, see LaDou (2004), Lauwerys (1998), Levy *et al*. (2000), Que Hee (1996), Rom (1998), Soule (2000c), Zenz (1994), and Chapter 4.

3.3.5 Health Examinations

Health evaluations are often warranted in the occupational setting. Two types of examinations are particularly appropriate. Preplacement examinations aim at protecting workers from exposures to which they would be especially sensitive. For example, workers with kidney disease may be excluded from work with toxic metals that are primarily excreted through the urine. Periodic evaluations, on the other hand, are designed to monitor biological response to exposure, to detect toxic effects of metals at an early stage, and to discover other relevant diseases that may weaken the worker's resistance to the workplace exposures. The frequency of periodic examinations is, in some instances, established by legislation or regulation and may vary depending on the degree of risk. Usually an annual examination is considered appropriate unless monitoring shows that the action level has been exceeded.

The routine use of chelating agents to control levels of metals in the bodies of workers is obsolete, dangerous, and deserves condemnation. This type of practice had previously been widespread in European countries and in the United States (Baker *et al.,* 1979). There are several reasons to prohibit this treatment as a form of prophylaxis. First, chelation is a poor substitute for good workplace hygiene. Second, chelating agents also mobilize and remove essential metals, such as zinc and copper. Third, all such agents have adverse side effects, such as nephrotoxicity (penicillamine and EDTA) or myelotoxicity (penicillamine), and they may be allergenic (penicillamine). For these reasons, an ethical physician would not consider the prophylactic use of chelating agents. The treatment of acute metal poisoning by chelation is, of course, quite another matter. In the United States, the OSHA Lead Standard (29CFR1910.1025) bans prophylactic chelation (OSHA, 2005).

3.4 Training

Education and training are important aspects of the prevention of metal toxicity. Most, if not all, preventive efforts will fail if the persons at risk are not informed about the hazards that they face in their work or in their community. The groups who need education include plant managers, health personnel, and engineers, as well as workers and the general public. All of these groups have a right to know about the materials to which they are at risk of exposure, the hazards of these materials, how to protect themselves, and how to detect early signs of overexposure. For further information see Lee *et al*. (1998) and Quinn *et al*. (2000). Information on occupational hazards is made available to workers through Material Safety Data Sheets and worker training, offered by management, unions, or both and/or by government agencies or community groups.

A classic example from heavy industry of the importance of education concerns lead oxide dust. If lead oxide dust is generated in a restricted area of a lead-using factory such as a battery plant, and its elimination is based on effective local exhaust ventilation, the workers should know about the dangers posed by this dust and how to prevent it from spreading to other parts of the plant. Also they need to be provided with changing and showering facilities so that they do not inadvertently transport lead dust home to cause lead poisoning in their children (Baker *et al.,* 1977a).

3.5 Authority

Most prevention programs, at least large-scale programs, are backed by some form of authority. This authority is often based in legislation, which is typically secured after many years of political struggle (examples being the Occupational Safety and Health Act in the United States and the Health and Safety at Work Act in

the United Kingdom). In other instances, the authority derives from regulation by governmental agencies at various levels (federal, state, or local) or from collective bargaining agreements. Before such authority is established, there may be a need to create political pressure.

Those engaged in research must not withdraw into an ivory tower. On the contrary, their responsibility is to provide politicians and policy makers with unbiased data and opinions on the basis of scientific judgment to assist the development of sound prevention programs. Researchers should also offer the public correct facts and should oppose any exaggeration created by mass media, misinformation inspired by industry, administrative indolence, and any forms of misinterpretation. These are core principles of the developing field of risk communication (Gheorghe and Seiler, 1998; Lee *et al.*, 1998; Sandman, 1993).

4 PREVENTION OF THE EFFECTS OF METAL TOXICITY IN THE GENERAL ENVIRONMENT

4.1 General Considerations

Industrial activity during the past 200 years, and especially in the past 50 years, has caused a sharp increase in the concentration of many metals in air, soil, water, and other environmental media worldwide (see Dick, 1991; Lockhart *et al.*, 1992; Mage *et al.*, 1996). Consequently, the likelihood that the general public will be exposed to toxic metals is greater than in the past and growing.

Exposure levels vary greatly, depending on geographical, cultural, and other circumstances (see WHO, 1973). Urban environments are typically more polluted than rural areas, and industrialization increases exposures directly through emissions and indirectly through its products. Local practices—for instance, the use of lead-glazed ceramic containers and battery recycling—may further cause exceptionally high exposures (Romieu and Hernandez-Avila, 2003). Finally, enrichment may take place in food chains, such as the bioaccumulation of methylmercury in marine food webs (Hansen and Gilman, 2005).

4.2. The Unique Vulnerability of Infants and Children to Poisoning by Metals

Children are especially susceptible to adverse health effects from exposure to metals dispersed in the environment. Infants and children have unique patterns of exposure and developmentally determined susceptibilities that differ qualitatively from those of adults and that generally increase their risk of toxicity (National Academy of Sciences, 1993a). The fetus that a pregnant woman carries to work has vulnerabilities that are very different from those of an adult worker.

Children's high degree of vulnerability to toxicity caused by metals reflects the fact that they have exceptionally heavy exposures coupled with unique biological vulnerabilities. Kilogram for kilogram of body weight, children drink more water, eat more food, and breathe more air than adults. In the first 6 months of life, children drink seven times as much water per day as the average adult. Children between 1 and 5 years of age eat three to four times as much food as an adult. The implication of these findings for health is that children have substantially heavier exposures than adults to any metals that are present in water, food, or air (National Academy of Sciences, 1993a).

Children's metabolic pathways, especially in the first months after birth, are immature. Their ability to metabolize, detoxify, and excrete many substances differs from that of adults. In some instances, children are better able to deal with chemical toxicants. More commonly, however, they are less well able to deal with toxic metals, such as lead.

Children are undergoing rapid growth and development, and these highly complex developmental processes are easily disrupted. Organ systems undergo very rapid change prenatally, as well as in the first months and years after birth. These developmental processes are very delicate and may be unable to repair damage caused by environmental toxicants. If chemicals such as lead, mercury, or solvents destroy cells in an infant's brain, the risk is high that the resulting dysfunction will be permanent and irreversible.

Because children have more future years of life than most adults, they have more time to develop chronic diseases triggered by early exposures. Many human diseases, including neurodegenerative diseases and many cancers, are now thought to arise through a series of stages that require years or even decades to evolve form the earliest initiation to the actual manifestation of disease (Landrigan *et al.*, 2005). Recent studies raise the hypothesis that exposures to metals in early life may increase later risk for Parkinson's disease—in relation to manganese exposure (Logroscino, 2005; Zatta *et al.*, 2003)— and for dementia—in relation to exposure to lead (Stewart *et al.*, 2002). Genetically mediated differences in susceptibility among individuals may modulate these effects.

4.3 Reduction of Exposure

4.3.1 Elimination or Reduction of Use

The most effective method for control of the spread of a metal into the general environment is the

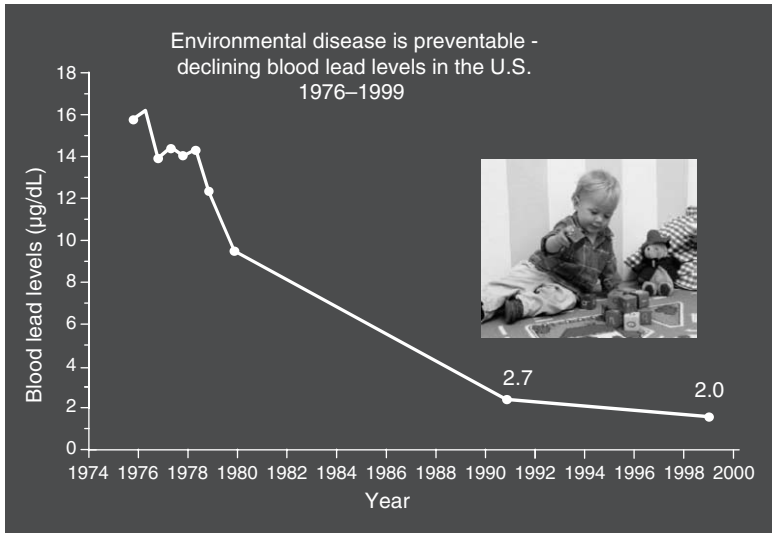

FIGURE 1 Decline in population blood lead levels in the United States (1976–1999). From CDC (2005).

discontinuation of dangerous and unnecessary uses. Such a step also reduces the need for production and, hence, simultaneously eliminates the exposure of workers. Generally, such a drastic measure requires a legislative ban, as in the case of a ban imposed on the use of methylmercury compounds for seed dressing in Sweden in 1966. Sometimes a practice is abandoned voluntarily, as in Finland in 1969, when the pulp industry agreed to discontinue the use of phenyl mercury acetate for slime control.

Major reductions in human exposure and in population blood lead levels have resulted from reduction of the content of tetraethyl lead in gasoline (Landrigan *et al.,* 2000). This advance has required legislation in some countries, while others nations have achieved promising results voluntarily. In the United States, the gradual removal of lead from gasoline produced a 90% decline in population blood lead levels between 1976 and 1997 (Figure 1) (CDC, 1997). In Chile, where lead in gasoline was first regulated in 1993, infants in Santiago experienced a decline of 29% in blood lead levels over the next 18 months (Pino *et al.,* 2004). More than 80 nations worldwide, including nearly all of the industrially developed nations, have now banned lead from gasoline.

In many nations undergoing the transition to industrialization, lead is still used in gasoline. In these nations, lead use in industry is often widespread, environmental contamination may be intense, and blood lead levels in workers, as well as in residents of communities near polluting industries, have been reported to be dangerously elevated (Landrigan, 2002; Landrigan *et al.,* 2000).

4.3.2 Source Control

If use of a metal cannot be eliminated, control of environmental contamination is achieved through prevention of escape as close as possible to the source of pollution. The most important method of preventing metallic emissions to the environment from industry is the purification of smoke, exhaust, and other emissions. The methods vary widely, but the following four principles are fundamental to most methods of particulate control (Johnson *et al.,* 1998):

1. Separation by inertial forces (e.g., cyclones)
2. Separation by dissolution (e.g., water scrubbers)
3. Separation by filters
4. Separation by electrostatic forces (electrofilters)

In addition, cooling traps and absorption into organic polysulfides are used for capturing mercury vapor.

4.3.3 Routes of Environmental Contamination by Metals

Metals can contaminate the general environment through many routes. Because of their stability, they may penetrate environmental compartments, in some cases, many years after the initial deposition.

Contamination of the air and then waterways by metals occurs as a consequence of anthropogenic emissions from coal-burning power facilities, chlor alkali plants, waste incineration, and other industrial activities, which contribute 70% of the mercury pollution into the earth's atmosphere annually (Trasande *et al.,* 2005). Large conventional power plants are the main polluters. In the United States, coal-burning

electricity–generating plants are major sources of atmospheric mercury emission and accounted for the release of 48 tons of mercury emissions in 1999, 41% of all anthropogenic mercury emissions in the United States that year (Trasande *et al.,* 2005).

Soil and water near large smelters or other major emitters are often heavily contaminated (Benin *et al.,* 1999; Liu, 2003; Sterckeman *et al.,* 2000). Vegetables and grain grown in such environments usually contain undesirably high concentrations of metal (Kalac *et al.,* 1996). Community and well water often become contaminated (Baker *et al.,* 1977b; Polissar *et al.,* 1990). Preventing undue exposure from such sources requires, in addition to reducing the emissions, the monitoring of food and water and a ban on the sale of foodstuffs containing excessive amounts of metals. Hence, the risk for adverse effects is highest among those living in these vicinities. Taller chimney stacks distribute the emissions over larger areas and thereby dilute the concentrations, but this practice is no substitute for efficient exhaust purification in the case of toxic metals and should, therefore, be condemned. The same can be said of the practice of leading unpurified sewage water containing, for example, mercury or cadmium far out into the sea.

The aquatic system becomes contaminated when industries—especially small factories—discharge untreated, metal-containing waste directly into the water, when rainwater washes away the metal-containing dust that has settled around industries, or when metal trapped in air-purification systems is carelessly handled and allowed to enter the waste water stream. Water scrubbers constitute a particular problem in the last respect. The purification of sewage water is based on recirculation and various precipitation processes. For example, mercury can be precipitated into sulfide and absorbed into aluminum hydroxide, which is then removed as foam. Iron oxide can also be used. Unfortunately, the control of the spread of dust from the surroundings of industries sometimes entails a liberal use of water, thereby placing an extra load on water-cleaning systems, and, in some cases, polluting rivers and lakes.

Pollution of the soil and water systems may also arise from the weathering of disposed metal products. The reuse of scrap decreases both these types of pollution and the need for exploiting ores. However, most scrap for reuse goes to iron and steel works, where the melting process releases impurities (e.g., cadmium and lead) stemming from alloys, paints, etc. The impurities are seldom regained but are emitted into the environment with the smoke. Unless effective exhaust purification is applied, such recycling, in fact, can increase rather than decrease exposure to toxic metals.

Careless dumping of metal waste can severely pollute water basins. For example, old gravel quarries are definitely unsuitable as dumps, because dissolved metal compounds then have easy access to the ground water. All dumps must be without any contact with water supplies. A specific problem has arisen caused by sewage sludge being used as a fertilizer. Such sludge may contain fairly high concentrations of many metals (e.g., cadmium), which may then enter the food chain. Because mercury is transformed into methylmercury by microorganisms in the environment, it is important to take care of mercury waste.

Pollution may arise acid when industrialized societies ship waste material containing toxic metals to developing nations. Severe episodes of contamination have resulted from uncontrolled export of products such as lead batteries.

Metal objects such as cans and batteries often end up in community incinerators together with other waste. Burning such waste may cause significant emissions of the most common toxic metals, especially lead.

Food and wine may be contaminated from lead-glazed ceramic ware, which releases large amounts of lead into food, especially in an acid environment, such as fruit juice. Canned food may contain lead that has been released from soldered joints in food containers. Several cases of cadmium poisoning have occurred because of the ingestion of food and drink into which this metal had dissolved from cadmium-plated articles (e.g., ice trays, juice makers, roasters). Toxic metals, such as cadmium and arsenic, have been found in high amounts in seafood in some areas. Preventing poisoning from such foods requires systematically organized analyses of their metal contents and the regular reporting of results to the public. Ceramic ware should be tested for lead and cadmium release during acid treatment before being allowed onto the market (Galvez *et al.,* 2004).

The consumption of illicit whiskey, which is often distilled in discarded car radiators or lead-soldered tubing, causes many cases of severe poisoning each year in the United States (Kaufmann *et al.,* 2003). The household use of mercury in religious Santeria rituals among some Latin American persons has produced high levels of mercury in the blood of these persons and their family members (EPA, 2003; Tarabar and Su, 2003). Folk medicines imported from developing to developed nations may be another source of lead poisoning that may come to be recognized only after extensive investigation. (Markowitz *et al.,* 1994; Silva, 2005) Effective consumer information seems to be the only method for preventing such poisoning.

4.4 Environmental Monitoring

The need for monitoring metal concentrations in food and drinking water, whenever there is reason to suspect excessive amounts, is critically important. In

addition, air and water monitoring help ensure that regulations are being met. In principle, two types of sampling procedures should be used. First, metal concentrations should be measured at representative sites, so that compliance with environmental standards can be ensured. Second, emissions from particular point pollution sources should be checked, so that individual polluters will comply with emission standards.

In public health, few regular monitoring programs for metals have existed until recently. Hence, most experience in the general environment derived from specific campaigns, such as lead screening programs in the United States, and studies of fish consumers in Sweden and Japan, to assess their exposure to mercury. However, in a number of countries in recent years, national health surveys have provided important new information on metal levels in the human body and in the diet. Data from these surveys have become an invaluable source of information about metals in the environment and their impact. For example, in the United States, the National Health and Nutrition Examination Survey (NHANES), a program of the Centers for Disease Control and Prevention, has been collecting data on various metal levels in adults and children for the past 30 years. These data helped guide and shape major public health programs related to these metals. Currently, NHANES samples blood levels for 13 different heavy metals, including lead, mercury, cadmium, and uranium, and reports the levels of these in ongoing reports through the National Center for Environmental Health (NCEH, 2005).

4.5 Public Education

Communities in the vicinity of toxic industrial facilities or hazardous waste disposal sites have a right to be informed of the nature of the hazards and of releases to the environment. In the United States, this right to know is protected by law, and the necessary information is available on the web site (*www.scorecard.org*). In Europe, the Aarhus Convention states that everybody has the right to access information on the quality of the environment in which they live (*www.unece.org/env/pp/*).

To protect the public against metal contamination of food, dietary advisories may be appropriate, such as those published by state health departments in the United States in regard to mercury in fish. Similarly, warnings are sometimes released by regulatory agencies (e.g., when contamination of food, toys, or consumer products has been discovered). Government agencies issuing such advisories have a special obligation to ensure that these are properly communicated to the affected communities in language that is understandable and culturally acceptable to the community.

Workers who live in communities surrounding metals plants are doubly exposed to these metals in their homes, along with their families, when plant emissions enter the community air, soil, and water. Thus, it is doubly important that they be aware of and avoid metal contamination in their food and exposures from consumer products. They urgently need to be aware of dietary metals health advisories and consumer warnings released by regulatory agencies.

4.6 Regulatory Authority

The possibilities for the accidental intake of toxic metals are almost unlimited. For example, although the rate of lead poisoning deaths has dropped sharply in the United States in recent decades, there were still an estimated 200 such deaths in the United States between 1979 and 1998, many of them caused by the ingestion of moonshine alcohol (Kaufmann *et al.*, 2003). Accidentally ingested lead shot has also been reported to cause lead poisoning (Gustavsson and Gerhardsson, 2005). There is also evidence of long-term mortality effects from instances of childhood lead poisoning (McDonald and Potter, 1996). When mercury thermometers are broken indoors, especially in bedrooms, hazardous mercury concentrations may result, unless the spilled droplets are meticulously removed. The preventive action lies in banning the use of toxic metals both as components and as paints, in such consumer goods as toys and ceramics, as well as requiring consumer warnings for products in which metal contamination cannot be avoided. In some communities, special lead abatement task forces, with both enhanced legislative and judicial powers, have been effective in reducing childhood residential exposures (Campbell *et al.*, 2005).

In some countries, campaigns specifically aiming to increase awareness in the general population not to spread trash have been initiated (e.g., to collect thermal thermometers with mercury, not to put Cd batteries in trash).

5 PERSPECTIVES ON PRECAUTION AND PREVENTION

5.1 Populations at Risk

Identification of populations at high risk of exposure is of paramount significance for the prevention of metal toxicity in the general population. Some of these groups have already been noted in this chapter (i.e., populations living around smelters and other emitters [all toxic metals], inner-city children living in deteriorated housing [lead], consumers of illicit

whiskey [lead], heavy fish consumers [mercury], dwellers in districts with lead-plumbing systems [lead], users of primitive ceramic ware [lead], children drinking well water in Bangladesh [arsenic], and children living near highways in Australia [manganese]). From time to time new hazards are recognized—for example, the recent recognition in the United States of the lead hazards that result from use of imported medications containing lead and mercury among Asian immigrants (Markowitz *et al*., 1994).

Special attention must always be directed to determining whether young children or women of childbearing age are at risk of exposure. In the United States, in certain states where there are large populations of young children at risk of exposure to lead-based paint and to the dust released from this paint, mandatory blood lead screening of all children is required at 12 months and 24 months of age.

5.2 Widening Implications of Subclinical Toxicity

If a metal is in wide use and has become extensively dispersed in the environment, the subclinical toxicity that it causes can be equally widespread and can affect the well-being and the security of entire societies. Moreover, such widespread subclinical toxicity can be extremely costly. Such population-wide toxicity occurred in nations around the world in the 20th century as a consequence of the nearly universal use of tetraethyl lead as an additive in motor fuel.

Between 1950 and 1975, approximately 100 million children in the United States and hundreds of millions more worldwide were exposed to lead emitted to the environment by the burning of leaded gasoline. This widespread exposure produced a mean national blood lead level in US children in the 1970s of nearly 200 μg/L (20 μg/dL) (today the mean is less than 20 μg/L [2 μg/dL]) and comparable elevations in children of many other nations. This widespread elevation of blood lead levels was associated with loss of intelligence that extended across entire populations and resulted in a 50% decline in the number of truly gifted children (IQ scores >130) and a corresponding increase in the number of children with mental retardation (IQ <70). This widespread loss of intelligence caused lifelong reduction in economic productivity in the affected birth cohorts, which, in the United States, is estimated to have cost between $110 billion and $319 billion (USD) in each annual birth cohort (Grosse *et al.*, 2002). The costs worldwide are many times that sum. Even today, continuing elevations of blood lead levels in many thousands of American children are estimated to cost $43 billion in each annual US birth cohort (Landrigan *et al.*, 2002). Decreased lifetime economic productivity, a consequence of the diminution in intelligence caused by lead, is the principal contributor to these great economic costs attributable to metal toxicity.

Widespread neurotoxicity could result again, if the organic manganese compound MMT (methylcyclopentadienyl manganese tricarbonyl) is allowed to be used extensively as a fuel additive (Weiss, 2005). Manganese is a known cause of parkinsonism in occupationally exposed adults (Hunter, 1969; Lucchini, 2005). Lower dose exposures to manganese have been associated with developmental neurotoxicity (Mergler *et al.*, 1999; Normandine *et al.*, 2004; Wasserman *et al.*, 2004). The potential consequences of population-wide exposure to manganese emitted to the environment from the combustion of MMT in motor fuels are poorly understood. Information is especially lacking on potential consequences of exposures to pregnant women and young children.

5.3 Precautionary Approaches

The history of metal toxicity provides a wealth of examples, in which opportunities for prevention were missed, despite early warnings (Hernberg, 2000). More than 300 years ago, a German state enacted capital punishment for adulteration of wine with lead, and the use of lead for water pipes was subsequently outlawed (Eisinger, 1982). Nonetheless, these uses continued elsewhere in the world and caused countless cases of lead poisoning. In the early 20th century, the introduction of tetraethyl lead as an octane booster of gasoline happened, despite early warnings of neurotoxicity that were disregarded (Needleman, 2000; Rosner and Markowitz, 1985). Although preventive measures are often initiated long after ample documentation of toxicity has been accumulated, long experience with metal toxicity would suggest that more stringent measures ought to be considered earlier. Typically, early studies of the health effects of metals document the existence of toxicity, whereas later research shows that metals possess toxic properties at low exposure levels that had previously been considered "safe" and that toxicity is more widespread than had originally been imagined. In the future, it would be prudent to compress this cycle and to intervene on the basis of early warnings (Harremoes *et al*., 2002).

To prevent recurrence of such disasters as the addition of tetraethyl lead to gasoline, the precautionary principle has recently been formally introduced into national and international law (Foster *et al.*, 2000; Grandjean, 2004). The key element of the precautionary principle is that it provides justification for acting in the face of uncertainty. It is a tool for acting on

the basis of early warnings to avoid possible future harm associated with suspected, but not yet conclusively proven, environmental risks. With the precautionary principle, the burden of proof is shifted from demonstrating the presence of risk to demonstrating the absence of risk (www.sehn.org/precaution.html). Scientific uncertainty should not be an excuse invoked to limit preventive efforts, and debates over minute details in risk assessments should not result in delayed action to protect the public health and the environment.

A recent application of the precautionary principle is to be found in the Declaration of Brescia on Prevention of the Neurotoxicity of Metals (Landrigan *et al*., 2006). This Declaration, which was developed under the auspices of the International Commission on Occupational Health (ICOH) states that intensified attention must be paid to early warnings of neurotoxicity. It calls for an immediate halt in all nations to the addition of manganese compounds to gasoline. This call is based on published data on the developmental neurotoxicity of manganese (Mergler *et al*., 1999; Wasserman *et al*., 2004). The call was specifically intended to prevent a recurrence of the worldwide tragedy of tetraethyl lead. The Declaration of Brescia is reproduced in full at the conclusion of this chapter.

The tools for preventive action outlined in this chapter will still be relevant in the future, and we should be prepared to use these tools vigorously and proactively. Although upper limits of exposure to some metals have unfortunately been set too high and later reduced, because of recognition of adverse health effects at the levels previously considered safe, the precautionary principle would suggest that the limits should initially be set well below the threshold for disease and later raised as safe experience with the exposure is documented in the literature. Although some would argue that such a practice would make most new metallurgical processes economically unfeasible, one cannot measure all of the potential negative human and economic consequences of an unmitigated overexposure in a large population.

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THE DECLARATION OF BRESCIA ON PREVENTION OF THE NEUROTOXICITY OF METALS

Brescia, Italia

17–18 June 2006

On 17–18 June 2006, the Scientific Committee on Neurotoxicology and Psychophysiology and the Scientific Committee on the Toxicology of Metals of the International Commission on Occupational Health (ICOH) convened an International Workshop on *Neurotoxic Metals: Lead, Mercury and Manganese—From Research to Prevention (NTOXMET)* at the University of Brescia. Scientists and physicians from 27 nations participated.

Data were presented for each of the three metals on environmental sources, fate, and distribution; human exposure; clinical, subclinical, and developmental neurotoxicity; epidemiology; risk assessment; and prospects for prevention. Ongoing and future studies were described and discussed.

For each of the metals, initial recognition of neurotoxicity occurred in the context of high-dose exposure. For example, lead poisoning was first recognized in miners, smelters, and typesetters, methylmercury poisoning in inhabitants of the fishing community of Minamata, and manganese poisoning in miners and ferroalloy workers. Subsequent development of more sensitive and sophisticated analytical instruments led to the recognition of subclinical toxicity and developmental neurotoxicity at progressively lower levels of exposure. In each case the extent of toxicity was much greater than initially appreciated and the size of the affected population much larger. Many decades typically elapsed between the initial recognition of neurotoxicity and the initiation of programs for prevention. Early warnings were frequently ignored and even actively resisted.

The historical observation that long delays had typically elapsed before the initiation of prevention prompted extensive discussion at the Workshop about the need to develop more effective strategies. From this discussion, a series of recommendations emerged on future directions for research and prevention of the neurotoxicity of metals.

At the closing session of the International Workshop at Brescia, the following recommendations on the Prevention of the Neurotoxicity of Metals were adopted by consensus:

1. *Intensified attention must be paid to early warnings of neurotoxicity*. Clinical observations or toxicological data suggesting the existence of neurotoxicity—including subclinical and developmental toxicity—must be taken very seriously. Such observations should prompt consideration of prudent preventive action.
2. *All uses of lead including recycling should be reviewed in all nations* and uses contributing to environmental and human exposures, such as uses in toys, paint, water pipes, building materials, solder, electronics, medications, and cosmetics ended. The transfer of these products from one country to another should also be avoided. This approach has been adopted successfully in the EU and needs to be extended worldwide.
3. In particular, *tetraalkyllead must be eliminated without delay from the gasoline supplies of all nations*. The removal of organic lead from gasoline has produced declines of >90% in population mean blood lead levels in industrially developed nations, and this success is now being repeated in some of the developing nations. This action represents one of the great

public health triumphs of the late 20th century and needs urgently to be extended to all nations.

4. *Current exposure standards for lead need urgently to be reduced*. Current standards were established many years ago and do not reflect recent advances in scientific knowledge about toxic effects at levels of exposure below these standards. The Brescia Workshop recommends that:
   - *For children*, the action level, which triggers community prevention efforts to reduce exposure sources, should be immediately reduced to a blood lead concentration of 50 µg/L in nations worldwide. This level is proposed as a temporary level that may need to be revised further downward in future years as new evidence accumulates on toxicity at still lower blood lead levels. This reduction of the blood lead action level will reduce the incidence of subclinical neurotoxicity in children as well as the delayed consequences of developmental toxicity.
   - *For industrial workers*, the standard for lead in blood should be reduced immediately to 300 µg/L in nations worldwide. Additional consideration should be given to further reducing this standard to 200 µg/L and below in the years ahead. This reduction in exposure standard will reduce the incidence of subclinical neurotoxicity and other toxic effects during the working life and responds to new documentation presented at the Workshop that long-term lead exposure increases the risk of dementia in later life.
   - *For female industrial workers of reproductive age*, the standard for lead in blood should be reduced immediately in nations worldwide to the lowest obtainable, preferably to 50 µg/L, a level consistent with the recommended blood lead standard for children. Lead passes freely across the placenta from the maternal to the fetal circulation to enter the developing brain where it causes prenatal brain injury. This recommended reduction in maternal lead exposure will reduce the incidence of fetal neurotoxicity in the offspring of women workers.
5. Exposures of pregnant women and women of reproductive age to methylmercury need to be reduced to prevent subclinical fetal neurotoxicity. Evidence is strong that prenatal exposure to methylmercury causes fetal neurotoxicity. Consumption of fish with high mercury concentration by pregnant women is the primary route of exposure. More than 50% of the mercury in fish may be of industrial origin.

   Strategies for reducing mercury exposure recommended by the Brescia Workshop are the following:
   - All industrial uses, recycling processes, and other industrial input of mercury into the environment should be reviewed in all nations, and non-essential uses should be eliminated and releases controlled. This approach has been successfully introduced in the EU and is actively promoted by the United Nations Environmental Program.
   - Mercury emissions from coal-fired power plants need to be curtailed.
   - All chlor alkali plants worldwide should be urgently converted to alternative technologies that are not based on mercury, and mercury stores and wastes must be safely deposited.
   - Gold mining with mercury must be controlled and enforced with safety guidelines, and alternative technology should be promoted.
   - Dietary advisories should be developed as effective, culturally appropriate means to limit childbearing women's consumption of fish contaminated with methylmercury. Taking into account nutrient contents and availability, healthy diets should be recommended with fish and seafood containing minimal levels of contamination.
6. *Exposures of pregnant women and young children to manganese need to be reduced to prevent subclinical neurotoxicity*. Important new data on the neurotoxicity of manganese were presented at Brescia. In adult workers, these data suggest that manganese produces subclinical neurotoxicity at levels of exposure below those that produce parkinsonism. In children, evidence from two recent epidemiological studies suggests that exposure to manganese in early life causes subclinical developmental neurotoxicity.
7. *The addition of organic manganese compounds to gasoline should be halted immediately in all nations*. The data presented at the Brescia Workshop raise grave concerns about the likelihood that addition of manganese to gasoline could cause widespread developmental toxicity similar to that caused by the worldwide addition of tetraalkyllead to gasoline. In light of this information, it would be extremely unwise to add manganese to gasoline.
8. *Exposure standards for manganese need to be reconsidered* .The drinking water standards for manganese in many countries are not based on health concerns, and those that are do not protect against developmental neurotoxicity resulting from exposures *in utero* and in early postnatal life. The current occupational exposure standard may not protect workers against subclinical neurotoxicity. The value for air manganese concentration in inhalable/total dust of 100 µg/m^3

should be adopted to protect the workers from prolonged exposure and consequent long-term effects.

9. *Economic impacts of the neurotoxicity caused by metals must be considered.* The costs of toxicity may be far greater than the costs of pollution control. The major contributor to these costs is damage to the developing central nervous system. Such injury can result in lifelong loss of intelligence and motor capacities, permanent psychological disturbances, and disruption of behavior. These effects can produce reduction of economic productivity, and when this reduction occurs widely across a society, the resulting economic impacts are great. The costs of pollution recur annually in each exposed birth cohort, adults, and elderly while the costs of control are one-time costs.
10. *Need is great for continuing research into the neurotoxicity of metals.* Recent studies of neurotoxicology of each of the metals discussed at the Brescia Workshop inform us that we can anticipate harmful effects of increasingly lower levels of exposure to metals previously considered safe as larger studies using sensitive measures of exposure and outcome, and better statistical techniques are conducted.
    a. For lead, mercury, and manganese, much remains to be learned about the delayed consequences of developmental toxicity and the prolonged exposure to low levels in the adults, as possible causes of neurodegeneration. This research is critical to guide both future research in metals as paradigms of neurotoxic pollutants and targeted programs of prevention.
    b. Prospective cohort studies from birth are needed, parallel to study on adults and elderly with a retrospective assessment of exposure
    c. Neurotoxicological research, including research on developmental neurotoxicology, is needed on metals not considered at the Brescia Workshop—arsenic and aluminum in particular, and on interactions with essential elements, pesticides and persistent organic pollutants.
    d. Research is needed into genetic and other factors that contribute to susceptibility to metal toxicity.
    e. Research is needed into various determinants of the rearing environment, including the social setting, that can modify the exposure indicators to neurotoxic metals and subsequently the magnitude of neurodevelopmental effects.
    f. Research is needed into the potential consequences of global warming for human exposures to neurotoxic metals—especially mercury.

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CHAPTER

17

Aluminum

BENGT SJÖGREN, ANDERS IREGREN, CARL-GUSTAF ELINDER, AND ROBERT A. YOKEL

ABSTRACT

Aluminum is ubiquitous in the environment. Its proportion of the earth's crust is about 8%. Aluminum can be absorbed from the gastrointestinal tract and from the lungs. Excretion is mainly by the kidneys, probably as aluminum citrate. Aluminum is a well-known neurotoxicant. Accumulation in the human body has been related to the presence of aluminum in dialysis fluids and the concomitant intake of aluminum-containing drugs. This accumulation has resulted in dialysis encephalopathy that was often fatal. Neurotoxic effects have been observed in welders with aluminum urine >100 μg/L. The upper reference limit among nonexposed individuals is 16 μg/L. Aluminum has been suggested to be one of several factors contributing to Alzheimer's disease, although this has not been satisfactorily demonstrated.

Occupational exposure to aluminum powder has resulted in pulmonary fibrosis.

Asthma has been associated with the inhalation of aluminum sulfate, aluminum fluoride, potassium aluminum tetrafluoride, and the complex environment in potrooms during aluminum production.

Cancer and coronary heart disease have been observed among aluminum production workers. However, it is unlikely that aluminum per se is responsible for these diseases.

1 PHYSICAL AND CHEMICAL PROPERTIES

Aluminum (Al): Atomic weight, 26.98; atomic number, 13; density, 2.7 g/cm^3; melting point, 660.4°C; boiling point, about 2400°C.

Pure aluminum is a light ductile metal with a density of approximately one third that of iron. Aluminum is a good conductor of both heat and electricity. It is easy to weld. These properties make it especially suitable for industrial purposes. When aluminum is exposed to air or water, a thin film of oxide (about 0.01 μm) is formed on the surface, creating a protective coating that is resistant to corrosion. By further forced oxidation, this coating can be made thicker to increase corrosion resistance. Aluminum is used in alloys with copper, zinc, manganese, and magnesium.

Aluminum is the third most abundant element in the earth's crust and constitutes about 8% by mass. In nature, it only exists in very stable combinations with other materials (particularly as silicates and oxides).

The chemical form (chemical speciation) has a great influence on aluminum toxicokinetics and toxicodynamics. As with most metals, soluble forms of aluminum are better absorbed and better able to distribute to target organs of its toxicity. In the absence of associating ligands, aluminum, as the hydroxide, is least soluble at pH 6.2 (Harris *et al.*, 1996). The rate constant for water (~sec^{-1}) and ligand exchange with aluminum are slower than for most metals (Burgess, 1992).

2 METHODS AND PROBLEMS OF ANALYSIS

Because of the ubiquitous distribution of aluminum in nature, contamination may be a serious problem during sampling and sample preparation. In the 1970s, reported "normal" values of aluminum in serum or plasma were around 100 μg/L, which was a reflection of contamination (Versieck and Cornelis, 1980).

In 1994, we reported on a case of aluminosis, an aluminum-induced condition that was first recognized in 1946. Many years after exposure, the patient developed a dementia with motor disturbances. His cerebrospinal fluid aluminum level was found to be 259 µg/L (Sjögren *et al*., 1994). In 1998, he died, and his cerebrospinal fluid contained 7 µg Al/L, which is normal. The first reported high concentration of aluminum was most probably caused by contamination during sampling or storage (Sjögren *et al*., 1999). This case illustrates the importance of avoiding contamination.

Instrumental methods most commonly applied to the determination of aluminum include inductively coupled plasma-atomic emission spectroscopy (ICP-AES) and graphite furnace-atomic absorption spectroscopy (GF-AAS). ICP can also be combined with a mass spectrometer to further increase sensitivity (WHO/IPCS, 1997).

Certified reference materials for aluminum are available in some useful matrices but are generally lacking in mammalian tissue, including brain (Yokel, 2004).

3 PRODUCTION AND USE

In 1825, the Danish chemist Örsted produced minute quantities of aluminum metal by using dilute potassium amalgam to react with anhydrous aluminum chloride. Bauxite is the most commercially important form of weathered igneous minerals that contain aluminum. It is abundant in the earth's crust in large areas of the world. The Bayer process of obtaining aluminum oxide (alumina) from bauxite was invented in the 1880s and remains the most economical means to do so. In 1886, Héroult from France and Hall from the United States, invented, simultaneously and independently, an electrolytic process for aluminum production. The Hall–Héroult process is still the basis for aluminum production. They discovered that molten aluminum was produced when an electric current was passed through a bath of molten cryolite (Na_3AlF_6) with dissolved alumina. The presence of cryolite in the Hall–Héroult process enables electrolysis to proceed at a temperature <1000°C, lower than the melting point of alumina (Abramson *et al*., 1989). A modern aluminum smelter contains a series of reduction cells or a potline. There are two types of pots: prebaked and Söderberg. In prebaked pots, anodes are prepared in a carbon plant from calcined petroleum pitch and coal pitch. This mixture is baked in solid blocks and subsequently attached to conductive rods and inserted into the pots. In the Söderberg process, the carbon paste is dropped into a steel casing hanging above the pot and baked *in situ*.

Enormous quantities of energy are necessary for the electrolytic reduction of aluminum, almost 16,500 kWh per ton. Because of this fact, aluminum plants are usually located close to relatively cheap power sources (e.g., in the vicinity of hydroelectric power plants). In the beginning, aluminum production was very modest, but it increased rapidly in the 20th century, especially after the Second World War. Annual aluminum production in the beginning of the 21st century was 20–22 million tons (*www.world-aluminium.org*). The metal and its alloys are used in airplanes, trains, trucks, cars, in the construction industry, and in food preparation and storage. Aluminum powders are used in paints, explosives, and fireworks.

The adjuvant effect of aluminum compounds was first described by Glenny and coworkers (1926). They investigated the immunogenicity of various diphtheria toxoid precipitates, including those precipitated by addition of potassium alum ($KAl(SO_4)_2.12H_2O$). Alum precipitates led to a significant increase in the toxoid-induced immune response. Vaccines prepared by this method are referred to as alum-precipitated vaccines. Another procedure is antigen adsorption onto preformed aluminum hydroxide hydrated gels. Such gels can be preformed in a standardized way. They adsorb protein antigens from an aqueous solution. These vaccines are called aluminum-adsorbed vaccines. Aluminum hydroxide ($Al(OH)_3$) and aluminum phosphate ($AlPO_4$) are now the most commonly used adjuvants and have almost completely replaced the alum precipitation method in vaccine preparation (Lindblad, 2004). Their safety has been questioned by some, but alternatives to replace aluminum as adjuvants are not likely soon (Baylor *et al*., 2002).

Some aluminum-containing drugs, such as hydroxide and sucralfate, have been used as antacids and phosphate binders (Yokel and McNamara, 2001). Alum is used to treat urinary bladder hemorrhage. Aluminum salts are used in antiperspirants; styptic pencils; in products for dermatitis, diaper rash and prickly heat, insect stings, and bites and for athlete's foot (tinea pedis); in antidiarrheal products; in vaginal douches; and as a keratolytic in anorectal preparations (Allen *et al*., 2000).

Aluminum silicate functions as an abrasive, anticaking agent, bulking agent, and opacifying agent in cosmetics. Magnesium aluminum silicate is also used as a viscosity-increasing agent in aqueous cosmetics. According to the FDA, it was used in 629 formulations in 1998 (Elmore, 2003). The use of aluminum-containing food additives is approved by many governments. These are major contributors to dietary aluminum intake.

Substantial amounts of aluminum salts are commonly used as flocculents during the treatment of drinking water.

4 DIETARY, ENVIRONMENTAL, AND OCCUPATIONAL EXPOSURE

The primary normal source of aluminum for the human is food. Daily aluminum intake averages 5–10 mg. The aluminum content of food is highly variable. The average aluminum content of spices and herbs ranged from less than 0.005 mg/100 g for salt to 200 mg/100 g for thyme. Although the quantities of spices and herbs used in foods is generally very small, a diet high in spicy foods may be high in aluminum (e.g., a teaspoon of cayenne powder could contribute about 4 mg of aluminum). Baking powder containing aluminum additives was extremely high in aluminum, providing more than 2 g of aluminum per 100 g. The most commonly used foods that may contain substantial amounts of aluminum-containing food additives except baking powder are processed cheese, cake mixes, frozen dough, pancake mixes, and pickled vegetables (Saiyed and Yokel, 2005). Concentrations of aluminum in tea leaves and tea powder can be quite high, up to 140 mg/100 g. Based on these values, a cup of tea could contain 1 mg of aluminum (Pennington, 1987). Tea and aluminum utensils were estimated to increase aluminum in the diet by approximately 4 and 2 mg per day, respectively (Jorhem and Haegglund, 1992).

Beverages from aluminum cans contained generally higher levels of aluminum than beverages from glass bottles. Noncola and cola soft drinks averaged 900 and 660 µg/L from cans and 150 and 240 µg/L from glass bottles, respectively, whereas beer in cans or bottles averaged about 160 µg/L. The highest concentration in a noncola soft drink can was 10,000 µg/L (Duggan *et al*., 1992).

The contribution of aluminum from drinking water is about 100 µg/day (Yokel and McNamara, 2001).

Air aluminum concentrations vary between 20 and 500 ng/m^3 in rural settings and 1000 and 6000 ng/m^3 in urban settings. Humans exposed to ambient aluminum concentrations of 2000 ng/m^3 and particle size <5 µm and who have a normal ventilatory volume of 20 m^3/day would inhale 40 µg aluminum/day (WHO/IPCS, 1997).

Occupational exposure to aluminum particles reached 100 mg/m^3 during production of stamped aluminum powder in the 1950s (Mitchell *et al*., 1961). However, in the 1990s reported levels were 5–21 mg/m^3 during production of aluminum powder and 1–4 mg/m^3 during production of aluminum paste (Letzel *et al*., 1996). Aluminum welding (Figure 1) produced levels of 0.2–5 mg/m^3 (Sjögren *et al*., 1988). Powder production and aluminum welding are generally associated with the highest occupational aluminum exposure.

FIGURE 1 Metal Inert Gas (MIG) welding of aluminum.

5 METABOLISM

5.1 Absorption

There have been several studies in animals and humans dosed with ^{27}Al using atomic absorption spectroscopic analysis and dosed with ^{26}Al using accelerator mass spectrometric analysis to estimate the percentage of aluminum absorbed from drinking water. The results suggest ~0.3% is orally absorbed (Priest *et al*., 1998; Stauber *et al*., 1999; Yokel *et al*., 2001). On the basis of daily aluminum dietary consumption and urinary aluminum excretion, the oral bioavailability of aluminum from the diet has been estimated to be 0.1–0.3% (Ganrot, 1986; Priest, 1993; Nieboer *et al*., 1995). Bioavailability of ^{26}Al that was incorporated into two FDA-approved food additives, acidic and basic sodium aluminum phosphate, which were then incorporated into a biscuit and a processed cheese, respectively, was ~0.02% from the biscuit and ~0.05% from the cheese (Yokel *et al*., 2005). Several studies demonstrated increased aluminum absorption when taken with citrate. Aluminum hydroxide is still used as an antacid and a phosphate binder. The recommended dose can deliver up to 1.6 g of aluminum per day. Thus, the absorption from this dose may exceed 1 mg/day (Yokel and McNamara, 2001). The site of aluminum absorption is the upper intestine. The mechanisms of gastrointestinal aluminum absorption have been suggested to include passive (diffusion) and active (carrier- and vesicular-mediated) transport across intestinal cells and paracellular diffusion between these cells. There is evidence for an energy-dependent component of uptake. Aluminum absorption is enhanced by processes that mediate calcium uptake. Citrate may enhance aluminum absorption through the paracellular pathway by increasing permeability between cells

(Provan and Yokel, 1988; Whitehead *et al.*, 1997; Zhou *et al.*, 2005).

Aluminum-containing welding fume consists of particles smaller than 1 μm (Ulfvarson, 1981). Aluminum flake powders vary from 5–200 μm in diameter and from 0.05–1 μm in thickness (Ljunggren *et al.*, 1991). Volunteers previously unexposed to welding fume inhaled welding fume for 1 day and excreted 0.1–0.3% of the inhaled aluminum during the following 2 days (Sjögren *et al.*, 1985). Daily urinary aluminum excretion by aluminum welders, who may have been approaching a steady state for their lung aluminum burden, averaged 0.1 mg. Calculated daily aluminum lung deposition was estimated to be 4.2 mg, suggesting base ~2.4% absorption (Sjögren *et al.*, 1997). Results from workers exposed to the air suggest ~0.2–0.5 mg soluble Al/m^3 in ~2% absorption (Pierre *et al.*, 1995). Estimated aluminum absorption was similar in the workers in another study (Gitelman *et al.,* 1995). In an experiment, a solution of aluminum nitrate, containing ^{26}Al, was dispersed to produce an aerosol, which was dried and calcined in a furnace. The produced oxide particles had a mean aerodynamic diameter of 1.2 μm and were inhaled by two subjects. The ^{26}Al levels were determined in urine. Measurements were made at different times after inhalation and had ceased 900 days after exposure. The calculated fraction of the respiratory deposit of aluminum that was transferred to the blood was 1.9% (Priest, 2004). Despite this relatively low absorption, occupational exposure to aluminum-containing particles has resulted in increased aluminum concentrations in urine (Table 1).

TABLE 1 High, Intermediate, and Low Aluminum Exposures Based on Urine Levels in Different Occupations

High aluminum exposure (urine concentrations often >100 μg/L)t
Aluminum powder production (Iregren *et al.*, 2001; Letzel *et al.*, 1996 and 2000)
Welding (Buchta *et al.*, 2005; Kallio *et al.*, 1999; Sjögren *et al.*, 1985)
Cryolite production (Grandjean *et al.*, 1990)
Intermediate aluminum exposure (urine concentrations sometimes >100 μg/L)
Electrolytic production (Röllin *et al.*, 1996)
Corundum production (Valentin *et al.*, 1976)
Low aluminum exposure (urine concentrations seldom >100 μg/L)
Electrolytic production with better technology (Röllin *et al.*, 2001)
Aluminum sulfate production (Sjögren *et al.,* 1983)
Grinding (Elinder and Sjögren, 1990; Harwerth *et al.*, 1987)
Melting and foundry (Elinder and Sjögren, 1990; Sinczuk-Walczak *et al.*, 2003)
Optoelectronic industry (Liao *et al.,* 2004)

Salts of aluminum such as chlorohydrate are extensively used as antiperspirants. The estimated dermal absorption of aluminum is about 0.01% (Flarend *et al.*, 2001). Some results suggest aluminum might be absorbed from the nasal cavity directly into the brain through the olfactory neurons. The extent of absorption by this route is unknown (Yokel, 2000).

5.2 Distribution

The total body burden of the normal human is 30–50 mg. The skeleton contains about 50% and the lungs 25% of the body burden. In the blood, aluminum is approximately equally distributed between plasma and erythrocytes. The brain has lower concentrations than many other tissues (Priest, 2004; Yokel and McNamara, 2001). Increased brain aluminum is seen in patients with dialysis encephalopathy (Alfrey and Froment, 1990). Studies of Alzheimer's disease victims inconsistently show elevated brain aluminum levels (Yokel, 2000). The primary site of brain entry of aluminum is through the blood–brain barrier. It has been suggested that brain aluminum influx is mediated by transferrin receptor–mediated endocytosis of aluminum transferrin (Roskams and Connor, 1990) and transferrin-independent mechanisms influxing aluminum citrate (Yokel, 2001). There is also evidence for carrier-mediated efflux of aluminum from the brain, probably as aluminum citrate (Yokel, 2001).

5.3 Excretion

Urine accounts for more than 95% of aluminum excretion. Reduced renal function markedly increases the risk of aluminum accumulation. Biliary aluminum accounts for less than 2% of the elimination. Chelators can increase aluminum clearance, and citrate seems to act as a chelator (Yokel, 2002). Renal clearance of aluminum may be as the citrate.

After intravenous injection of aluminum citrate, the half-lives for blood, trabecular, and cortical bone were about 1 hour, 1.4, and 29 years, respectively. Thus, a small fraction of the absorbed aluminum could be retained in the body for years (Priest, 2004).

5.4 Biological Monitoring

Accurate measurements of aluminum in human biological fluids such as blood and urine are difficult because of risks for contamination. Aluminum contamination can derive from airborne particles, reagents, glass lab ware, and insufficiently cleaned lab ware that comes into contact with the sample. Plasma aluminum of occupationally nonexposed

humans was most often <5 µg/L (House, 1992). In Finland, the upper reference limit among nonexposed humans was 2.7 µg/L (0.1 µmol/L) in serum and 16 µg/L (0.6 µmol/L) in urine (Kallio *et al.*, 1999; Valkonen and Aitio, 1997). A linear relationship has been observed between the air concentration of aluminum and the urinary concentration at the end of a work shift among electrolytic production workers (Pierre *et al.*, 1995) and welders (Sjögren *et al.*, 1988). In welders, urine aluminum levels are also related to the years of exposure. A welder exposed to aluminum for 20 years had an average urine aluminum of 300 µg/L (Figure 2). The highest aluminum exposures on the basis of urine levels have been reported among welders and aluminum powder production workers (Table 1). Normalization of urinary aluminum clearance to creatinine clearance is usually not done and does not seem to be useful, because aluminum clearance relates to urine output, not creatinine clearance (Alessio *et al.*, 1985; Schlatter and Steinegger, 1992).

Biological exposure limits are established in some countries (e.g., Finland and Germany).

6 EFFECTS

6.1 Gastrointestinal Symptoms

On July 6, 1988, at the Lowermoor water treatment works near Camelford, Cornwall, England, 20 tons of 8% aluminum sulphate solution was inadvertently deposited directly into the chlorine contact tank where chlorine disinfects the water and which is immediately upstream of the treated water reservoir from which water is distributed to consumers, rather than into the storage tank. The domestic water supplies of an estimated 20,000 people in the area were affected. The pH plunged to as low as 3, and the aluminum rose as high as 109 mg/L at one location. Flushing of the water system led to the death of thousands of fish in the rivers (*www.advisorybodies.doh.gov.uk/cotnonfood/lowermoor.htm*). Two patients, who had acute symptoms including mouth and nasal ulcers, still felt unwell 6–7 months later. Bone biopsy specimens showed aluminum staining (Eastwood *et al.*, 1990). The first Lowermoor Incident Health Advisory Group report, published in July 1989, concluded that: "Early symptoms, which were mostly gastrointestinal disturbances, rashes and mouth

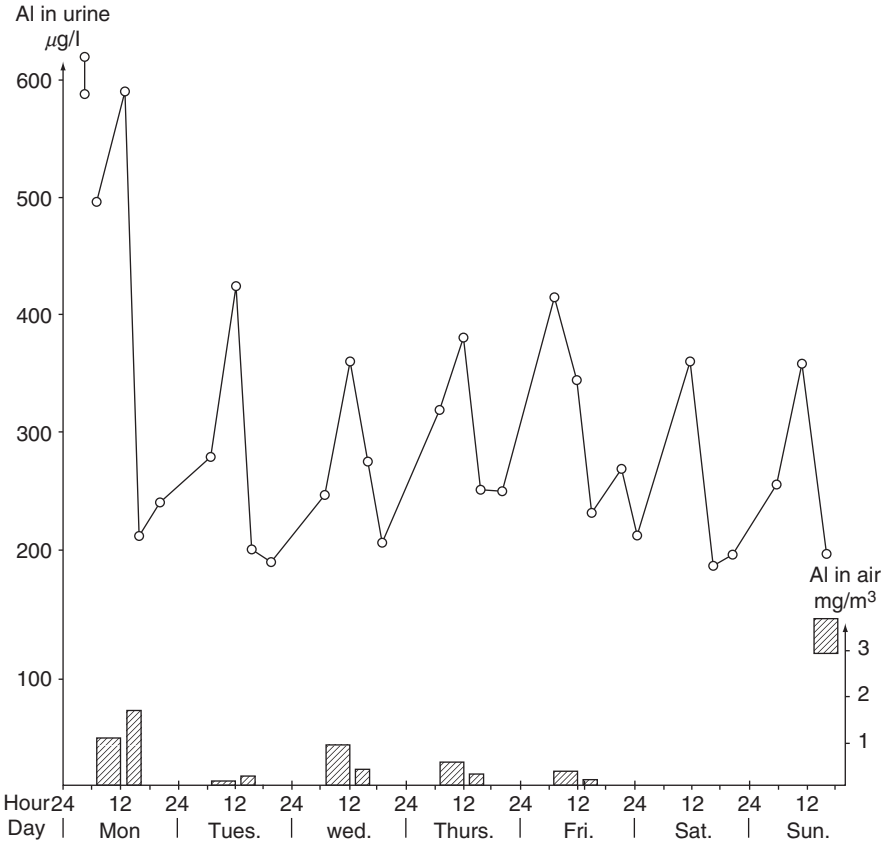

FIGURE 2 Concentrations of aluminum in air and urine in a welder exposed to aluminum-welding fumes for 20 years.

ulcers, can most probably be attributed to the incident. It would appear that symptoms were mostly mild and short lived, as general practitioners experienced no increase in consultation rates at the time and in the subsequent month. A substantial number of residents and holiday makers are known to have complained later of continuing or new symptoms after the incident. These symptoms have included joint and muscle pains, memory loss, hypersensitivity and gastrointestinal disorders. We consider it unlikely in the extreme that long-term effects from copper, sulphate, zinc or lead would result from exposures of the degree and short duration that occurred after this incident. Although the possibility of effects because of the interaction of these chemicals cannot be wholly excluded, we can find no supportive evidence. Increased absorption of aluminum may have occurred in some individuals who persisted in drinking the heavily contaminated water. However, all the available evidence suggests that such increases would have been transient, with most of the aluminum being excreted rapidly and only trace amounts being deposited in tissue, chiefly bone. All the known toxic effects of aluminum are associated with chronically elevated exposure and we have concluded therefore that delayed or persistent effects following such brief exposures are unlikely. In our view it is not possible to attribute the toxic effects of the incident except insofar as they are a consequence of the sustained anxiety naturally felt by many people." In 2005, a subgroup basically agreed with these previous conclusions but recommended further study of those who were exposed bottle-fed infants at the time of the incident (i.e., <1 year of age) (*www.advisorybodies.doh.gov.uk/cotnonfood/lsgreportjan05.pdf*). A summary of the subgroup's report and some dissenting opinions have been published (Khanna, 2005).

6.2 Restrictive Pulmonary Disease

Exposure to aluminum-containing minerals such as bauxite and corundum is often accompanied by silica. This combined exposure may lead to the development of fibrosis of the lung, Shaver's disease (Shaver and Riddell, 1947). The ability of industrial exposure to aluminum, which did not contain silica, to cause pulmonary fibrosis is somewhat contradictory.

Cases of severe lung fibrosis caused by aluminum exposure were reported from Germany during the 1930s and 1940s (Doese, 1938; Koelsch, 1942; Meyer and Kasper, 1942), from Sweden (Ahlmark *et al*., 1960; Swensson *et al*., 1962), and from England (Jordan, 1961; McLaughlin *et al*., 1962; Mitchell *et al*., 1961). Similar cases have rarely been reported from North America (Gross *et al*., 1973). German, Swedish, and British occupational health studies have shown that stamped aluminum powder, even in the absence of silica, causes lung fibrosis. Stamped aluminum powder is produced by crushing and grinding hard unmelted aluminum metal. This aluminum powder is mainly used in the manufacture of pyrotechnical products but also in the production of some aluminum metal dyes. Ninety-five percent of stamped aluminum powder particles are smaller than 5 μm. Stamped aluminum powder has a rather large surface area because of the flake-like form of the particles. The powder reacts with water to form hydrogen and aluminum hydroxide (Corrin, 1963). Granular aluminum particles, which are manufactured from melted aluminum, display a more regular particle structure and are not as reactive as stamped aluminum powder (Corrin, 1963). Different types of lubricating agents are used in the production of the powder, generally stearin or mineral oils. Cases of lung fibrosis were first reported from industries using mineral oil. Cases of the disease have, however, been reported from at least one industry where only stearin was used (McLaughlin *et al*., 1962).

The first signs of lung fibrosis caused by stamped aluminum powder exposure were respiratory problems, occasionally in conjunction with coughing. X-ray of the lungs revealed fibrosis accompanied by atrophy and secondary emphysema (Edling, 1961). Pneumothorax was a typical complication. The fibrosis, also known as aluminosis, could progress rapidly and in severe cases lead to death within a few years after onset (Ahlmark *et al*., 1960; Swensson *et al*., 1962). No clear LOAEL could be estimated, because cases of fibrosis occurred after exposure to 4–50 mg aluminum/m^3 (Elinder and Sjögren, 1986).

Since the beginning of the 1990s, a few new cases of fibrosis from the aluminum powder industry in Germany were identified and compensated (Kraus *et al*., 2000). A 40-year-old worker was employed as a stamper for 14 years in a plant producing aluminum powder. He was exposed to high levels of aluminum and had a urine concentration of 400 μg aluminum per liter. He also had signs of pulmonary fibrosis on high-resolution computed tomography and a vital capacity of 58% of expected. Between 1960 and 1989, only a few individuals were compensated because of lung disease induced by aluminum dust in Germany (Kraus *et al*., 2000). Thus, it seems that this type of fibrosis is not yet eradicated. Aluminum powder has also been associated with a case of sarcoid-like lung granulomatosis (De Vuyst *et al*., 1987).

In North America, exposure to McIntyre powder, consisting of finely ground aluminum and aluminum oxides, has been used as a prophylactic (Denny *et al*., 1939; Godin, 1955) and in the treatment of silicosis (Crombie *et al*., 1944). A comprehensive evaluation of the studies that used inhalation of aluminum particles in the treatment of silicosis has, however, resulted in total rejection of this form of treatment (Kennedy,

1956). The British Medical Council concurred with this evaluation and did not recommend the use of aluminum powder in the treatment or prevention of silicosis. Despite this recommendation, aluminum powder was used as a prophylactic agent until 1979 in the mines in northern Ontario, Canada (Rifat *et al.,* 1990).

Grinding and polishing aluminum have sometimes been associated with an extremely dusty work environment. One case of pulmonary fibrosis (De Vuyst *et al*., 1986) and one case of alveolar proteinosis (Miller *et al*., 1984) have been reported after long-term exposure.

In a factory producing aluminum oxide abrasives employing about 1000 workers, nine workers had abnormal chest X-rays. The mean exposure duration was 25 years. In each of three lung biopsies, interstitial fibrosis with honeycombing was found with an absence of asbestos bodies and silicotic nodules. The authors concluded that aluminum oxide dust was the most likely cause, although mixed dust exposure may explain the findings (Jederlinic *et al*., 1990).

Long-term exposure to aluminum welding fumes in three case reports has been associated with chronic interstitial pneumonia (Herbert *et al*., 1982), pulmonary granulomas (Chen *et al*., 1978) and pulmonary fibrosis (Vallyathan *et al*., 1982). However, in a group of 64 aluminum-exposed welders with a duration of exposure between 1 and 24 years, no signs of pulmonary fibrosis were observed (Sjögren and Ulfvarson, 1985).

Aluminum-containing fibers have been detected in lung parenchyma and bronchial alveolar lavage in aluminum smelter workers despite a nonexposure period of several years (Voisin, 1996). Small opacities or accentuations of the bronchopulmonary markings on chest X-ray were more common (29%) among 119 potroom workers exposed for more than 10 years from two plants in northern Italy compared with 15% of a similar sized referent group made up of unexposed clerical and maintenance workers from the same factories. Signs of pneumoconiosis were more common among long-term than short-term exposed workers (Saia *et al*., 1981).

In a cross-sectional study, 670 aluminum workers from Alabama were compared with 659 pipe fitters. Both groups were asbestos exposed. Irregular opacities observed on X-rays were more common among aluminum workers (21% vs. 15%) and pleural abnormalities less common (2% vs. 12%) than the referents. These findings suggest an influence of particles or fibers other than asbestos (Kilburn and Warshaw, 1992). Some cases of pulmonary fibrosis have also been reported after long-term exposure during electrolytic aluminum production (Akira, 1995; Al-Masalkhi and Walton, 1994; Gaffuri et al., 1985; Gilks and Churg, 1987).

To conclude, exposure to high levels of small particles of aluminum powder has caused pulmonary fibrosis, also called aluminosis. Sometimes a mixed dust exposure can explain pulmonary fibrosis among exposed workers. It is not clear whether these workers are very sensitive or susceptible regarding exposure to particles or belong to a small fraction of heavily exposed workers. Aluminum-containing fibers have been detected in lung parenchyma and bronchial alveolar lavage fluid in aluminum smelter workers; however, it is not clear whether these fibers play a causal role in the development of pulmonary fibrosis.

6.3 Obstructive Pulmonary Disease

In 1936, asthma was described among Norwegian workers in electrolytic aluminum production (Frostad, 1936). This is often called potroom asthma. Symptoms of asthma, airflow limitation, and increased bronchial responsiveness have been described from aluminum smelters around the world. The likely causes are irritant airborne particulates and fumes contributed by cryolite, gaseous hydrogen fluoride, and other agents that may be adsorbed onto aluminum (WHO/IPCS, 1997). In a cross-sectional study of 1760 Norwegian potroom workers, 11% reported work-related asthmatic symptoms (Kongerud *et al*., 1990). A relationship between the levels of fluoride exposure and work-related asthmatic symptoms was also observed among such workers (Kongerud and Samuelsen, 1991). A cross-sectional study comprised 1615 male employees of two Australian aluminum smelters. After adjusting for smoking and age, workers with a cumulative exposure for fluoride >0.16 mg/m^3-years or inspirable dust >2.9 mg/m^3-years were two to four times more likely to report work-related wheeze and chest tightness than were unexposed subjects. The effect of these two exposures could not be separated, because there was only one subject with work-related chest tightness and no subjects with work-related wheeze who were exposed to fluoride without being exposed to inspirable dust (Fritschi *et al*., 2003).

Production of aluminum fluoride and aluminum sulfate has been associated with reversible bronchial obstruction or asthma (Simonson *et al.,* 1985). In 1975 and 1976, six and seven cases of asthma occurred, respectively, in a Swedish plant producing aluminum fluoride. The number of exposed workers was 35–40, and the mean concentrations of aluminum fluoride during these two years were 3–6 mg/m^3. In 1977, environmental improvements were made, and the exposure was reduced to 0.4–1.0 mg/m^3. During the years 1978–1982, only two cases of asthma occurred (Simonson *et al*., 1985).

During 1971–1980, an average of 37 workers produced aluminum sulfate in a Swedish plant. Four workers had short-lasting asthma develop, mainly in

connection with heavy dust exposure during rinsing and repair work. The mean aluminum sulfate concentrations varied between 0.2 and 4 mg/m^3 (Simonsson *et al*., 1985).

Potassium aluminum tetrafluoride is sometimes used as a flux for aluminum soldering. Exposure to this flux has been associated with asthma and bronchial hyperreactivity (Hjortsberg *et al*., 1986 and 1994).

One case of asthma related to aluminum welding has been reported. Challenge exposure to aluminum welding with flux-coated electrodes and electrodes without flux elicited marked asthmatic reactions. However, welding on mild steel did not cause a significant bronchial response (Vandenplas *et al*., 1998). Exposure to aluminum powder has been observed to cause asthma in a worker after mixing aluminum and minimal amounts of cement powders to make safes in a closed room. He reacted with a decrease of FEV_1 after inhalation of aluminum powder or aluminum chloride but not after inhalation of lactose powder (Park *et al.*, 1996). A 46-year-old smoker had cough and chest tightness starting 3 h after coming to work. He had worked as a caster of molten aluminum for 19 years. The challenge test showed a dual reaction to aluminum chloride with a 28% immediate fall in FEV_1 and a 34% late fall in FEV_1 after 7 hours (Burge *et al*., 2000).

A historical cohort study comprised 5627 men who had worked in two Norwegian aluminum plants. An increased number of deaths from chronic obstructive lung diseases (bronchitis, emphysema, and asthma) was observed, which related to fluoride exposure (Romundstad *et al*., 2000b).

In conclusion, potroom asthma is a well-known disease associated with the complex exposure in the potrooms of the aluminum electrolytic production industry. Exposure to several other aluminum salts such as aluminum fluoride, aluminum sulfate, and potassium aluminum tetrafluoride has also been associated with asthma. In some case reports, aluminum welding fume, foundry fume, and aluminum powder were also linked to asthma.

6.4 Central Nervous System

6.4.1 Dialysis Encephalopathy

Dialysis encephalopathy is a complication of prolonged hemodialysis first described in 1972 (Alfrey *et al*., 1976). The main symptoms are speech disorder followed by the development of dementia, convulsions, and myoclonus. The mean duration of dialysis was 48 months. The dialysis fluids were made from untreated tap water (WHO/IPCS, 1997). Death occurred within 12 months of symptom onset, and recovery was exceptional (Bugiani and Ghetti, 1990). Between 1968 and 1976, 55 patients with dialysis encephalopathy were identified from six dialysis centers in the United States. The overall attack rate of dialysis encephalopathy was 4%. Encephalopathy was the cause of death in most cases (Schreeder *et al*., 1983). Dialysis encephalopathy was related to the presence of aluminum in dialysis fluids and the concomitant intake of aluminum-containing drugs used to decrease serum phosphate levels. Elevated aluminum was found in the brain, muscle, and other tissues of the affected patients. Serum aluminum >80 µg/L has been associated with dialysis encephalopathy (Nieboer *et al*., 1995). Many outbreaks of encephalopathy have been described in association with the use of dialysis fluids containing aluminum levels >200 µg/L (WHO/IPCS, 1997). Today, aluminum exposure of dialysis patients is minimized, because the dialysis fluid water is monitored at most dialysis centers and the aluminum level is kept <10 µg/L. However, accidents happen, causing occasional incidences of aluminum-associated dialysis encephalopathy (de Wolff *et al*., 2002). Thus, overt dialysis encephalopathy is rare, but less obvious intoxications may occur. An aluminum-associated low turnover bone disease and a hypochromic, microcytic anemia can occur at lower aluminum exposures and body burden than those producing encephalopathy.

6.4.2 Other Medical Aluminum Exposures

During the last decades, there were several reports describing aluminum accumulation and toxicity in patients without chronic renal failure. These include preterm infants, largely fed intravenously, patients on total parenteral nutrition, patients with severe burns, patients undergoing cranial bone reconstruction, and patients receiving alum irrigation of their urinary bladder (Flaten *et al*., 1996).

Premature infants with gestational ages of less than 34 weeks were assigned to receive either standard or aluminum-depleted intravenous-feeding solutions. When tested 18 months later, those who received more aluminum exposure had impaired neurological development (Bishop *et al*., 1997). Some patients with severe burns experience reduced bone formation at least partially attributed to aluminum from human serum albumin, calcium gluconate, and other sources (Klein *et al*., 1994). An aluminum-containing cement, with calcium aluminum fluorosilicate, has been used as a bone reconstruction material in neurosurgical operations. In five reported cases, symptoms and signs of encephalopathy developed, including seizures. The concentrations of aluminum in the cerebrospinal fluid were typically >100 µg/L (Renard *et al*., 1994). Two of the five patients died of brain failure with persistent

convulsions (Hantson *et al*., 1994) and a third from septic complications (Reusche *et al*., 2001).

Intravesical alum instillation is a treatment for hemorrhagic cystitis. This treatment has resulted in acute aluminum intoxication among patients with some degree of renal insufficiency (Phelps *et al*., 1999).

6.4.3 Neurobehavioral Effects of Occupational Aluminum Exposure

Several studies of welders have been performed in Finland to assess the potential for occupational aluminum exposure to produce adverse neurobehavioral effects. The first, comprising 17 participants, did not use a control group but compared data for subgroups of welders with differing exposures. Dose-effect relationships between aluminum levels in serum and urine and performance on four tests of memory function (symbol learning, memory-for-designs, digit span, and associative learning) were found (Hänninen *et al*., 1994). The mean level of aluminum in urine was 75 µg/L. The second article (Akila *et al*., 1999) reported data from 51 aluminum welders and 28 controls, who were divided into three groups according to the levels of aluminum in urine. The low exposure group had a mean of 12 µg/L, the medium exposed group had 60 µg/L, and the high exposure group had 269 µg/L. Dose-related impaired performance attributed to aluminum was found on the digit symbol, synonyms, embedded figures, memory for designs, and block design tests. The third article from this research team reported on the same worker groups, with the addition of further welders (Riihimäki *et al*., 2000). Welders were assigned to one of three groups according to a combined measure of aluminum in serum and in urine to provide an indication of aluminum body burden. The three groups were composed of 25, 29, and 30 workers. The median urinary aluminum levels were 11, 49, and 192 µg/L, respectively. Significant group differences were found with respect to symptoms of fatigue, memory and concentration, and emotional lability. Six of 18 psychological tests of memory and concentration also showed significant group differences, with a lower performance in the highly exposed groups. Furthermore, there were EEG differences between the exposure groups. A number of subjects in the exposed groups exhibited mild or moderate diffuse or epileptiform abnormalities. To evaluate dose-response relationships, the prevalence of findings in six critical domains (symptoms, visual accuracy, attention, verbal, visuospatial memory, and EEG) was examined. The proportion of deviant findings in these domains was between 15 and 20%. A plot of the number of deviant findings against the measure of aluminum body burden suggested a critical level of aluminum was 110–160 µg/L in urine.

A Swedish study compared 38 aluminum welders with a control group of 39 steel welders (Sjögren *et al*., 1996). The median employment time as aluminum welder was 15 years. Aluminum welders performed less well than the reference group on four tests of motor function: finger tapping, two tasks of the Luria-Nebraska battery, and a pegboard test. For the former three tests, this effect was dose-related and observed in a subgroup of 19 highly exposed welders who had a median urine concentration of 59 µg aluminum/L. In five of the 19 welders, the urine aluminum exceeded 100 µg/L.

A Norwegian study compared a group of 20 aluminum welders with a reference group of construction workers (Bast-Pettersen *et al*., 2000). The welders performed better than the construction workers in several of the tests used. However, when performance of the welders was analyzed separately, significant correlations were found between exposure duration in years and poorer performance on the tremor test. Performance on a test of reaction time was found to be worse for workers exposed to higher levels of aluminum in the air. The median urine aluminum for this group was 41 µg/L, and the range was 19–129 µg/L.

In two German longitudinal studies of aluminum welders' psychometric measurements were performed 2 years apart. One study had 98 aluminum welders from the car industry and 50 nonexposed car production workers (Buchta *et al*., 2003). The median urinary aluminum was 58 µg/L in 1999 and 52 µg/L in 2001, and the median exposure time was 6 years. No significant differences in psychomotor performance or other neurobehavioral tasks, except for reaction time, were observed. A second study compared 44 aluminum welders in the train body and truck trailer industry with 37 nonexposed production workers (Buchta *et al*., 2005). Median urinary aluminum was 130 µg/L in 1999 and 150 µg/L in 2001. Mean exposure time was 11 years. Welders showed significantly poorer performance in symbol-digit substitution, block design, and, to some extent, in switching attention, but not in reaction time. The first study (Buchta *et al*., 2003) revealed a small, but statistically insignificant, increase in the reaction-time difference between the first and second examination. In the second study (Buchta *et al*., 2005), no trend differences compared with the controls were seen. The lack of any differences between the two examinations might be explained by the short period for follow-up. A third examination, which is planned for both studies, may clarify this picture, unless further nonparticipation biases the group comparison.

There are two studies of psychometric performance by workers exposed to aluminum powder. Neither of

the studies observed any differences compared with referents. A German study composed of 32 exposed subjects with a mean aluminum of 110 µg/L urine (Letzel *et al.*, 2000). A Swedish study contained 16 subjects with a mean urine aluminum of 83 µg/L (Iregren *et al.*, 2001). A study of Canadian miners, inhaling aluminum powder as a prophylactic against silicosis, reported a higher proportion of workers with impaired cognitive functions among those being treated for longer periods of time (Rifat *et al.*, 1990). However, neither blood nor urine concentrations of aluminum were measured.

In summary, neurotoxic effects have been observed in welders with urine aluminum >100 µg/L compared with welders with lower levels. The consistency in these observations is probably explained by the fact that the welders were compared with other welders, being similar in most respects except the aluminum exposure. It should be emphasized that it is unknown whether these effects are reversible. Most studies of exposed groups other than welders compare one occupational group with another occupational group. Comparison of workers with different training and occupations may well bias the results, especially when psychological outcome variables are used.

6.4.4 Alzheimer's Disease

Alzheimer's disease (AD) is a progressive deterioration of brain function, initially characterized by cognitive deficits, with loss of recent memory and language ability, impairment of orientation, problem solving, and abstract thinking. The typical neuropathological signs of the disease are neurofibrillary tangles and senile plaques.

A large number of studies have been performed to replicate the first observation that the concentration of aluminum is generally higher in the brains of patients with AD than in other patients at autopsy (Crapper *et al.*, 1973). Some investigations found higher levels of aluminum and some did not (Yokel, 2000).

The results of several epidemiological studies suggest a small increased risk of dementing illnesses, including Alzheimer's disease, for people living in areas of high water aluminum concentrations. However, there are also several reports of no associations.

The inconsistent findings from autopsy and epidemiological studies of water aluminum contribute to the controversy concerning the role of aluminum in the etiology of AD.

Even if the level of aluminum is elevated in the brain in AD, it would not prove a cause–effect relationship, because AD might produce plaques and neurofibrillary tangles that may bind the metal. Thus, aluminum may be one of several factors contributing to AD, although this has not been satisfactorily demonstrated (Gupta *et al.*, 2005; Yokel, 2000).

6.5 Bone

Clinical and experimental research has shown that high doses of aluminum inhibit bone remodeling. Aluminum slows osteoblast and osteoclast activities and produces osteomalacia and an adynamic bone disease (Jeffery *et al.*, 1996).

A survey of 1293 patients from 18 dialysis centers in the United Kingdom showed a correlation between the dialysate aluminum concentration and the incidence of fracturing dialysis osteodystrophy (Parkinson *et al.*, 1979).

Serum aluminum >30 µg/L has been associated with dialysis-associated bone diseases (Nieboer *et al.*, 1995). Bone biopsy is needed for definitive diagnosis (Cronin and Henrich, 2004).

Persons without renal failure who take several grams of aluminum per day as antacids on a long-term basis may have osteomalacia. This is attributable to phosphate imbalance secondary to aluminum intake (Insogna *et al.*, 1980; Woodson, 1998). A marked decrease of gastrointestinal phosphate absorption can be observed after a rather small antacid dose (Spencer *et al.*, 1982).

Thirty-two aluminum powder–producing workers were compared with 29 nonexposed workers from the same factory. Lumbar spine bone density did not differ between the groups. The median preshift urine aluminum of exposed workers was 110 µg/L and the referents 7 µg/L (Schmid *et al.*, 1995).

6.6 Hematopoietic Tissue

Some patients receiving dialysis treatment may develop a hypochromic, microcytic anemia. This anemia is caused by aluminum and will not respond to iron therapy (Jeffery *et al.*, 1996).

6.7 Skin

Applications of 2 g magnesium aluminum silicate to human skin onto a 6 cm^2 (1-inch) area daily for 1 week did not cause any observable effects. Magnesium aluminum silicate was tested on rabbits and was regarded as a weak primary skin irritant (Elmore, 2003).

6.8 Allergic Effects

Allergic contact dermatitis from aluminum is rare. Two routes of sensitization have been described. Aluminum can be administered on the surface of the skin in antiperspirants, medicaments, and the aluminum disc used in routine patch testing (Finn Chamber®), or injected in aluminum-adsorbed vaccines, hyposensitization extracts, and other forms. The risk of sensitization is higher from the latter route (Peters *et al.*, 1998). Cutaneous granulomas after immunization

may take some years to heal or decrease (Kaaber *et al*., 1992). Itching nobulus were found in 645 children out of 76,000 vaccines (0.8%) after both subcutaneous and intramuscular injections. A majority of the children (75%) had symptoms after a month duration of 4 years, and 77% of the children with nodulus showed contact hypersensitivity to aluminum (Bergfors *et al.*, 2003). Sensitization has also occurred after aluminum particles were accidentally impelled into the skin from a compressed air pistol (Peters *et al*., 1998). Tattooing with aluminum silicate to create blepharopigmentation has resulted in a delayed-hypersensitivity granulomatous reaction (Schwarze *et al*., 2000).

6.9 Coronary Heart Disease

Some studies of aluminum production workers have observed an increased risk of coronary heart disease (Rönneberg, 1995; Theriault *et al*., 1988). This effect might be explained by the possible association between exposure to air pollutants and the occurrence of coronary heart disease mediated by an inflammatory response that increases blood coagulation (Sjögren, 2004). It is unlikely that aluminum per se is responsible for this effect, but more likely that it is due to air pollutants in general. Slightly higher levels of fibrinogen were observed among aluminum smelter workers at Söderberg pots exposed to high levels of air pollutants compared with those working with prebaked electrodes and exposed to lower levels of air pollutants (Sjögren *et al*., 2002). A high plasma fibrinogen level is an established risk factor for coronary heart disease (Danesh *et al*., 1998). However, there are studies that do not support the association between potroom exposure and coronary heart disease (Romundstad *et al*., 2000a).

6.10 Carcinogenic Effects

Most animal studies have failed to demonstrate carcinogenicity attributable to aluminum powder or several aluminum compounds (Léonard and Gerber, 1988).

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area classifies aluminum oxide as a category 2 carcinogen because of the unequivocally positive results of intrapleural injections in animals (Deutsche Forschungsgemeinschaft, 2004).

In 1987, the International Agency for Research on Cancer (IARC) concluded that there is sufficient evidence that certain exposures occurring during aluminum production cause cancer of the lung and bladder (Group 1). Pitch volatiles have fairly consistently been suggested in epidemiological studies as possible causative agents (IARC, 1987).

Synthetic abrasive materials containing aluminum oxide, silicon carbide, and different additives have been used for more than 70 years (Wegman and Eisen, 1981). Exposure to these compounds occurs during production and when they are used for metal grinding and polishing. An increased risk for stomach cancer has been observed in two studies (Järvholm *et al*., 1982; Wegman and Eisen, 1981), and an increased risk for lung cancer has been observed in one (Siemiatycki *et al*., 1989). During the past decade, studies observed an increased risk of lung cancer (Infante-Rivard *et al.*, 1994; Romundstad *et al*., 2001) and stomach cancer (Romundstad *et al*., 2001) associated with silicon carbide production. These workers are, however, also exposed to crystalline silica. Thus, further studies are needed to explore whether components in synthetic abrasive materials are carcinogenic.

7 OTHER ALUMINUM COMPOUNDS

Aluminum phosphide is used as a fumigant to protect stored grain from insects and rodents. In the presence of moisture, aluminum phosphide releases phosphine, which is highly toxic. The fatality rate was 59% among 195 patients admitted to a hospital in Northwest India. The fatal dose was 1.5 g, and the predominant clinical feature was hypotension (Singh *et al*., 1996). Two children and 29 of 31 crew members aboard a grain freighter became acutely ill after inhaling phosphine originating from aluminum phosphide. One of the children died (Wilson *et al*., 1980).

8 RECOMMENDATIONS

Aluminum is used as a coagulant in water treatment. According to the WHO, a health-based guideline value cannot be derived; however, practical levels based on optimization of the coagulation process in drinking-water plants using aluminum-based coagulants were derived: 0.1 mg/L or less in large water treatment facilities, and 0.2 mg/L or less in small facilities (WHO, 2004). Similarly, Health Canada established an operational guidance value of <0.1 mg/L for drinking water from treatment plants using aluminum-based coagulants and <0.2 mg/L for other types of treatment systems (*http://www.hc-sc.gc.ca/ewh-semt/pubs/water-eau/doc_sup-appui/aluminum/aluminum-aluminium_e.html#1*).

The occupational exposure limits for aluminum have decreased during the last decade in many countries (Table 2). Occupational biological exposure limits are established in some countries. Finland has a urine biological action level (BAL) of 160 µg/L (6 µmol/L) (Kallio *et al*., 1999). Germany has a biological tolerance value for occupational exposure of 200 µg aluminum/L

TABLE 2 Occupational Exposure Limits for Aluminum in Some Countries Expressed as Time-Weighted Averages (TWA, mg/m^3)

Form of aluminum	Country or organization	TWA	Source
Metal dust	ACGIH	10	ACGIH (2005)
	NIOSH	10	NIOSH (2005)
	Sweden	5	Swedish Work Environment Authority (2005)
Respirable metal dust	Germany	1.5	Deutsche Forschungsgemeinschaft (2006)
	NIOSH	5	NIOSH (2005)
	Sweden	2	Swedish Work Environment Authority (2005)
Pyro powder as Al	ACGIH	5	ACGIH (2005)
	NIOSH	5	NIOSH (2005)
Welding fume as Al	Germany	1.5	Deutsche Forschungsgemeinschaft (2006)
Soluble salts	ACGIH	2	ACGIH (2005)
	NIOSH	2	NIOSH (2005)
	Sweden	1	Swedish Work Environment Authority (2005)

of postshift urine (Deutsche Forschungsgemeinschaft, 2006).

Physicians determine dialysate aluminum levels twice a year. It has been recommended that dialysate aluminum be <10 µg/L (AAMI, 2001) and stated that it must be <5 µg/L but should be <2 µg/L. Serum aluminum should be <20 µg/L (Rob *et al*., 2001).

Aluminum compounds are the only adjuvants used in the manufacture of currently licensed vaccines in the United States. Chapter 21 of the US Code of Federal Regulations governs the amount of aluminum permitted in the recommended single human dose of a product. The amount of aluminum is limited to 0.85 mg/dose if the level is assayed or 1.14 mg if determined by calculation on the basis of the amount of aluminum compound added (Baylor *et al*., 2002).

The daily intravenous intake of more than 4–5 µg aluminum/kg from total parenteral nutrition solutions can produce aluminum accumulation and central nervous system and bone toxicity. To avoid this problem, the US FDA established a labeling requirement that large and small volume additives used in parenteral nutrition must state the maximum aluminum concentration (US FDA, 2002; Canada, 2005).

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CHAPTER

18

Antimony

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ABSTRACT

Antimony is a silvery white brittle metal of medium hardness and exists in four valence states: 0, −3, +3, and +5. Most absorbed antimony is excreted rapidly through urine and feces. Elimination and route of excretion depend on the type of antimony compound. Urinary excretion is higher for pentavalent than for trivalent antimony compounds, whereas the gastrointestinal excretion is higher for trivalent than for pentavalent antimony. Some data on humans, as well as on animals, indicate that a small part of absorbed and retained antimony may have a long biological half-life, especially in the lung. After acute or chronic oral or parenteral exposure to antimony, highest concentrations are found in the thyroid, adrenals, liver, and kidney.

Industrial exposure may give rise to symptoms of irritation in the respiratory tract. Pneumoconiosis, occasionally in combination with obstructive lung changes, has been reported after long-term exposure in humans. Focal fibrosis of the lung has been seen in animal trials. Effects on the heart, even fatal, have been related to long-term industrial exposure to antimony trioxide. Secondary cardiovascular effects as a result of treatment of parasitic disease with antimony compounds have also been reported. In addition, cardiovascular effects have been observed in animal experiments. Rats exposed to antimony trioxide through inhalation for long periods of time showed a high frequency of lung tumors.

1 PHYSICAL AND CHEMICAL PROPERTIES

Antimony (Sb): atomic weight, 121.8; atomic number, 51; density, 6.7; melting point, 631°C; boiling point, 1750°C; crystalline form silvery white metal; oxidation states: 0, −3, + 3, and + 5. Compounds to be referred to in this chapter are antimony trioxide (Sb_2O_3), CAS No. 1309-64-4, antimony trisulfide (Sb_2S_3)', CAS No. 1345-04-6, antimony trichloride ($SbCl_3$), CAS No. 10025-91-9, antimony pentasulfide (Sb_2S_5), antimony potassium tartrate ($C_8H_4K_2O_{12}Sb_2.3H_2O$), CAS No. 28300-74-5, antimony pentoxide (Sb_2O_5), stibine (SbH_3), and sodium antimony dimercaptosuccinate.

Antimony belongs to the same periodic group as arsenic, which it resembles both chemically and physically. Arsenic is, however, much more toxic. Antimony exists in both organic and inorganic complexes. Stibine is an odorless gas. Antimony potassium tartrate, or tartar emetic, has a relatively high solubility in water (83 g/L), whereas most other inorganic antimony compounds have a low solubility.

2 METHODS AND PROBLEMS OF ANALYSIS

The main methods used to analyze antimony in both biological and environmental samples are atomic absorption spectroscopy (AAS) and inductively coupled plasma-atomic emission spectroscopy (ICAP-AES), with or without preconcentration or

separation steps. Instrumental neutron activation is also used but has the disadvantages of being slow, costly, and requiring special facilities (ATSDR, 1992). Alimonti *et al*. (2005) compared two types of inductively coupled plasma mass spectrometry, quadrupole and sector field, in analyzing urinary Sb in the general population. Detection limits were 1.09 and 0.43 ng/L, respectively.

The United States Occupational Safety and Health Administration (OSHA) publishes several fully validated methods for analyzing for antimony (U.S. Department of Labor, 2005). The principal method (OSHA ID-121) uses atomic absorption spectroscopy to analyze antimony in air samples collected on a mixed-cellulose ester membrane contained in a styrene cassette using a calibrated personal sampling pump to filter 480–960 liters of air. Maximum flow rate is 2 L/min. Wipe samples and bulk samples can also be analyzed with this method. The analytical detection limit is 0.1 µg/mL. Advantages to this method are that it is specific for antimony, the equipment is inexpensive, and no special training is required. Disadvantages are that the method does not distinguish different compounds and cannot distinguish different particle sizes. Alternative digestion procedures are used if samples are not digested using the standard protocol. Alternative methods, also fully validated, use ICAP-AES (OSHA ID-125G and OSHA ID206). An advantage to using ICAP-AES is the rapid simultaneous analysis of a number of elements, as opposed to the need for separate analyses that use conventional atomic absorption techniques.

Other methods are available. Antimony in air and biological materials has been determined polarographically, spectrographically, and colorimetrically with rhodamine B (Tabor *et al*., 1970). A spectrophotometric method based on the liberation of iodine from acidified potassium iodide by antimony (III) has a detection limit of 6 µg Sb/L. The method has been used successfully to analyze the antimony content of silicate rocks, natural water, waste water, plant leaves, soil, and biological samples (Tiwari *et al*., 2006). A stibine generation technique, with subsequent AAS, was reported to have a detection limit of about 0.5 µg Sb/L in blood (Bencze, 1981). A method that uses inductively coupled plasma-mass spectrometry after nitric acid/hydrogen peroxide digestion of blood samples has a detection limit of 0.03 µg Sb/L (Bazzi *et al*., 2005). A colorimetric method used for determination of stibine in air had a detection limit of 50 µg Sb/m^3 (Short and Wheatley, 1962).

For the speciation of antimony in algae and animal and plant tissues, extraction methods have been studied (Miravet *et al*., 2005). It was noted that commonly used solvents for metal extraction are not appropriate for antimony speciation (Foster *et al*., 2005).

3 PRODUCTION AND USES

3.1 Historical Background

Although antimony was not described in detail as an element until the 17th century, it is believed to have been recognized in various compounds as early as 4000 BC, when it was used by the Chaldeans for its fine casting qualities. It was also used in coloring glass.

Antimony compounds have historically been used as cosmetics. Antimony sulfide was found in an Egyptian cosmetic case from 2500 BC, intended for use as a rouge for lips. In Roman times, black mineral containing antimony (III) sulfide was applied as makeup to eyelashes and around women's eyes to embellish them (McCallum, 1977).

Antimony was used for medicinal purposes since ancient times for its emetic and diaphoretic-inducing properties and was a favored remedy in the Middle Ages for the treatment of a wide variety of conditions. Paracelsus, a physician in 16th century Europe, and a proponent of alchemy, and his followers, favored the use of metals, including antimony, as drugs. The view of the Paracelsus followers diverged from the teachings of Galen (ca AD 130–201), a preeminent Roman physician who considered metals to be poisonous. The period of ca 1560–1660 was termed the "antimony war" and reflects the conflict in medicine between Galenic medicine and the practices of Paracelsus. (Koehler, 2001). Cups made of antimony were popular in the 17th and 18th centuries as a means to induce therapeutic sweating, vomiting, and purging. Wine was poured into the cup and allowed to sit for many hours during which time antimony leached from the cup into the wine, presumably combining with the tartaric acid found in most wines. Small amounts of the wine were swallowed at intervals until the intended effect was achieved. Although antimony was recognized as a poison and claimed many fatalities among patients administered antimony-containing medicinals, it was believed that a poison would attract other poisons and rid the body of them (McCallum, 1977; 2001).

3.2 Production

Antimony is most commonly found in sulfides and sulfo salts such as stibnite, tetrehedrite, bournite, boulangerite, and jamesonite, as well as in some oxides such as valentinite, stibiconite, and semarmontite. Of

the more than 100 minerals of antimony that are found in nature, the predominant one is stibnite (Sb_2S_3). The principal world resources of antimony are Bolivia, China, Mexico, Russia, and South Africa. The global mine production of antimony in 2003 was 81,600 metric tons and in 2004 it was 112,000 metric tons (estimated) (U.S. Geological Survey, 2005).

3.3 Uses

Antimony is a common constituent of metal alloys (e.g., with lead and copper). In fact, the most important use of antimony metal is as a hardener in lead storage batteries (U.S. Geological Survey, 2005). Antimony trioxide is used in fire retardants formulations for plastics, rubbers, textiles, paper, and paints; as an additive in glass and ceramic products; and as a catalyst in the chemical industry. Antimony trisulfide is used in explosives, pigments, antimony salts, and ruby glass (IARC, 1989). Antimony is a component of important thermoelectric materials, which are being synthesized and studied in the form of nanoparticles (Quarez *et al*., 2005; Schlecht *et al*., 2006; Xie *et al*., 2004).

Pentavalent antimony has been used for more than 50 years to treat both visceral and cutaneous leishmaniasis, parasitic diseases. One proposed mode of action is the inhibition of energy metabolism and macromolecular biosynthesis by means of inhibition of glycolysis and fatty acid β-oxidation (Berman *et al*., 1985; 1987). Wyllie *et al*. (2004) demonstrated that antimony affects both the thiol-buffering capacity and the thio redox potential within the cells of *Leishmania donovani*. The authors suggest that Sb(V) in pentavalent antimonial drugs is reduced to Sb(III), which is the biologically active form.

A major problem with pentavalent antimony drug therapy is that increasingly larger doses for longer durations are needed to be effective. In India, where more than half the cases of leishmaniasis occur, large-scale unresponsiveness to antimony treatment is occurring (Murray, 2000; Thakur *et al*., 2004). In addition, treatment is limited because of the toxicity of the drugs. The results of one study suggest that residual Sb (III) in the pentavalent meglumine antimonate drug is responsible for the cytotoxicity of the drug, and the multiple resistance-associated protein 1 (MRP-1)–mediated resistance (Dzamitika *et al*., 2006). Although alternative therapies have become available (Blum *et al*., 2004; Murray, 2000; 2004; Sundar 2002), treatment with pentavalent antimony compounds is still an accepted form of therapy (Laguna, 2003; MMWR, 2003; 2004). Uzun *et al*. (2004) described a technique of intralesional application of meglumine antimonate solution to treat 890 patients with cutaneous leishmaniasis and reported an efficacy of 97.2% with no serious adverse side effects.

4 ENVIRONMENTAL LEVELS AND EXPOSURES

4.1 General Environment

4.1.1 Food and Daily Intake

Meat, freshwater fish, poultry, cereal, fruit, and vegetables contain about 1–10 ng/g wet weight (Health Canada, 1997). A study of 12 table-ready foods showed antimony content to range from 0.22–2.81 µg/kg (Cunningham, 1987). Other reports on antimony levels in food include pooled human milk, 13 µg/kg (Iyengar *et al*., 1982); dorsal muscle of white suckers and brown bullheads (fish) from two acidified Adirondack, New York, lakes, <0.014–0.20 µg/g, and <0.04–<3.0 (dry wt.), respectively (Heit and Klusek, 1985); crayfish, redbreast sunfish, stonerollers from a stream in Tennessee that receives effluent from a U.S. Department of Energy facility, 20.24 µg/kg, 8.73 µg/kg, and 18.51 µg/kg, respectively (Rao *et al*., 1996); bivalves from the Gulf of Mexico, <0.05 µg/g (wet wt.) (Reish, *et al*., 1983); mollusk tissue, crustacean tissue, and fish tissue, 0.031–0.060, 0.018–0.116, and <0.009–0.010 µg/g (dry wt.) (Maher, 1986).

A study of nutrients in the diet conducted by the U.S. Food and Drug Administration estimated the average daily intake of antimony from food and water to be 4.6 µg/day (Iyengar *et al*., 1987). Using data on levels of antimony in the United States, Health Canada estimated a daily intake for adults of 7.44 µg of antimony per day, with about 38% coming from drinking water (Health Canada, 1997).

4.1.2 Air, Soil, and Water

Antimony is present in the earth's crust at a concentration of about 0.2–0.5 mg/kg (McCutcheon *et al*., 1995) and is released naturally into the atmosphere in the form of particulate matter or adsorbed to particulate matter. It is estimated that 41% of antimony present in air is due to natural sources, including wind-borne soil particulates (32.5%), volcanoes (29.6%), sea salt spray (23.3%), forest fires (9.2%), and biogenic sources (12.1%) (Nriagu, 1989). Anthropogenic sources include nonferrous metal mining, nonferrous metal primary and secondary smelting and refining (Crecelius *et al*., 1974, Pacyna *et al*., 1984; Shotyk *et al*., 2005b), coal combustion (Gladney *et al*., 1978), and refuse and sludge combustion (Greenberg *et al*., 1978). Data regarding levels of antimony in ambient air are insufficient to report mean or median levels (ATSDR, 1992). The EPA (1992 revised 2000) has established a respiratory benchmark dose (BMD) for antimony of 0.087 mg/m^3 and an RfC for antimony trioxide of 0.0002 mg/m^3.

Studies have found antimony concentration in air particulate matter in remote, rural, and urban areas in the United States to be 0.00045–1.19, 0.6–7, and 0.5–171 ng/m^3, respectively (Arimoto *et al*., 1987; Austin *et al*., 1988). Peirson *et al*. (1973) reported levels of 1.4–55 ng/m^3 in air from seven sites in the United Kingdom. Emissions from antimony-containing ore-processing facilities can reach levels greater than 1000 ng antimony/m^3 (ATSDR, 1992).

In soil, antimony usually ranges from 0.1–10 mg/kg dry weight (Bowen, 1966; Khatamov *et al*., 1967). Soil around industrial sites that process antimony-containing ores can be much higher (ATSDR, 1992). A study of soil from eight orchards in the state of Washington found levels of antimony ranging from 0.4–1.5 mg/kg. The authors attributed these antimony levels to the use of insecticides containing antimony as an impurity, because the levels were higher than nearby areas where insecticide was not used.

Because antimony is naturally present in soil, it would be expected to be found in runoff. In a study of runoff samples from 19 cities in the United States, 14% contained antimony in concentrations ranging from 2.6–23 µg/kg (Cole *et al*., 1984). Levels in other areas include groundwater in Switzerland, 0.3–1.0 µg/kg; Canadian surface water, range 0.001–9.1 mg/L (Health Canada 1997); open ocean water, 1×10^{-9} moles/L; filtered and unfiltered seawater from the North Adriatic Sea, 0.31–0.45 µg/kg, respectively (Johnson *et al*. 1992); and surface water samples from Gniezo city, Poland, 1.61 ng/ml (Niedzielski, 2006). Sediments can be significant sinks for antimony (ATSDR, 1992). Levels up to 900 µg/g of antimony have been found in sediment in areas where antimony-containing ore was mined or processed (ATSDR, 1992). By using an analytical method developed for the determining trace element concentrations in polar snow and ice, which gave a detection limit of 30 pg Sb/L, Shotyk *et al*. (2005a) measured the antimony level of water from natural flows and wells in Springwater Township, Ontario, Canada. The average concentration in 34 samples was 2.2 ± 1.2 ng/L, and the maximum concentration 5.0 ng/L.

The World Health Organization recommends that drinking water contain no more than 0.02 mg antimony/liter (World Health Organization, 1993). This equates to 10% of a total recommended maximum daily intake (TDI) of 6 µg/kg of body weight.

4.1.3 Tobacco

Antimony in cigarettes has been studied by means of neutron activation (Nadkarni and Ehman, 1970; Wyttenbach *et al*., 1976). The tobacco contained an average of 0.1 mg Sb/kg dry weight. The amount of inhaled antimony was estimated to be 20% of the total amount of antimony in one cigarette.

4.2 Working Environment

Air concentrations of antimony on the order of 1–10 mg/m^3 have been reported from different smelter operations. Renes (1953) found average working zone concentrations of antimony ranging from 4.7–10.2 mg/m^3 in smelting works. In an abrasives plant studied by Brieger *et al*. (1954), the average air concentration was 3.0 mg/m^3. It should be mentioned in this context that arsenic frequently occurs as a contaminant in the production and handling of antimony. Thus, risks for simultaneous exposure to arsenic, when antimony and its compounds are used, are always present. Some of the health effects that were previously thought to be primarily related to antimony have, indeed, subsequently been questioned by some researchers and considered to be an effect of simultaneous exposure to arsenic rather than antimony (Brooks, 1981). Stibine (SbH_3) was found to evolve during charging of lead storage batteries in which antimony was a compound of the negative grid (Haring and Compton, 1935). Because of improved working conditions over the years, there are relatively few instances of high exposure to antimony over long periods of time (McCallum, 2005). The OSHA permissible exposure limit (PEL) is 0.5 mg Sb/m^3 (8-hour time-weighted average) (U.S. Department of Labor, 2006).

5 METABOLISM

Antimony is considered a nonessential metal.

5.1 Absorption

No correlation was found between soil levels of antimony and antimony levels in urine, blood, or hair of area residents (Gebel *et al*., 1998a,b). On the other hand, in another study, significant correlation was found between antimony concentrations in air and in blood and urine (Kentner *et al*., 1995).

5.1.1 Inhalation

Quantitative data on the absorption of inhaled antimony compounds are not available. It can, however, be deduced from the experiments on rats by Djuric *et al*. (1962) that inhaled trivalent antimony is, to a great extent, absorbed from the lungs, distributed to various organs, and excreted in urine and feces. It has been shown that absorption of antimony compounds

from the respiratory tract is influenced by particle size. One study showed that 1.6 µm particles were absorbed in the upper respiratory tract to a greater extent than 0.7 or 0.3 µm particles (Felicetti *et al*., 1974b). The 1.6 µm particles were cleared by mucociliary clearance within a few hours, whereas the smaller particles were cleared over several weeks (Thomas *et al*., 1973). No differences were seen in body burden between trivalent and pentavalent antimony tartrate 1 day after exposure (Felicetti *et al*., 1974a). In a study of workers in the lead battery production industry, pulmonary absorption of antimony trioxide and stibine were found to be virtually equal (Kentner *et al*., 1995). Although no differences in the rate of absorption of antimony compounds were noted, it is likely that there are differences related to solubility (ATSDR, 1992).

5.1.2 Ingestion

No quantitative data on absorption of antimony from the gastrointestinal tract in humans is available. In animals, absorption rates for antimony tartrate and antimony trichloride are estimated at 2–7% (Felicetti *et al*., 1974a; Gerber *et al*., 1982). At least 15% of a single oral dose of labeled antimony potassium tartrate (trivalent) given to mice is absorbed (i.e., recovered in urine and tissues) (Waitz *et al*., 1965). Absorption may, however, be higher, because gastrointestinal excretion starts immediately after the metal is taken up from the gut. Thus, Gellhorn *et al*. (1946) observed a fecal excretion of 50% of the given dose (0.43 mg) within 24 hours after intraperitoneal administration of antimony potassium tartrate to hamsters.

The therapeutic doses in humans of antimony and potassium tartrate that produce a diaphoretic/expectorant or emetic response are 2.8 mg and 30–60 mg, respectively; see Section 3.1. (Martindale, 1941).

5.2 Distribution

5.2.1 Animals

Antimony given as a single dose or by repeated injections is found in kidney and liver (Boyd *et al*., 1931 [pentavalent antimony to monkeys]; Hassan, 1938 [trivalent antimony to dogs and monkeys]; Goodwin and Page, 1943 [pentavalent antimony to mice]) as well as in thyroid (Brady *et al*., 1945 [trivalent antimony to dogs]). There are remarkable differences in the distribution of trivalent and pentavalent antimony in blood of rats. Two hours after an intraperitoneal injection of trivalent antimony (3 µg), more than 95% of the amount of antimony in blood was incorporated in the red blood cells. On the other hand, about 90% of pentavalent antimony was mainly found in plasma (Edel *et al*., 1983). In hamsters, trivalent antimony levels increased more rapidly in the liver than pentavalent antimony levels, whereas pentavalent antimony was taken up more rapidly by the skeletal system than trivalent antimony (Felicetti *et al*., 1974a). In rats fed a diet containing 2% antimony trioxide for 1.5 months, the highest concentrations of antimony were found in the thyroid and adrenal glands, values of 88.9 and 67.8 mg/kg, respectively (wet or dry weight not stated). Spleen, lungs, liver, and kidneys had concentrations between 6.7 and 18.9 mg/kg (Westrick, 1953). The highest concentrations of antimony in rats given antimony in drinking water (38 µg/L) for 26 months were found in blood and spleen; 185 µg/kg and 31.5 µg/kg wet weight, respectively (Edel *et al*., 1983). As is the case for arsenic (Chapter 19), there seem to be important species differences with regard to the metabolism of antimony. Djuric *et al*. (1962) noticed that in rats exposed to antimony trichloride by inhalation, a large proportion of the body burden of antimony was recovered in blood. A couple of weeks after exposure, about 10% of the body burden of antimony was found in blood. This was a higher proportion than in any other organ. Comparative studies with rabbits and dogs intratracheally exposed to antimony trichloride revealed that these animals had less than 1% of the blood antimony concentrations found in rats.

5.2.2 Humans

A study conducted in Ireland to establish a reference range for antimony in serum and urine of infants in the first year of life found levels of 0.09–0.25 µg/L in serum. Urinary antimony concentration for 95% of the infants was <2.6 ng/mg creatinine (Cullen *et al*., 1998). An earlier study also detected antimony in very low levels in both serum and urine of infants in the United Kingdom (Dezateux *et al*., 1997). A Japanese autopsy study examined the levels of antimony in tissues in unexposed humans. The mean body burden was 0.7 mg, with the highest levels of antimony found in skin and hair (Sumino *et al*., 1975). Surface body scanning of people given intravenous injections of labeled antimony as sodium antimony dimercaptosuccinate revealed the highest amounts in liver, thyroid, and heart. Forty-three days after the last injection, the liver still showed values of about 1/6 of the maximum, which was reached 1 day after the last injection (Abdallah and Saif, 1962; Abdel-Wahab *et al*., 1974). Certain inhaled antimony compounds seemed to be retained in the lung for long periods. Gerhardsson *et al*. (1982) found that former smelter workers occupationally exposed to

several different types of metals, of which antimony was one, on an average had 12 times higher lung concentrations of antimony (315 µg/kg) than persons not previously occupationally exposed (26 µg/kg).

5.3 Excretion

5.3.1 Animals

Rate and route of excretion are dependent on the valency of the compound. Certain species differences are also seen. In general, pentavalent organic antimony is mainly excreted in urine, trivalent mainly in feces (Edel *et al*., 1983; Otto and Maren, 1950).

Cows given antimony orally as $^{124}SbCl_3$ at an average dose of 21.1 mg Sb/kg body weight excreted 82% of the antimony in feces. Milk and urine accounted for 0.008 and 1.1%, respectively. Highest tissue concentrations were found in the spleen, liver, bone, and skin. A single cow administered $^{124}SbCl_3$ intravenously at a dose of 1.5 mg Sb/kg body weight showed fecal excretion of only 2.4%, whereas milk and urine accounted for 0.08 and 51%, respectively (Von Bruwaene *et al*., 1982).

Six hours after intravenous and intraperitoneal injections of pentavalent antimony compounds to mice, about 50–60% was found in urine (Goodwin and Page, 1943). The initial excretion rate was less rapid for the trivalent form, but after 48 hours, the differences between the forms declined; total urinary excretion in 48 hours was around 70%.

Twenty-four hours after intraperitoneal administration of trivalent and pentavalent antimony to hamsters, about 15% of the trivalent and 65% of the pentavalent antimony were found in urine. The fecal excretion over the same period of time was about 50% for trivalent antimony and less than 10% for pentavalent antimony (Gellhorn *et al*., 1946). Likewise, Edel *et al*. (1983) found that 6% and 88% of a given dose of trivalent and pentavalent antimony in rats was eliminated in urine within 24 hours of the administration. The fecal elimination of trivalent and pentavalent antimony during the same period was 33% and 1%, respectively.

5.3.2 Humans

Healthy subjects in the general population in central Italy were found to have a urinary antimony level of 60.8 ng/L (Alimonti *et al*., 2005).

Median exposure to antimony in air of workers in a lead battery production facility ranged from 4.5 µg Sb/m^3 for casting workers to 12.4 µg Sb/m^3 for formation workers. Average urine values were 3.9 µg Sb/g creatinine for the casters and 15.2 µg Sb/g creatinine for the formation workers. Characteristics of pulmonary absorption and urinary excretion for antimony trioxide and stibine were very similar (Kentner *et al*., 1995). Single intravenous or intramuscular injections to volunteers produced higher 24-hour urinary excretion of pentavalent (80%) than of trivalent (25%) antimony compounds, a pattern similar to the one found in animals (Abdallah and Saif, 1962; Boyd and Roy, 1929; Goodwin and Page, 1943).

Bartter *et al*. (1947) studied the elimination of a single intravenously administered dose of labeled antimony potassium tartrate (trivalent). The urinary excretion was about four times higher than the fecal. In one patient, 73% of a total single dose was eliminated within 4 weeks. Rees *et al*. (1980) studied the renal clearance of pentavalent antimony. When pentavalent antimony in the form of sodium stibogluconate was given intramuscularly to patients, about 95% was recovered in urine within 6 hours after the administration.

In workers exposed to air containing around 3 mg Sb/m^3, a colorimetric method showed urinary values ranging from 0.8–9.6 mg/L (Brieger *et al*., 1954), which are highly elevated compared with normal values (see Section 6). Likewise, Smith and Griffiths (1982) found considerably higher urinary concentrations of antimony (10–220 µg/L) in urine samples from workers exposed to antimony compared with urine from nonexposed subjects (1–5 µg/L).

5.4 Biological Half-Life

5.4.1 Animals

5.4.1.1 Inhalation

A study of beagles exposed to labeled antimony aerosols indicated an initial fast clearance of up to 80% of the initially deposited material within a few days (whole-body measurements). This fast excretion phase was followed by a slow clearance, with a biological half-life in whole-body on the order of 36–100 days (Felicetti *et al*., 1974b). The retention in the lung of initial lung burden, after 4 months, ranged from 0.0–6%, depending on the type of particles. Excretion of antimony took place through urine and feces, with a ratio of 0.8. The particles had been nebulized from an antimony potassium tartrate solution passing through a heating column at 100, 500, and 1000°C, which yielded particles with aerodynamic diameters of 0.3, 1.0, and 1.3 µm, respectively. Similar results have also been reported by Djuric *et al*. (1962), who exposed rats, and by Thomas *et al*. (1973), who exposed mice under conditions similar to those used by Felicetti *et al*. (1974a). After a rapid elimination of about 80% of the initially deposited amount

of antimony in the lung, the whole-body burden biological half-life was found to be in the order of 30–140 days in the two different species. It is possible that the half-life would have been even longer if the animals had been followed up for an extended period of time.

Leffler *et al*. (1984) studied lung retention of antimony and arsenic after intratracheal instillation of industrial dust—which had been neutron activated—from a Swedish copper smeltery. Comparison was made with pure substances of antimony trioxide and arsenic trioxide. There were two phases in the clearance curves. The approximate half-life for the initial phase was about 40 hours for Sb_20_3, and about 30 hours for antimony dust. The second phase had an approximate half-life of 20–40 days for Sb_20_3 and antimony dust. The low solubility of antimony in factory dust combined with a long biological half-life may, according to the present authors, be of importance in explaining the observed lung accumulation of antimony in exposed workers (see Section 5.2.2).

5.4.1.2 Parenteral Administration

Most data indicate an initial rapid clearance of antimony, involving gastrointestinal and urinary excretion (see Section 5.3.1). Twenty-four hours subsequent to an intraperitoneal injection in hamsters, Gellhom *et al*. (1946) found about 70% of trivalent and pentavalent antimony compounds in feces and urine together.

5.4.2 Humans

The initial excretion of antimony in humans is rapid (see Section 5.3.2). When pentavalent antimony compounds are given intravenously or intramuscularly, more than 90% of the dose will be found in the urine within 24 hours (Rees *et al*., 1980). There might, however, be a long-term component according to a study by Mansour *et al*. (1967). They studied antimony in blood and urine (neutron activation) in patients who, 1 year earlier, had been treated with antimony for bilharzia. They found average blood and urine values of 6.7 and 27.6 µg/L, respectively, in three patients. Three untreated subjects had averages of 3.4 and 6.2 µ/L in blood and urine, respectively. Data from measurements of antimony in lung tissue obtained from former occupationally exposed smelter workers show that inhaled antimony may be deposited and retained in the lung for extended periods of time (several years) (Gerhardsson *et al*., 1982). In a study of workers in a lead battery production facility, who were exposed to antimony trioxide and stibine in air, the half-life of renal excretion was found to be approximately 4 days (Kentner *et al*., 1995).

6 BIOLOGICAL MONITORING

Relatively high antimony concentrations (around 0.5 mg/kg dry weight about 0.1 mg/kg wet weight) are found in the lung. Liver and kidney contain about one third of the lung concentration (Kennedy, 1966; Nixon *et al*., 1967; Schicha *et al*., 1972, all using neutron activation). More recently, Gerhardsson *et al*. (1982) using the same technique arrived at somewhat lower normal values: 26, 7, and 5 µg Sb/kg wet weight in lung, liver, and kidney, respectively. Japanese autopsies have shown that skin and adrenal glands contain slightly higher concentrations of antimony than lung and liver, 100 µg/kg and 70 µg/kg wet weight compared with 60 µg/kg and 20 µg/kg. The total body burden of antimony in an average Japanese was estimated to be about 0.7 mg by use of the rhodamine B method (Sumino *et al*., 1975).

Blood and serum concentrations of antimony in normal subjects have been reported to be around 3 and 1 µg/L, respectively (Mansour *et al*., 1967; Wester, 1973—both studies used neutron activation; Bencze, 1981—AAS used). The 24-hour urinary excretion of 16 subjects ranged from 0.5–2.6 µg (Wester, 1973). Smith and Griffiths (1982) found a median antimony concentration of less than 1 µg/L for persons not occupationally exposed to antimony and 54 µg/L for occupationally exposed workers. Because quality control programs were not reported in any of the studies cited, the results concerning normal levels of antimony in biological materials, especially in urine and blood, must be regarded as tentative only.

More recent studies on workers occupationally exposed to antimony in a variety of industries and using modern analytical methods have found that determination of urinary antimony is useful for biological monitoring of exposure (Bailly *et al*., 1991; Kentner *et al*., 1995; Ludersdorf *et al*., 1987). Significant correlation was found between the concentration of antimony in air and in the blood and urine of workers in a lead battery factory. In this study, levels of exposure to antimony-containing dust and stibine gas were 10 times lower than threshold limit values (Kentner *et al*., 1995). A study of workers in a textile factory that uses antimony-containing flame retardants compared urinary antimony levels in two groups of workers and controls. Levels of antimony in ambient air were very low (1.45 µg/m^3). A statistically significant ($P < 0.001$) difference was observed in the mean antimony urinary level between workers in a "lower" exposure group and a "higher" exposure group and between the two groups and the control group. The "high" exposure group had a urinary antimony level of 0.39 ± 0.26 µg/L compared with 0.10 ± 0.06 µg/L in the controls. Thus,

increases in urinary antimony levels are seen in cases of exposure to very low levels of antimony in air (Iavicoli *et al*., 2002).

7 EFFECTS AND DOSE-RESPONSE RELATIONSHIPS

7.1 Animals

The major sites of accumulation of antimony in animals after oral exposure, other than the gastrointestinal tract, are the liver, kidney, bone, lung, spleen, and thyroid. This accumulation, however, is not dose-related (Sunagawa, 1981). With peroral exposure, both acute and chronic, antimony potassium tartrate is more toxic than antimony trioxide and pentoxide. In dogs and cats, acute symptoms such as vomiting and diarrhea were produced by antimony potassium tartrate in doses on the order of 10 mg/kg. Antimony trioxide and pentoxide could be given to the same animals in doses above 100 mg/kg for months without toxic manifestations (Bradley and Fredrick, 1941; Flury, 1927). In a long-term study, rats given 5 mg/L as antimony potassium tartrate in drinking water showed a significantly shortened average length of life (about 15%) compared with controls (Schroeder *et al*., 1970). Rats given a diet containing 0.5–2% antimony or antimony trioxide had a decreased weight gain (Sunagawa, 1981).

7.1.1 Local Effects and Dose-Response Relationships

After inhalation of antimony trioxide at an average concentration of 45 mg/m^3, 2–3 hours daily for several weeks, guinea pigs showed interstitial pneumonitis (Dernehl *et al*., 1945). Rats and rabbits exposed to antimony trioxide (90–125 mg Sb_20_3/m^3 during 100 hour/month) for periods of up to 14 months, in addition to pneumonitis, also developed lipoid pneumonia, fibrous thickening of alveolar walls, and focal fibrosis (Gross *et al*., 1951; 1955). Rabbits seemed to be more susceptible than rats (Gross *et al*., 1955). Watt (1983) used 148 female CDF-rats and 8 miniature swine to examine the long-term toxicity of inhaled antimony trioxide. Both species were divided into a control group, a low-exposure (1.6 mg Sb/m^3), and a high-exposure group (4.2 mg Sb/m^3). The animals were exposed for 6 hours a day, 5 days per week, for about 1 year. In the miniature swine, there were no histopathological changes in the lungs, but in the rats, there were pronounced morphological changes in the lung with focal fibrosis, adenomatous and pneumonocytic hyperplasia, and cholesterol clefts. The histopathological changes were most pronounced in the high-exposure group.

7.1.2 Systemic Effects and Dose-Response Relationships

In a study of the effects of subchronic oral exposure of Wistar rats to antimony or antimony trioxide, growth retardation was observed. The addition of 0.1% or 1.0% antimony or 1.0% antimony trioxide to the diet for 12 weeks resulted in a decrease in body weight that returned to normal after 12 weeks on an antimony-free diet (Hiraoka, 1986). A decrease in body weight was also observed in a study of Sprague–Dawley rats exposed to potassium ammonium tartrate in drinking water at concentrations of 0.5, 5, 50, or 500 mg/L for 13 weeks. As with the study by Hiraoka, the rats returned to their normal weight within several weeks post exposure (Poon *et al*., 1998).

7.1.2.1 Circulatory System Effects

Intravenous injections of antimony produce an acute circulatory response with a drop in blood pressure (Chopra, 1927; Cotten and Logan, 1966). Pathological ECG changes have been observed. In dogs injected for 4 days with antimony potassium tartrate, 5 mg/kg body weight, one of the most prominent features was inversion of the T wave (Girgis *et al*., 1970). Several of the dogs died as a result of the treatment.

Chronic effects with parenchymatous degeneration in the myocardium were observed on histopathological examination of hearts from rats and rabbits exposed to 3.1 and 5.6 mg/m^3 as antimony trisulfide for 6 weeks (Brieger *et al*., 1954). On the other hand, Watt (1983), in his long-term study on swine exposed to antimony trioxide (1.6 and 4.2 mg Sb/m^3), found no evidence of cardiovascular effects.

7.1.2.2 Liver and Kidney Effects

Fatty degeneration occurred in the convoluted tubules of the kidney and the liver after a single administration of 60 mg antimony potassium tartrate solution to rabbits. The kidney changes appeared a few hours after the administration, and preceded the changes in the liver (Franz, 1937). Fatty degeneration in the liver, but no effects in the kidney, was recorded in an inhalative exposure study on guinea pigs (Dernehl *et al*., 1945). Long-term peroral exposure to high doses of antimony may also induce signs of liver toxicity. Sunagawa (1981) gave rats 0.5–2% antimony and antimony trioxide in the diet. After 24 weeks, the most exposed animals had elevated serum levels of a liver enzyme, GOT.

7.1.2.3 Endocrine Effects

Antimony is among a group of metal ions, termed metalloestrogens, shown to bind to estrogen receptors and cause estrogen agonist responses *in vitro* and

in vivo. Thus, these metalloestrogens can mimic the actions of physiological estrogens (Darbre, 2006).

7.2 Humans

Data on acute and chronic toxicity of antimony come essentially from results found in connection with industrial atmospheric exposure. Adverse effects from treatment of tropical diseases with antimony compounds are discussed only briefly. Stibine (SbH_3), like arsine (AsH_3), when inhaled, damages the red blood cells and causes hemolysis. Signs of poisoning include symptoms of shock and hemoglobinuria (Stokinger, 1963).

7.2.1 Local Effects and Dose-Response Relationships

7.2.1.1 Gastrointestinal Effects

Acute antimony poisoning, manifested as vomiting, nausea, and diarrhea, was reported in 150 children who drank a contaminated lemon drink (about 30 mg/L) (Werrin, 1963). In former times, wine and other drinks stored in antimony cups were used to induce vomiting (Fairhall and Hyslop, 1947; McCallum, 1977), the emetic dose for adults being in the range of 30–60 mg (Martindale, 1941).

7.2.1.2 Respiratory Effects

Acute respiratory exposure to antimony trichloride (73 mg/m^3) caused irritation and soreness of the upper respiratory tract in seven workers (Taylor, 1966). Three cases, two of them fatal, of severe pulmonary edema evoked by antimony pentachloride have been described by Cordasco and Stone (1973); concentrations in air were not available.

Chronic respiratory effects related to antimony were reported by Renes (1953). He examined 78 workers engaged in smelting processes, for periods exceeding 2 weeks. Exposure concentrations ranged from 4.7–11.8 mg/m^3, 20% of the workers suffered from rhinitis, 8% from pharyngitis, 5.5% from pneumonitis, and 1% from tracheitis. Soreness in the nose and nosebleed were experienced by more than 70%. The workers were, however, also exposed to arsenic at concentrations around 0.7 mg/m^3. In view of the more severe toxic properties of arsenic (Chapter 19), several of the effects that were related to antimony by Renes (1953) could actually have been caused by exposure to arsenic (ACGIH, 1983). Brieger *et al*. (1954) did not mention respiratory tract irritation in their extensive study discussed in Section 7.2.2 in which workers were exposed to antimony trisulfide at levels ranging from 0.6–5.5 mg Sb/m^3.

Several authors have remarked on pneumoconiosis-like X-ray pictures obtained from workers with long-term occupational exposure to antimony (Browne, 1968; Cooper *et al*., 1968; Karajovic, 1958; Le Gall, 1969; McCallum, 1963; Parkes, 1982; Potkonjak and Pavlovich, 1983). It is likely that silica was present in some of these instances. McCallum *et al*. (1970) have developed an X-ray method for measurement of inhaled antimony trioxide. On examination of 113 antimony process workers, they found a significant correlation between estimated lung antimony and period of employment (Figure 1). Parkes (1982), in his textbook on occupational lung disorders, regards the antimony pneumoconiosis as being benign, having no detrimental effect on health or life expectancy.

Potkonjak and Pavlovich (1983) reported on the examination of 51 workers with X-ray changes indicating antimoniosis, in a Yugoslavian antimony smelter. The men had been exposed to a total dust concentration of 17–86 mg/m^3 for 9–31 years. The dust mainly consisted of antimony trioxide (40–90%) but also contained lesser amounts of antimony pentoxide (2–8%) and free silica (1–5%). The X-ray findings were characterized by the presence of diffuse, densely distributed punctate opacities, having a diameter of less than 1 mm. Confluence of opacities was not seen. Pulmonary function tests revealed obstructive changes in 17.6% and mixed restrictive-obstructive changes in 9.8% of the examined workers with X-ray changes. Increased airway resistance was seen in 26.3%, and a decreased

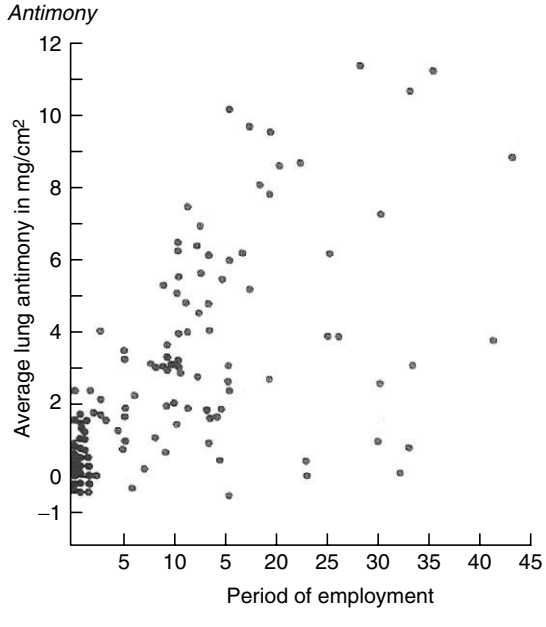

FIGURE 1 Lung antimony (mg/cm^2) in 113 antimony workers, measured with an X-ray spectrometry method, in relation to period of employment in year. Mc Callum *et al.* (1970).

forced expiratory flow rate in 16.7%. In addition, 61% of the workers complained of chronic coughing.

Two other groups of investigators have also observed lung changes among antimony workers (Karajovic, 1958; Klucik *et al*., 1962). Klucik *et al*. (1962) reported on workers exposed to antimony trioxide for up to 28 years (concentration and number of workers were not given). The prevalence of pneumoconiosis and symptoms of emphysema were given as 21 and 42%, respectively.

It thus seems that antimoniosis is commonly regarded as a relatively benign condition, but nevertheless chronic respiratory effects have been reported in a number of studies. Therefore, heavy antimony exposure cannot, according to our interpretation, be regarded as harmless.

7.2.1.3 Skin Effects

Pustular skin eruptions, "antimony spots," are sometimes seen in persons working with antimony and antimony salts. These eruptions are transient and mainly affect skin areas exposed to heat and those areas where sweating occurs (McCallum, 1963; Paschoud, 1964; Potkonjak and Pavlovich, 1983; Renes, 1953; Stevenson, 1965). Thirty-two (62.7%) of the antimony smelter workers examined by Potkonjak and Pavlovich (1983) had "antimony dermatosis."

7.2.2 Systemic Effects and Dose-Response Relationships

7.2.2.1 Circulatory System Effects

Antimony is associated with ECG abnormalities and an increased risk of sudden death that is likely due to arrhythmias (Sullivan and Krieger, 1992). Brieger *et al*. (1954) reported hypermortality and morbidity among workers in an abrasive industry. In all, 124 workers were exposed to air concentrations of antimony trisulfide ranging from 0.6–5.5 mg/m^3 for 8–24 months. During this period, six workers died suddenly and two others died of chronic heart disease. Of the deceased, four were under 45 years of age. ECG changes, mostly of the T wave, were seen in 37 of 75 examined. During the preceding 16 years before introduction of antimony in the plant, only one death had occurred in this department. No control group was examined.

Renes (1953) reported abdominal cramps, diarrhea, and vomiting among certain workers subject to heavy exposure to antimony fumes in a smelter. A higher prevalence of ulcer, 6% of 111 examined antimony workers compared with that of the total plant population, 1.5% of 3912 employees, was seen by Brieger *et al*. (1954).

7.2.2.2 Immunological Effects

Kim *et al*. (1999) reported altered cytokine and immunoglobulin levels in workers exposed to antimony. The sera from exposed workers showed lower levels of IgG1 and IgE compared with the sera from controls. A positive correlation between IgG4 and urine serum Sb levels in the exposed group was noted.

7.2.2.3 Hemolytic Effects of Stibine Gas

Stibine gas (SbH_3) is another important chemical form of antimony (Andrewes *et al*., 2004; Gallicchio *et al*., 2001; Hussain *et al*., 1998) that has toxic properties similar to those of arsine gas. Information on the carcinogenic or other toxic potential of stibine is currently very limited (Andrewes *et al*., 2004; Gallicchio *et al*., 2001; Husain *et al*., 1998) relative to that of arsine gas. Stibine releases have been reported in the production of lead automobile batteries (ATSDR, 1992), and it is also currently used in the production of some III–V semiconductors. Stibine has been reported to produce hemolysis and hemoglobinuria, although it seems to be less acutely toxic than arsine gas (Gallichio *et al*., 2001).

7.2.3 Adverse Effects During Antimony Treatment

Various side effects, and in several studies cases of sudden death, have been recorded in connection with clinical treatment of parasitic diseases with antimony (El Halawani, 1968; Rugemalila, 1980). Nausea and vomiting are common features (Hamad, 1969; Pedrique *et al*., 1970; Zaki *et al*., 1964). Effects on the liver, with rises in serum concentrations of two liver enzymes (GOT and GPT) at the onset of therapy, were reported by Woodruff (1969). As in animal studies, ECG changes, particularly in the T wave, were frequently reported during long-term treatment (Abdalla and Badran, 1963; Davis, 1961; Mainzer and Krause, 1940; Sapire and Silverman, 1970; Schroeder *et al*., 1946). Mansour and Reese (1965) have proposed that the influence of tartar emetic is an augmenting factor in the development of schistosomal myopathy.

8 CARCINOGENIC AND GENOTOXIC EFFECTS

There are few studies on the carcinogenicity of antimony (Gebel, 1997; Hayes, 1997). One reason is that the environmental distribution of antimony is low. In an inhalation study using Wistar rats, antimony ore concentrates and antimony trioxide have been linked to lung cancer. After inhalation of 45 or 36–40 mg/m^3 Sb_2O_3 or Sb, 7 hours per day, 5 days per week, for 52 weeks, followed by a 5-month observation period,

female rats, but not male rats, developed lung neoplasms. The authors attributed this difference to a lower immunological responsiveness in female rats (Groth *et al*., 1995). A subchronic and chronic inhalation study of Sb_2O_3 in rats did not lead to increased frequencies of neoplasms (Newton *et al*., 1994). Watt (1983) found that female rats exposed to antimony trioxide at concentrations of 1.6 and 4.2 mg Sb/m^3 for 6 hours per day, 5 days per week for 1 year followed by a 15-month observation period, showed a high frequency of lung neoplasias (see also Section 7.1.1). The lung neoplasias were either scirrhous carcinomas, squamous cell carcinomas, or bronchioalveolar adenomas. There was evidence of local invasion, but no metastasis was observed.

Human carcinogenicity of antimony is difficult to evaluate because of the frequent coexposure to arsenic (De Boeck *et al*., 2003). In a study of U.S. antimony workers, excess mortality from cancers of the liver, biliary tract, and gallbladder was found in a cohort of antimony smelter workers (Schnorr *et al*., 1995). The American Conference of Governmental Industrial Hygienists (ACGIH, 1983) refers to unpublished data on mortality among workers in a large antimony smelter in the U.K. In the 1960s, exposure to antimony, mainly in the form of antimony trioxide, ranged from 0.5–40 mg/m^3. Apart from antimony, the workers were also exposed to zirconium. The data cited are difficult to interpret, but there seems to be an increased proportional mortality from lung cancer among the most heavily exposed men. On the basis of these data, the ACGIH (1983) concluded that antimony oxide should be regarded as a suspected carcinogen.

Overall, the International Agency for Research on Cancer (IARC) finds the available data on the carcinogenicity of antimony to be inconclusive. IARC classifies antimony trioxide as *possibly carcinogenic to humans* (Group 2B) and antimony trisulfide as *not classifiable as to its carcinogenicity to humans* (Group 3) (IARC, 1989).

Antimony compounds display genotoxic activity *in vivo* and *in vitro*. They behave clastogenically (Gurnani *et al*., 1992a; 1992b; 1993) but are not directly mutagenic (Kuroda *et al*., 1991). Genotoxicity is valence-dependent. The trivalent species (Sb_2O_3 and $SbCl_3$) were genotoxic in the sister chromatid exchange test with V79 cells, whereas the pentavalent species (Sb_2O_5 and $SbCl_5$) were not (Kuroda *et al*., 1991). Gebel *et al*. (1997) used the sister chromatid test to assess the genotoxicity of extracts of soil containing high concentrations of antimony and arsenic, both in the pentavalent state. They found low genotoxicity, as well as partial antagonism. Andrewes *et al*. (2004) tested five antimony compounds for genotoxicity. Stibine and trimethylstibine were genotoxic when tested using a pBR 322 plasmid DNA-nicking assay, whereas potassium antimony tartrate, potassium hexahydroxyantimonate, and trimethyl antimony dichloride were not. There is a scarcity of data on the mechanism of action. A biomonitoring study of 23 textile workers exposed to Sb_2O_3 assessed genotoxicity using the sister chromatid assay and cytokinesis-block micronucleus (MN) tests and the enzyme (Fpg)-modified comet assay. Results support the theory that genotoxicity is due to oxidative DNA damage (Cavallo *et al*., 2002; USEPA, 2004).

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CHAPTER

19

Arsenic

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ABSTRACT

Arsenic in the environment occurs in both organic and inorganic compounds in its trivalent or pentavalent state. Certain fish and crustaceans contain very high levels of organic arsenic, often as arsenobetaine. In most other foodstuffs, levels of arsenic are low, and the form is not known. The total daily intake of arsenic in the general population is reported to be approximately a few tenths of a milligram but varies to a great extent, depending on the amount of fish consumed.

Both organic arsenic in seafood and inorganic arsenic in water, beverages, and drugs have been shown to be readily absorbed (70–90%) by the gastrointestinal tract. Some reports also indicate a fairly high degree of absorption after inhalation of arsenic.

Absorbed arsenic, irrespective of form, is widely distributed in the body. After exposure to inorganic arsenic, clearance of arsenic from the skin, upper gastrointestinal tract, epididymis, thyroid, and skeleton is slower than from other organs. The highest levels of arsenic in humans are normally found in hair, nails, and skin. The main route of excretion is through the kidneys. After ingestion of arsenite or arsenate, approximately 35% of the dose is excreted within 2 days. From animal experiments it seems that insoluble inorganic arsenic inhaled through the airway is deposited and retained in lung tissue for a relatively long time. Animal data indicate accumulation of arsenobetaine in cartilage, testes, epididymis, and muscle. Of ingested arsenobetaine, 50–80% is excreted by the urine within 2 days.

Biotransformation of inorganic arsenic has been shown to occur in both animals and humans. Methylated compounds, such as methylarsonic acid and dimethylarsinic acid, have been detected in urine after ingestion or inhalation of inorganic arsenic. Reduction of arsenate and oxidation of arsenite *in vivo* have been demonstrated in experimental animals. Recently, a human arsenic methyl transferase has been identified.

Medications, contaminated food, beverages, and drinking water have given rise to a number of episodes of arsenic poisoning.

Inorganic arsenic-induced skin lesions such as dermatoses, which may include eruption, pigmentation, or leukodermal hyperkeratosis, may ultimately lead to the development of skin cancer and Bowen's disease. Effects on the nervous system (e.g., peripheral nervous disturbance), as well as on the heart and circulatory system (e.g., abnormal electrocardiograms, and peripheral vascular disturbances with gangrene of the extremities), have also been reported after chronic exposure to inorganic arsenic. Hematological changes after inorganic arsenic exposure are characterized by anemia and leukopenia. Chronic oral ingestion of inorganic arsenic in drinking water has also been reported to cause internal cancers (lungs, bladder, kidneys, and liver).

Arsenic poisoning among industrial workers is characterized by perforation of the nasal septum, skin changes, and peripheral neuritis. There is substantial epidemiological evidence of an excessive risk of lung cancer among workers exposed to arsenic.

Arsine gas is a powerful hemolytic poison encountered under some industrial conditions. Arsine poisoning is characterized by nausea, vomiting, headache, shortness of breath, and hemoglobinuria. The literature on toxicology and environmental aspects of

arsenicals has recently been reviewed by the World Health Organization (1981; 2001), in International Agency for Research on Cancer (IARC) Monographs (1982; 2004; 2006), Fowler (1983), and by the National Research Council (NRC) (1999).

1 PHYSICAL AND CHEMICAL PROPERTIES

Arsenic (As): atomic weight, 74.9; atomic number, 33; metalloid; allotropes: crystalline (hexagonal-rhombic) grey arsenic (stable); density, 5.727 (25°C/4°C); melting point, 818°C (36 atm); boiling point, 615°C (sublimate); vapor pressure, 1 mmHg (372°C), yellow arsenic (metastable); density, 1.97; melting point, 815°C (under additional pressure), and black arsenic; valence states −3, 0, +3, and +5. When elemental arsenic is heated at atmospheric pressure without oxygen, it sublimes without melting and forms a yellow gas. Heating arsenic in air will yield a white smoke consisting of arsenic trioxide.

From both the biological and the toxicological points of view, arsenic compounds may be classified into three major groups: (1) inorganic arsenic compounds; (2) organic arsenic compounds; and (3) arsine gas. The toxicology of this last compound, which has special properties, will be dealt with separately in Section 10 of this chapter. The most common inorganic trivalent arsenic compounds are arsenic trioxide, sodium arsenite, and arsenic trichloride. Pentavalent inorganic compounds are arsenic pentoxide, arsenic acid and arsenate (e.g., lead arsenate and calcium arsenate). Common organic arsenic compounds are arsanilic acid, methylarsonic acid, dimethylarsinic acid (cacodylic acid), and arsenobetaine (NRC, 1999).

Arsenic trioxide is only slightly soluble in water; in sodium hydroxide it forms arsenite, and with concentrated hydrochloric acid it forms arsenic trichloride. Sodium arsenite and sodium arsenate are highly soluble in water. Interchanges in valence state may occur in water solutions, depending on the pH of the solution, as well as the presence of other substances that can be reduced or oxidized (Reay and Asher, 1977).

2 METHODS AND PROBLEMS OF ANALYSIS

Until a few decades ago, the only available methods for analyzing arsenic, such as Reinsch's method, Marsh's test, and Gutzeit's test, were qualitative rather than quantitative in nature. Because of this limitation, results of studies based on these methods must be evaluated with caution.

The molybdenum blue method and the silver diethyldithiocarbamate method are two reasonably good quantitative colorimetric methods that have a limit of detection in the range of 1–50 µg/L in a 5-mL solution (Sandell, 1959; Vasak and Sedivec, 1952). Atomic absorption spectrophotometric (AAS) measurement of generated arsine gas (Holak, 1969) has been widely used for determination of total arsenic and has a detection limit of 0.04 µg. Atomic fluorescence spectrometry (AFS) with online hydride generation (Karthiheyan and Hirata, 2003) has the advantage of less scattering and matrix effects and has a detection limit of 0.03 ug/L. Neutron activation analysis (NAA) has a detection limit of 0.1 ng for total arsenic (Liebscher and Smith, 1968). Proton-induced X-ray emission analysis (PIXEA) with a detection limit of 0.1 mg/kg has been used for the simultaneous determination of arsenic and a number of other elements in biological samples (Fowler *et al.*, 1975; Walter *et al.*, 1974). Advancements in inductively coupled plasma spectroscopy (ICP) have resulted in the general use of either ICP optical emission spectroscopy (ICP-OES) or the mass spectroscopy (ICP-MS) detection method. Although the ICP-OES method showed promise (Hueber and Winefordner, 1995), it has been largely replaced with the ICP-MS method with its higher sensitivity and less interferences than ICP-OES with the ICP-MS detection limits of 0.002 ug/L (Marawi *et al.*, 1993). The primary drawback of ICP-MS is the generation of the Ar-Cl interference in the plasma, which can be overcome by either high-resolution ICP-MS (Townsend, 1999), the use of a dynamic reaction cell (Nixon *et al.*, 2004; Tanner *et al.*, 1999) or collision cell (B'Hymer *et al.*, 2004; Nakazato *et al.*, 2002) technology. The ICP-MS is the most widely used method for the determination of total arsenic (Hung, 2004). All these methods are suitable for the measurement of total arsenic.

Several methods for the quantitative determination of the various valence states and chemical forms of arsenic have also been developed. Pulse polarography (detection limit of 0.3 µg As/L water) is capable of distinguishing the polarographically active As(III) from the inactive As(V) (Myers and Osteryoung, 1973). A method to collect generated arsines in a cold trap, evaporate them at different temperatures, and measure them by atomic emission spectroscopy has been found to have a detection limit in the order of 1 ng (Braman and Foreback, 1973). With this method, it is possible to distinguish As(III), As(V), methylarsonic acid, and dimethylarsinic acid.

Speciation of arsenic in water and other matrices like urine has seen major advances because of coupled techniques like ion chromatography (IC)

coupled to ICP (Sheppard *et al*., 1992). In this technique, the IC separates the species of arsenic before the introduction into the ICP-MS (or other detection method). This allows for the automation of the speciation method(s) for the different matrices. The method most used for speciation of the various arsenic species is high-performance liquid chromatography (HPLC) in various forms. Arsenic(III), As(V), monomethylarsonic acid (MMAA), dimethylarsinic acid (DMAA), and arsenobetaine (AsB) are separated by HPLC and determined on-line by inductively coupled plasma mass spectrometry (ICP-MS). Two forms of HPLC may be used: ion pairing and ion exchange, with absolute detection limits for arsenic ranging between 50 and 300 pg (Beauchemin *et al*., 1989). An ICP-MS detector in combination with HPLC has been used for the analysis of six arsenic compounds with detection limits for arsenic species in the range from 10–30 pg. (Demesmay *et al*., 1994).

Methods and problems of arsenic analysis, including speciation in various matrices, have been reviewed by Braman (1977, 1983), Brinckman *et al*. (1977), Hung *et al*. (2004), Munoz and Palmero (2005), Gong *et al*. (2002), and Rose *et al*. (2001).

3 PRODUCTION AND USES

3.1 Production

Arsenic is widely distributed in the earth's crust, which contains about 3.4 ppm arsenic (Wedepohl, 1991). It usually exists in nature in sulfide ores. Arsenopyrite is by far the most common arsenic-containing mineral. There are more than 150 arsenic-bearing minerals (Budavari *et al*., 2001; Carapella, 1992). Arsenic trioxide (white arsenic) is principally obtained as a by-product in the smelting of copper, lead, or gold ores. When these ores are smelted, the arsenic becomes gaseous and is trapped by electrostatic precipitators as a crude dust that is then roasted, whereby the arsenic trioxide is driven off. The purified arsenic trioxide is collected in a cooling chamber. The principal impurity is Sb_2O_3 (Pinto and McGill, 1953). Most commercially available arsenic compounds are produced from arsenic trioxide. The average annual world production of arsenic on the basis of limited data (1975–1977) was approximately 60,000 tons, and this production seems to be stable (WHO, 1981). In 2003, China was the world leader in the production of commercial-grade arsenic, followed by Chile and Peru (Brooks, 2003). The United States is the world's leading consumer of arsenic; however, the United States ceased production in 1985.

3.2 Uses

Arsenic was known as a therapeutic agent as early as 400 BC and has since been widely used as such. From the 19th century onward, an inorganic arsenic compound known as Fowler's solution (liquor arsenicalis B.P. 1963, arsenite solution containing 7.6 g As/L) has been used for the treatment of leukemia, psoriasis, and chronic bronchial asthma, and organic arsenic compounds have been extensively used in the treatment of spirochetal and protozoal disease (Martindale, 1977; NRC, 1999). Many countries have now banned the use of Fowler's solution. Recently, there has been reported use of arsenic trioxide in the treatment of acute promyelocytic leukemia (APL) (Gallagher, 1998; Wang, 2001).

The major current uses of arsenic are in pesticides (e.g., lead arsenate, calcium arsenate, and sodium arsenite), herbicides (e.g., monosodium arsenate and cacodylic acid [dimethylarsinic acid]); cotton desiccants (e.g., arsenic acid); and wood preservatives (e.g., copper chromium arsenate). Arsenic is also used as a bronzing or decolorizing agent in the manufacture of glass and in the fabrication of opal glass and enamels. It has also been used in the manufacture of dyestuffs and chemical warfare gases and is still used in the purification of industrial gases (removal of sulfur). Elemental arsenic is used as an additive in the production of several alloys to increase hardness and heat resistance. In the livestock industry, organic arsenical is sometimes added to swine and poultry feed as antimicrobial medicine. In 1999–2000, approximately 70% of the broiler industry added roxarsone to broiler poultry feed (Garbarino *et al*., 2003). Recently, gallium arsenide and indium arsenide, artificial crystals, have become important materials in semiconductors, solar cells, and space research (Brooks, 2005; IARC, 2006; Yamauchi *et al*., 1992).

4 ENVIRONMENTAL LEVELS AND EXPOSURES

4.1 Food and Daily Intake

With the exception of some kinds of fish, crustaceans, and seaweed, most food contains low levels of arsenic, normally below 0.25 mg/kg (Jelinek and Corneliussen, 1977). The degree of arsenic uptake in plants is apparently related to the concentration of soluble arsenic in the soil, the chemical composition of the soil, and plant species (Walsh *et al*., 1977). Surface contamination with insecticides may increase the concentrations of arsenic in plants. Extensive lists of arsenic concentrations in foodstuffs have been published by the National Academy of Sciences (NRC,

1999). However, all the chemical forms of arsenic in food have not yet been established. Recently, Adair *et al*. (2005) reported on two chemical and enzymatic extraction methods followed by IC-ICP-MS analysis for analysis of dimethylarsinic acid (DMA) and inorganic arsenic iAs from rice cooked in arsenic contaminated water. Measured concentrations of DMA ranged from 22–270 ngAs/g rice and for iAs, 31–108 ngAs/g rice.

Arsenic concentrations in marine organisms and seaweed are, in general, considerably higher than those in other foods. Many species of bony fish contain between 1 and 10 mg/kg, whereas certain bottom-feeding fish, crustaceans, and seaweed may contain more than 100 mg As/kg (Lunde, 1973). Both lipid-soluble and water-soluble organic arsenic compounds have been isolated from marine organisms (Kurosawa *et al*., 1980; Lunde, 1975; Penrose *et al*., 1977). Certain algae grown in the presence of arsenate may contain arsenophospholipids (Cooney *et al*., 1978; Irgolic *et al*., 1977), whereas other studies have shown that brown kelp contains mainly arsenosugars (Edmonds and Francesconi, 198la). Arsenosugars are also found in mussels, oysters, and clams (Le *et al*. 2004). Anaerobic decomposition of the brown kelp produced dimethyloxarsylethanol, a possible precursor of arsenobetaine (Edmonds *et al*., 1982). Arsenobetaine, the arsenic analog of betaine, seems to be the most commonly occurring water-soluble arsenical in fish and *Crustacea* (Cannon *et al*., 1981; Edmonds and Francesconi, 1981b; Edmonds *et al*., 1977; Kurosawa *et al*., 1980; *Luten et al*., 1982). Indications of the presence of arsenocholine in some species have also been reported (Norin and Christakopoulos, 1982). Arsenobetaine does not seem to be harmful to humans and is excreted rapidly and unchanged in urine (Cullen, 1998).

The amount of arsenic ingested daily by humans through food is greatly influenced by the amount of seafood in the diet. Jelinek and Corneliussen (1977) noted that foods belonging to the meat, fish, and poultry groups contributed most of the arsenic ingested. They estimated the average daily intake of arsenic from food in the United States in the early seventies to be 0.01–0.02 mg. The daily intake of total arsenic in Japan is reported to be 0.07–0.37 mg per person (Ishinishi *et al*., 1974).

Schoof and colleagues (1999b) reported on the analysis of 40 commodities accounting for 90% of dietary inorganic arsenic intake. Average inorganic arsenic in seafood ranged from <1–2 ng/g. The highest inorganic arsenic concentrations were found in raw rice (74 ng/g), followed by flour (11 ng/g), grape juice (9 ng/g), and cooked spinach (6 ng/g). Schoof *et al*. (1999a) estimated that intake of inorganic arsenic in the U.S. diet ranges from 1–20 µg/day, with a mean of 3.2 µg/day. Nriagu and Lin (1995) analyzed 26 brands of wild rice sold in the United States and found arsenic levels ranging from 0.006–0.142 µg/g dry weight. At a mean level of chicken consumption of 60 g/person/day, Lasky *et al*. (2004) estimated that people may ingest 1.38–5.24 µg/day of inorganic arsenic from chicken, assuming ingestion of 60 g/per person/per day of chicken.

Wine made from grapes sprayed with arsenic-containing insecticides may contain high levels of arsenic. Levels up to 0.5 mg/L have been reported, most of the arsenic being in the trivalent inorganic form (Crecelius, 1977a). Zoeteman and Brinkmann (1976) reported a mean concentration of 0.02 mg/L (range, 0.001–0.19 mg/L) in bottled mineral water. The arsenic in mineral water is probably of natural origin.

4.2 Water

Arsenic is widely distributed in surface water, groundwater, and finished drinking water. Seawater generally contains 0.001–0.005 mg As/L (Ferguson and Gavis, 1972). The arsenic concentrations of rivers and lakes vary considerably. Most levels are well below 0.01 mg/L, but in some instances, they may even be as high as about 1 mg/L (Andreae, 1978; Durum *et al*., 1971; Sagner, 1973). A survey of 293 stations in two nationwide sampling networks on major U.S. rivers found median arsenic levels to be 1 µg/L; the 75th percentile level was 3 µg/L (Smith *et al*., 1987).

The natural concentration of total arsenic in groundwater depends on the arsenic content of the bedrock. Arsenic levels in groundwater average about 1–2 µg/L, except in some western states with volcanic rock and sulfide mineral deposits high in arsenic, where arsenic levels up to 3.4 mg/L have been observed (Robertson, 1989; Welch *et al*., 1988). Approximately 13% of groundwater samples from 800 wells in an area in Nova Scotia, Canada, where the arsenic content in the bedrock is high, had concentrations exceeding 0.05 mg As/L (Grantham and Jones, 1977). Arsenic was detected in 1298 of 3452 surface water samples recorded in the STORET database for 2004 at concentrations ranging from 0.138–1700 µg/L (EPA, 2005a). In Japan, concentrations of up to 1.7 mg As/L have been recorded in hotspring water (Kawakami, 1967). In Cordoba, Argentina, groundwater levels of up to 3.4 mg As/L have been reported (Arguello *et al*., 1938). In Taiwan, artesian well water has been shown to contain up to 1.8 mg/L (Kuo, 1968).

Braman and Foreback (1973) and Crecelius (1974) noted several different forms of arsenic in natural waters: arsenate, arsenite, methylarsonic acid, and dimethylarsinic acid, the methylated forms generally being in lower concentrations than the inorganic

ones. Andreae (1978) reported that arsenate is generally the dominant form in seawater. Clement and Faust (1973) found that only approximately 8% of the total arsenic in well-aerated stream water was in the form of As(III), whereas all of the arsenic in anaerobic reservoirs seemed to be in the form of As(III). The chemical form of arsenic in different ground waters is largely unknown. Clement and Faust (1973) found that 25–50% of the total arsenic in a few groundwater samples was in the form of As(III).

The average daily intake of arsenic through drinking water can vary widely, depending on the source of the water. McCabe *et al*. (1970) reported that less than 1% of more than 18,000 community water supplies in the United States had concentrations exceeding 0.01 mg As/L. Engel and Smith (1994) investigated the levels of arsenic in drinking water throughout the United States between 1968 and 1984. They found that 30 counties in 11 states had mean arsenic levels of >5 µg/L, 15 counties had mean levels from 5–10 µg/L; 10 counties had mean levels from 10–20 µg/L; and 5 counties had levels >20 µg/L. The highest levels were found in Churchill County, Nevada, where 89% of the population was exposed to a mean arsenic concentration of 100 µg/L and 11% to a mean of 27 µg/L. More recently, investigators at the USGS (Focazio *et al*., 2000) have developed an extensive map of the occurrence of arsenic in ground water in the United States on the basis of 18,850 sample locations; 2262 of these were identified as public water supplies. The results of these studies indicate a number of states in specific regional areas (e.g., New England, the Midwest, Rocky Mountain states, and the Pacific Coast with elevated concentrations (>10.0 µg/L) in the ground water supplies, so the issue of elevated arsenic concentrations in groundwater is national in scope. In January 2001, the EPA lowered the MCL for arsenic from 50–10 µg/L (EPA, 2001). With an assumed daily intake of 1.5 L drinking water, a concentration of 0.01 mg/L will result in the daily ingestion of 0.015 mg arsenic.

Some developing countries, such as Mexico, Bangladesh, India, and Vietnam, have highly elevated levels of arsenic in drinking water in some regions (Bagla and Kaiser, 1996; Berg *et al*.. 2001; Tondel *et al*., 1999; Wyatt *et al*., 1998a; 1998b). In Bangladesh and West Bengal, India, it is estimated that more than 1 million people are drinking arsenic contaminated water and tens of millions more could be at risk in areas that have not been tested for contamination. Analysis of 20,000 tube-well waters indicated that 62% have arsenic levels in drinking water above the WHO permissible exposure limit of 10 µg/L, with some as high as 3700 µg/L (Bagla and Kaiser, 1996). Arsenic concentrations in water samples from private small-scale tube-wells in Hanoi, Vietnam, averaged 159 µg/L, ranging from 1–3050 µg/L (Berg *et al*., 2001).

4.3 Soil

Arsenic is widely distributed in the earth's crust, which contains about 3.4 ppm (Wedepohl, 1991). It is mostly found in nature as minerals, and in its elemental form only to a small extent. Typical arsenic concentrations for uncontaminated soils range from 1–40 ppm (µg/g), with the lowest concentrations in sandy soils and soils derived from granites. Higher arsenic concentrations are found in alluvial soils and soils with high organic content (Mandal and Suzuki, 2002).

Soils in mining areas or near smelters may contain high levels or arsenic. Arsenic concentrations up to 27,000 mg/kg were reported in soils contaminated with mine or smelter wastes (EPA, 1982). Soils at an abandoned mining site in the Tamar Valley in southwest England have arsenic concentrations that may exceed 50,000 mg/kg (Erry *et al*., 1999).

Soil on agricultural lands treated with arsenical pesticides may retain substantial amounts or arsenic. A study reported an arsenic concentration of 22 mg/kg in treated soil compared with 2 ppm for nearby untreated soil (EPA, 1982). Soil samples from 13 old fruit orchards in New York state, where lead arsenate was used for pest control for many years, had arsenic concentrations from 1.6–141 mg/kg (Merwin *et al*., 1994).

Natural concentrations of arsenic in sediments are usually <10 µg/g dry weight but can vary widely around the world (Mandal and Suzuki, 2002). Contamination by heavy metals is a serious problem in some developing countries. Sepetiba Bay, a semi-enclosed coastal lagoon in Brazil, had sediment arsenic concentrations up to 80,000 µg/g in an area adjacent to a plant that produced zinc and cadmium (Moreira, 1996). The literature regarding the cycling and effects of arsenic in coastal marine environments has been reviewed by Sanders *et al*. (1994).

It has recently been suggested that the wood preservative, chromated copper arsenate (CCA), commonly used in dock pilings and bulkheads, can be toxic to estuarine organisms. Wendt *et al*. (1996) measured arsenic in surface sediments from creeks with high densities of docks and from nearby reference creeks with no docks. The average concentrations in the sediments ranged from 14–17 µg/g throughout the study area, which is within the range of natural background levels.

4.4 Air

Arsenic naturally occurs in soil and is present in the atmosphere as airborne dust. It is also emitted

from volcanoes and in areas of dormant volcanism. Arsenic naturally occurs in seawater and vegetation and is released into the atmosphere in sea salt spray and forest fires (ATSDR, 2005). Smelting of nonferrous metals, burning of coal, oil and wood combustion, and the use of arsenicals as pesticides are major anthropogenic sources of airborne arsenic. Chilvers and Peterson (1987) estimated global natural and anthropogenic arsenic emissions to the atmosphere as 73,500 and 28,100 metric tons per year, respectively. Copper smelting and coal combustion accounted for 65% of anthropogenic emissions. There is evidence that anthropogenic emissions from smelters are lower than they had been in the 1980s. The National Air Toxics Assessment reported that total anthropogenic emissions for arsenic compounds in the United States in 1996 were 355 tons/year (EPA, 2005c).

Arsenic in ambient air is usually a mixture of particular arsenite and arsenate (EPA, 2005). Mean levels in ambient air in the United States have been reported to range from <1–3 ng/m^3 in remote areas and from 10–20 ng/m^3 in urban areas. The 133 stations of the U.S. National Air Sampling Network reported in 1964 that the annual average concentrations of arsenic in air ranged from nondetectable (<0.01 µg/m^3–0.75 µg/m^3) (Sullivan, 1969). The overall average was approximately 0.02 µg/m^3. The highest value (1.4 µg/m^3) was a quarterly average in El Paso, Texas, where a large copper smelter is located. Daily mean concentrations of up to 1.6 µg/m^3 have been found near a smelter in Romania (Gabor and Coldea, 1977).

Vondracek (1963) reported a summer mean air concentration of 0.07 µg/m^3 and a winter mean of 0.56 µg/m^3 in Prague, Czechoslovakia, probably because of the use of coal with a high arsenic content for heating purposes.

Inorganic arsenic, primarily as arsenic trioxide, has been reported from fossil fuel and industrial processes (Pacyna, 1987). Arsenic trisulfide has also been reported from coal combustion, organic arsines from oil combustion, and arsenic trichloride from refuse incineration.

Measurements of arsenic concentrations in cases of occupational exposure have been rare. Patty (1962) identified the highest exposure in insecticide manufacturing was usually found in the mixing, screening, drying, bagging, and drum-filling operations. He reported concentrations of arsenic in air ranging from 0.5–45 mg/m^3 during these operations. High exposure to arsenic fumes and dusts may occur in smelting industries. Lundgren (1954) reported an average concentration ranging between 0.06–2 mg/m^3 in a Swedish copper smelter. In a Japanese copper refinery, arsenic concentrations in the air of nine workshops were measured at 0.006–0.011 mg/m^3 under normally ventilated conditions and 0.08–0.19 mg/m^3 under unventilated conditions. Average arsenic concentrations in air of 0.001–0.012 mg/m^3 were found around furnaces in copper and ferronickel smelters (Kodama *et al*., 1976).

When the airborne arsenic in a U.S. copper smelter was measured during the years 1943–1965, 8-hour average levels in the arsenic roaster, arsenic refinery, and main flue ranged between 6.9 and 20 mg/m^3 (Welch *et al*., 1982). Enterline and Marsh (1982) summarized some reports of airborne arsenic concentrations in the area around an arsenic plant in the United States for the period 1938–1957. They found that the levels varied but were very high, particularly before 1951 (e.g., 0.8–62.4 mg/m^3 in 1938). All levels were very high in relation to the current permissible exposure level of 0.01 mg/m^3.

Substantial exposure to airborne arsenic may also take place in work with arsenic-containing insecticides and wood preservatives. Handling, including burning, of preserved wood could constitute a risk for the general population as well (Peters *et al*., 1984). Nygren *et al*. (1992) investigated the occupational exposure to airborne dust, chromium, copper, and arsenic in six joinery shops in Sweden where impregnated wood was used for their production. The mean airborne concentration of arsenic around various types of joinery machines ranged from 0.54–3.1 µg/m^3.

Workers at three microelectronics industries where gallium arsenide was used have been reported to have exposure at or above the OSHA action level for arsenic of 5 µg/m^3, with a maximum exposure of 8.2 µg/m^3 (Sheehy and Jones, 1993). The toxicological literature and carcinogenic potential of gallium arsenide (GaAs) have been recently reviewed by IARC (2006), and GaAs was identified as a Class I human carcinogen on the basis of the inorganic arsenic component.

The current OSHA PEL is 0.010 mgAs/m^3 (TWA). The ACGIH TLV value is 0.01 mgAs/ m^3 (TWA) (NIOSH, 2006a). For arsine, the OSHA PEL is 0.05 mgAs/m^3 (TWA). ACGIH TLV for arsine is 0.05 mg/m^3 (NIOSH, 2006b).

4.5 Tobacco

Arsenic in tobacco originates from insecticides, especially lead arsenate. In the past, the arsenic content of various brands of American cigarettes averaged 12.6 µg/cigarette in 1932–1933 and increased to 42 µg/cigarette in the early 1950s (Satterlee, 1956). After the ban of arsenical pesticides, the maximum arsenic levels were reduced to 3 µg/g (Kraus *et al*., 2000). An international literature survey reports arsenic yields of 0–1.4 µg/cigarette from mainstream (inhaled) cigarette

smoke (Smith *et al*., 1997). Arsenic emission of 0.015–0.023 µg/cigarette have been measured for sidestream smoke from a burning cigarette (Landsberger and Wu, 1995). In Japan, cigarettes have been reported to contain less than 1 µg/g (Maruyama *et al*., 1970).

5 METABOLISM

This section regarding arsenical metabolism is focused on historical descriptive *in vivo* information related to biological monitoring for arsenic in humans and animal models. Section 5.6.1 that follows reviews more mechanistic studies of arsenical metabolism and the relationships between formation of methylated arsenic species and mechanisms of toxicity at the cellular and molecular levels of biological organization.

5.1 Absorption

5.1.1 Inhalation

Airborne arsenic is usually in the form of As_2O_3. More than 23% of the particles (by particle count) in samples of arsenic-polluted air were reported to be larger than 5.5 µm (Pinto and McGill, 1953). Particles of this nature will undergo a high deposition in the upper respiratory tract. Because of deposition in the nasopharyngeal region and because of mucociliary clearance in the airways, much of the inhaled arsenic may be swallowed and then absorbed from the gastrointestinal tract. Analytical data on fly ash emitted from a coal-fired power plant have indicated a pronounced concentration of arsenic in particles of 1.1–2.1 µm diameter (Davison *et al*., 1974).

Very few data are available on the deposition and absorption of inhaled arsenic in humans. In eight terminal lung cancer patients, Holland *et al*. (1959) found that deposition was estimated to be approximately 40%, of which 75–85% was absorbed; thus, the overall absorption (expressed as a percentage of inhaled arsenic) was approximately 30–34%. Great caution must be exercised in relating these data to healthy humans. In workers exposed to arsenic trioxide dusts in smelters, the amount of arsenic excreted in the urine (the main route of excretion) was approximately 40–60% of the estimated inhaled dose (Pinto *et al*., 1976; Vahter *et al*., 1986). Absorption of arsenic trioxide dusts and fumes (assessed by measurement of urinary metabolites) correlated with time-weighted average arsenic air concentrations from personal breathing zone air samplers (Offergelt *et al*., 1992). In contrast, arsenic sulfide and lead arsenate were cleared slowly (Marafante and Vahter, 1987), indicating that the rate of absorption may be lower if the inhaled arsenic is in a highly insoluble form.

5.1.2 Ingestion

Both human and animal data indicate that more than 90% of an ingested dose of dissolved inorganic trivalent or pentavalent arsenic is absorbed from the gastrointestinal tract (Bettley and O'Shea, 1975; Charbonneau *et al*., 1978; Crecelius, 1977b; Marafante *et al*., 1981; Pomroy *et al*., 1980; Vahter and Norin, 1980). The most direct evidence is from a study that evaluated the 6-day elimination of arsenic in healthy humans who were given water from a high-arsenic sampling site and that reported approximately 95% absorption (Zheng *et al*., 2002). In contrast, ingestion of arsenic triselenide did not lead to a measurable increase in urinary excretion (Mappes, 1977), indicating that gastrointestinal absorption may be much lower if highly insoluble forms of arsenic are ingested. Organic arsenic compounds in seafoods are also readily absorbed (< 80%) from the gastrointestinal tract, both in animals and humans (Charbonneau *et al*., 1978; Marafante *et al*., 1984; Munro, 1976; Tarn *et al*., 1982; Vahter *et al*., 1983). On the basis of urinary excretion studies in volunteers, it seems that both MMA and DMA are well absorbed (at least 75–85%) across the gastrointestinal tract (Buchet *et al*., 1981a). Animal experiments indicate that growth promoters, such as arsanilic acid, are absorbed to only 15–40% (Calvert, 1975).

In the case of arsenic trioxide, which is only slightly soluble in water, the gastrointestinal absorption will depend on factors such as particle size and pH of the gastric juice.

5.1.3 Skin Absorption

Systemic toxic effects have resulted from occupational accidents where arsenic acid or arsenic trichloride have been splashed on workers, indicating that the skin is a possible route for absorption of arsenic (Garb and Hine, 1977). However, no quantitative studies were located on absorption of inorganic arsenicals in humans after dermal exposure. Percutaneous absorption of ^{73}As as arsenic acid has been measured in skin from cadavers (Wester *et al*., 1993). Labeled arsenic was applied to skin in diffusion cells, and transit through the skin into receptor fluid measured. After 24 hours, 0.93% of the dose passed through the skin, and 0.98% remained in the skin after washing. Dermal absorption of arsenic has been measured in Rhesus monkeys (Wester *et al*., 1993). After 24 hours, 6.4% of ^{73} As as arsenic acid was absorbed systemically.

5.2 Transport and Distribution

After absorption by the lungs or the gastrointestinal tract, arsenic is transported by the blood to other parts of the body. After exposure to arsenite or arsenate, most arsenic is cleared from the blood at a very high rate (Charbonneau *et al*., 1978; Marafante *et al*., 1981; Mealey *et al*., 1959). In rats, however, a substantial part of the absorbed arsenic is accumulated in the red blood cells, where it is firmly bound to hemoglobin (Hisanaga, 1982; Marafante *et al*., 1982). Data from a number of investigators indicate that the main form of arsenic bound to rat hemoglobin is dimethylarsinic acid (Lerman and Clarkson, 1983; Rowland and Davies, 1982; Vahter *et al*., 1984). As a consequence of the efficient biotransformation of both trivalent and pentavalent inorganic arsenic *in vivo* (see later), the tissue partitioning is somewhat similar for the different exposure forms. In man, as well as in most animal species, exposure to either arsenite or arsenate leads to an initial accumulation in the liver, kidneys, and lungs (Bertolero *et al*., 1981). Tissue analysis of organs taken from an individual after death from ingestion of 3 g of arsenic (in the form of arsenic trioxide) showed a much higher concentration of arsenic in liver (147 μg/g) than in kidney (27 μg/g) or muscle, heart, spleen, pancreas, lungs, or cerebellum (11–12 μg/g) (Benramdane *et al*., 1999). Small amounts were also found in other parts of the brain (8 μg/g), skin (3 μg/g), and hemolyzed blood (0.4 μg/g). The clearance from these tissues is, however, rather rapid, and a long-term retention of arsenic is seen in hair, skin, squamous epithelium of the upper gastrointestinal tract, epididymis, thyroid, lens, and skeleton (Lindgren *et al*., 1982). Human autopsy data have also shown high arsenic levels in hair, nails, teeth, bone, and skin (Kadowaki, 1960; Liebscher and Smith, 1968; Sumino *et al*., 1975; Yukawa *et al*., 1980).

Arsenic passes through the placenta in hamsters after intravenous injections of high doses of sodium arsenate (Ferm, 1977). Kadowaki (1960) reported an increase in arsenic levels in the human fetus as pregnancy progressed. The arsenic levels in bone, liver, skin, and brain were 2–4 times higher in newborn babies than in 7-month-old fetuses. In a population of Andean women exposed to high concentrations (about 200 ppb) of inorganic arsenic in drinking water, concentrations of arsenic in breast milk ranged from about 0.0008–0.008 ppm (Concha *et al*., 1998).

Although the distribution pattern after exposure to different chemical forms of inorganic arsenic is similar, recent studies in mice and rabbits have shown that arsenite gives rise to higher concentrations than arsenate in most tissues (Lindgren *et al*., 1982; Vahter and Marafante, 1983; Vahter and Norin, 1980). Only the kidneys and the skeleton had higher levels in the arsenate-treated than in the arsenite-treated animals. In mice, radiolabel from orally administered ^{74}As was widely distributed to all tissues, with the highest levels in skin, kidney, and liver (Hughes *et al*., 2003). The major part of injected radioactive arsenic accumulating in the tissues of mammals has been found in the protein fraction (Marafante *et al*., 1981). Trivalent arsenicals can combine chemically with sulfhydryl groups, which may explain the inhibition of enzymatic reactions (Webb, 1966), as well as the accumulation in keratin-rich tissues such as hair, skin, and epithelium of the upper gastrointestinal tract.

It has also been found that the toxic effects of arsenic can be reversed by parenteral administration of thiol compounds of the reduced type (e.g., glutathione, cysteine, and BAL (British antilewisite) (Peters, 1955; Vallee *et al*., 1960; Webb, 1966). Arsenate, on the other hand, may interfere with metabolic phosphorylation reactions, probably because of its chemical similarities with phosphate (Fowler *et al*., 1975; Mitchell *et al*., 1971). Such chemical similarities can also explain the accumulation of arsenate in the skeleton (Lindgren *et al*., 1982; 1984).

When particles of inorganic arsenic compounds such as arsenic trioxide, calcium arsenate, and arsenic trisulfide were intratracheally instilled into the lung of rat or hamster, arsenic trioxide and arsenic trisulfide rapidly disappeared from the lung decreasing to a level of approximately 0.1–1% of the given dose over a time period of 24 hours to 1 week. However, half the given dose of calcium arsenate given to Syrian Golden hamsters was retained in the lung for 1–5 weeks (Inamasu *et al*., 1982; Pershagen *et al*., 1984).

In hamsters, MMA and DMA formed *in vivo* by methylation of inorganic arsenic seem to be distributed to all tissues (Takahashi *et al*., 1988; Yamauchi and Yamamura, 1985). This is supported by studies in animals in which MMA and DMA were found in all tissues after acute oral doses (Yamauchi and Yamamura 1984; Yamauchi *et al*., 1988). Studies on the tissue distribution of arsenobetaine in mice and rabbits have shown that this arsenical is rapidly cleared from most tissues (Vahter *et al*., 1983). The longest retention was observed in cartilage, testes, epididymis, and muscles. Animal data indicate that arsenocholine, like choline, may be incorporated into phospholipids (Marafante *et al*., 1984).

5.3 Biotransformation

The biotransformation of inorganic arsenic is complicated by the different metabolites induced and by differences among species. Two basic processes

are involved: (1) reduction/oxidation reactions that intervert As(III) and As(V), and (2) methylation reactions, which convert arsenite to MMA and DMA. The resulting series or reactions result in the reduction of inorganic arsenate to arsenite (if necessary), methylation to MMA(V), reduction to MMA(III), and methylation to DMA(V). These processes seem to be similar whether exposure is by the inhalation, oral, or parenteral route.

That trivalent inorganic arsenic is oxidized *in vivo* is indicated by the finding of pentavalent arsenic in urine of both animals and humans exposed to arsenite (Bencko *et al*., 1976; Mealey *et al*., 1959; Vahter and Envall, 1983). Also, the opposite reaction (i.e., the reduction of arsenate to arsenite) has been demonstrated in mice and rabbits (Vahter and Envall, 1983). Both arsenite and arsenate, after reduction to arsenite (McBride *et al*., 1978), are methylated *in vivo*. The major metabolite in urine of experimental animals exposed to inorganic arsenic is dimethylarsinic acid (Bertolero *et al*., 1981; Charbonneau *et al*., 1979; Inamasu, 1983; Tam *et al*., 1978; Vahter, 1981). The guinea pig, marmoset, and tamarin monkey do not methylate inorganic arsenic (Healey *et al*., 1998; Vahter and Marafante, 1985, Vahter *et al*., 1982; Zakharyan *et al*., 1996). Exposure of humans to either arsenites or arsenates results in increased levels of inorganic As(III), As(V), MMA, and DMA in urine (Aposhian *et al*., 2000a, 2000b). In man, the urinary excretion at low-dose levels consists of approximately 20–25% inorganic arsenic, 15–25% methylarsonic acid, and 40–75% dimethylarsinic acid (Buchet *et al*., 198l**a;** Crecelius, 1977b; Hopenhayn *et al*., 2003; Loffredo *et al*., 2003; Mandal *et al*., 2001; Smith *et al*., 1977; Tam *et al*., 1979; Yamauchi and Yamamura, 1979a,b). Methylation efficiency decreases with increasing dose levels (Mahieu *et al*., 1981; Vahter, 1981**).** The substrate for methylation is As(III), and As(V) is not methylated unless it is first reduced to As(III) (Buchet and Lauwerys 1985, 1988; Lerman *et al*., 1983). Reduction of arsenate to arsenite can be mediated by glutathione (Menzel *et al*., 1994). The main site of methylation seems to be the liver, where the methylation process is mediated by methyltransferases that use S-adenosylmethionine as cosubstrate (Buchet and Lauwerys, 1985; 1988). The relative proportions of As(III), As(V), MMA, and DMA in urine can vary, depending on the chemical administered, time after exposure, dose level, and exposed species. Arsenic derived from exposure to particles of GaAs or InAs is methylated in manner similar to that of As(III) (Yamauchi *et al*., 1986; 1992).With relatively constant exposure levels, these metabolic proportions remain similar over time (Concha *et al*., 2002) and seem to be similar among family members (Chung *et al*., 2002). It is expected that measurements of MMA and DMA in urine will provide useful information for risk assessment purposes once the relationships between *in vivo* formation of these major metabolites of inorganic arsenic and risk of toxicity or cancer are more fully delineated.

Arsenobetaine is apparently not biotransformed *in vivo* but excreted as such mainly in urine (Cannon *et al*., 1981; Vahter *et al*., 1983). Arsenocholine is, to a great extent, oxidized to arsenobetaine (Marafante *et al*., 1984).

Cacodylic acid and arsanilic acid are not converted to inorganic arsenic *in vivo* (Buchet *et al*., 1981a; Marafante *et al*., 1987; Stevens *et al*., 1977; Vahter *et al*., 1984; Yoshida *et al*., 2001).

5.4 Excretion

The major route of excretion after exposure to inorganic arsenic is through the kidneys. Only a low percent is excreted in feces (Apostoli *et al*., 1999; Bertolero *et al*., 1981; Ducoff *et al*., 1948; Hunter *et al*., 1942; Mealey *et al*., 1959). The rate of excretion in urine varies, depending on the chemical form of arsenic and the species exposed. The primary forms of arsenic found in the urine of inhalation-exposed humans are DMA and MMA, with inorganic arsenic making up <25% of the total urinary arsenic (Apostoli *et al*., 1999). In humans exposed to a single low dose of arsenite, approximately 35% was excreted in the urine over a period of 48 hours (Buchet *et al*., 1980; 1981a**).** At low exposure levels, urinary arsenic levels generally increase linearly with increasing arsenic intake (Calderon *et al*., 1999). Rabbits exposed to a similar low dose excrete approximately 80% (Bertolero *et al.,* 1981), mice as much as 90% (Vahter, 1981), and marmoset monkeys as little as 15% within the same period of time (Vahter *et al*., 1982). In the case of continuous human intake over a few days, 60–70% of the daily dose is excreted in urine (Buchet *et al*., 1981b; Mappes, 1977). After exposure to arsenate, the limited human data available indicate a rate of excretion similar to that for arsenite (Pomroy *et al*., 1980; Yamauchi and Yamamura, 1979b). Animal data indicate a somewhat faster excretion for arsenate than for arsenite (Charboneau *et al*., 1978; Hollins *et al*., 1979; Vahter, 1981).

During lactation, a very small percent of ingested arsenic may also be excreted in the breast milk (Concha *et al*., 1998a). Other routes of elimination of inorganic arsenic, although of less importance, include skin, hair, nails, and sweat (Molin and Wester, 1976).

Studies in humans indicate that ingested MMA and DMA are excreted mainly in the urine (75–85%), and this occurs mostly within 1 day (Buchet *et al*., 1981a). After ingestion, by humans, of organic arsenic

compounds present in seafoods, 50–80% of the dose was eliminated in the urine within 2 days (Tam *et al*., 1982). A similar rate of excretion was seen in rabbits exposed to arsenobetaine (Vahter *et al*., 1983).

Elimination of organic arsenic compounds used as drugs was noted by Hogan and Eagle (1944). They found that trivalent organic arsenicals were eliminated at a much slower rate than the pentavalent arsenicals in the urine of rabbits. More recent studies by Lin *et al*. (2005) reported that the tissue-specific distribution and elimination rates for inorganic and methylated arsenic species in rabbits after a single acute administration of arsenic trioxide (AsIII) over a dose range of 0.2–1.5 mgAs/kg for up to 30 and 60 days. The elimination pattern was nonlinear, and the liver and lung showed the highest concentrations of DMA, which was the major metabolite measured, on day 30. Similar organ-specific differences were observed in B6C3F1 mice after acute oral administration of arsenate (AsV). As with the rabbits, DMA was the predominant methylated species at later time points (Kenyon *et al*., 2005).

5.5 Biological Half-Time

Animals exposed to arsenic through inhalation or drinking water will have increased levels in tissue during the first weeks or months, but the levels will decrease even if the exposure is prolonged (Bencko and Symon, 1969; 1970; Hisanaga, 1982; Katsura, 1958). It has been suggested that the decrease of arsenic concentrations in internal organs, such as the liver, after long-term exposure may be due to a higher rate of excretion. Changes in tissue retention in the case of chronic exposure should be considered when evaluating biological half-times for arsenic compounds.

The biological half-time of arsenic in rats after a single exposure is long (approximately 60 days) because of the accumulation of arsenic in the blood. In humans and in other animals, the major part of the arsenic is eliminated at a much higher rate. Generally, whole body clearance is fairly rapid, with half-times of 40–60 hours in humans (Buchet *et al*., 1981b; Mappes, 1977). Three different phases of urinary excretion have been demonstrated in man after a single intravenous injection of radiolabeled arsenite, having half-times of approximately 2 hours, 8 hours, and 8 days, respectively (Mealey *et al*., 1959). After oral intake of radiolabeled pentavalent arsenic, 66% was excreted with a half-time of 2.1 days, 30% with a half-time of 9.5 days, and 3.7% with a half-time of 38 days in the three phases (Pomroy *et al*., 1980).

As stated previously, the major part of arsenic in ingested seafood is eliminated rapidly through the kidneys. The biological half-time for these organic compounds can be estimated to be less than 20 hours. Studies in humans indicate that ingested MMA and DMA are excreted mainly in the urine (75–85%), and this occurs mostly within 1 day (Buchet *et al*., 1981a).

The WHO/IPCS (2007) has examined a number of issues related to the roles of elemental speciation in human health risk assessment.

5.6 Mechanisms of Arsenical Toxicity

5.6.1 Mechanisms of Arsenical Metabolism and Toxicity

Arsenical inhibition of cellular respiration as a primary cause of cell death has been appreciated for a number of decades (ATSDR, 2005; NRC, 1999; 2001), and the methylation of inorganic arsenic to form methylated species such as methyl arsenic acid (MMA) and dimethylarsinic acid (DMA) has also been studied for a number of decades (Braman and Foreback, 1973; Challenger, 1945; 1951). In recent years, scientific attention has been focused on understanding the relationships that must exist between the *in vivo* methylation of inorganic arsenic and mechanisms of arsenical toxicity. This issue is of great practical importance, because the methylation of inorganic arsenic was originally thought to be a detoxification pathway, but more recent studies (NRC, 1999) have suggested that highly toxic reactive oxygen species (ROS) generated by MMA (III) and DMA (III) mitochondrial toxicity may also play an important role in both cellular toxicity and carcinogenicity of this element. The increased presence of MMA (III) in the urine of a Mexican population exposed to inorganic arsenic in drinking water on a chronic basis was found to be a basis for identifying subpopulations at greater risk for arsenic-induced toxicity and cancer (Steinmaus *et al*., 2005a,b; Valenzuela *et al*., 2005). These studies also reported a strong linkage between dietary intakes of protein and other nutrients and the ability to methylate arsenicals such that persons with low dietary intakes of these nutrients would be more susceptible than others to arsenic-induced cancers. The central role of oxidative stress induced by arsenic in cell death via apoptosis or necrosis and carcinogenicity via oxidative damage to cellular DNA cannot be underestimated, because it may permit attenuation of these deleterious cellular effects through nutritional interventions and stimulation of cellular antioxidant systems.

5.6.2 Metabolism

This section regarding arsenical metabolism is focused on reviewing mechanistic linkages between mechanisms of inorganic arsenical methylation and mechanisms of toxicity at the cellular and molecular levels of biological organization.

5.6.2.1 Arsenical Reduction/Oxidation

A key step in the methylation pathway for inorganic arsenicals is the reduction and oxidation of the arsenic between the ±3 and +5 oxidation states during the various steps in the methylation pathway. Studies by Styblo and Thomas (1995) reported the inhibition of glutathione reductase by arsenoglutathione *in vitro* and demonstrated that reduced glutathione (GSH) was required for the *in vitro* methylation of arsenic.

More recent studies by Nemeti and Gregus (2005) demonstrated reduction of As 5+ to As3+ via human erythrocyte lysates and rat liver cytosol through an arsenic reductase, which required glycolytic substrates, GSH, and nicotinamide diamide (NAD) for the activity of the enzyme. Zakharyan *et al*. (2005) studied the interaction of selenite with the omega class human glutathione transferase, which is an identical protein with MMA 5+ reductase, which catalyzes the reduction of As (V), MMA (V), and DMA(V). They found that selenite acted as a noncompetitive inhibitor of this enzyme. The tissue dosimetry of pentavalent and trivalent MMA was studied by Hughes *et al*. (2005), and they found that a much greater percentage of the administered dose of MMA (III) was converted to DMA than after administration of MMA (V).

5.6.2.2 Arsenical Methylation

The main characteristics of the pathway for inorganic arsenic methylation have been appreciated since the pioneering work by Challenger in the 1940s (1945). The pathway involves a series of reductions and oxidation steps which interconvert As 5+ to As 3+ to As 5+ during the methylation steps, ultimately resulting in the formation of MMA (V) and DMA (V). The further metabolism and excretion of DMA and trimethylarsine oxide has been extensively studied in rodents by Yamauchi and coworkers (Yamauchi and Yamaura, 1984; Yamauchi *et al*., 1989; 1990). The enzyme responsible for reduction of AsV to AsIII has not been identified, but a glutathione (GSH) and NAD-dependent AsV reductase activity that is linked to glycolysis has been recently identified in rat liver cytosol and human erythrocyte lysate by Nemeti and Gregus (2005). During this metabolism MMA (III) and DMA (III) are also formed, and MMA(III) and DMA(III) in particular have been demonstrated to be a highly toxic and reactive chemical species (Mass *et al*., 2001). The kinetics of *in vitro* arsenic methylation in mouse hepatocytes have been recently reported by Kedderis *et al*. (2006), who found a concentration-dependent formation of MMA(III) up to a concentration of about 2 μmol/L As(III) followed by a decline at higher concentrations. They also suggested a role for a glutathione complex–based mechanism for this methylation pathway. This hypothesis is consistent with observations from a number of other laboratories (Chang *et al*., 1991; Csanaky *et al*., 2003; Hayakawa *et al*., 2005; Hirano *et al*., 2004; Kala *et al*., 2004; Kobayashi *et al*., 2005; Waters *et al*., 2004). The formation of arsenic–glutathione complexes has also been reported (Cui *et al*., 2004; Suzuki *et al*., 2001) to play important roles in the biliary and urinary excretion of arsenic in rats.

In recent years, an arsenic methyl transferase (AsMT) has been identified (Drobna *et al*., 2005; Li *et al*., 2005; Wood *et al*., 2006) and demonstrated to exist as a number of polymorphic forms (Wood *et al*., 2006). Zakharyan *et al*. (2005) studied the interactions of arsenic species with selenite, glutathione, and the omega class of human glutathione transferase. They found that human monomethylarsenate reductase and human glutathione S- transferase omega 1-1 were identical proteins and that selenite inhibition of the MMA (V) was reversed by dithiothreitol but not GSH, which is the required substrate of this enzyme. They found that three selenium atoms and three GSH molecules were bound to the monomeric form of the enzyme. This enzyme and its demonstrated polymorphic forms may well explain observed differences in methylation patterns for both animals and humans (NRC, 1999) and differences in susceptibility to a variety of toxic effects including various cancers (Smith *et al*., 2005), hepatomegally (Guha Mazumder *et al*., 2005), and heme and porphyrin metabolism (Fowler *et al*., 2005; Garcia-Vargas *et al*., 1994; Ng *et al*., 2005; Woods and Fowler, 1978; Yamauchi *et al*., 1998).

5.6.3 Mechanisms of Arsenical Toxicity

5.6.3.1 Mitochondrial Inhibition and Oxidative Stress

Arsenical inhibition of mitochondrial respiration supported by NAD-linked substrates that use the lipoic acid cofactor for the pyruvate dehydrogenase complex is regarded as a primary mechanism by which arsenicals produce cell injury/cell death and cancer (Fowler and Woods, 1979; Fowler *et al*., 1979; NRC, 1999). Inorganic arsenicals, monomethyl arsenic acid, and dimethyl arsenic acid have each been shown to produce oxidative stress by means of inhibition of mitochondrial respiration resulting in the formation of reactive oxygen species (ROS), which may cause DNA mutations and ultimately play a role in the development of cancer (Liu *et al*., 2005). In addition, either acute or chronic exposure to arsenicals had been shown to produce a characteristic porphyrinuria pattern in experimental animals (Conner *et al*., 1993; 1995; Fowler *et al*., 2005;

Woods and Fowler, 1978) and humans (Garcia-Vargas *et al.*, 1994; Garcia-Vargas and Hernandez-Zavala, 1996; Ng *et al*., 2002; 2005; Yamauchi *et al*., 1998) dominated by elevated levels of uroporphyrins with lesser amounts of coproporphyrin. This pattern is, hence, conserved across species and may serve as a useful biomarker for early arsenical toxicity to target organs such as the liver and hematopoietic system. It should also be noted that porphyrins are also toxic and capable of catalyzing formation of ROS, thus exacerbating direct arsenical toxicity.

5.6.3.2 Reactive Oxygen Species

The reactive oxygen species generated by inhibition of mitochondrial respiratory function and ATP depletion (Chen *et al*., 1987) produce oxidative stress in a number of major organ systems (Flora *et al*., 2005; Li *et al*., 2002; Ramos *et al*., 1995) and elicit a number of cellular responses, including up-regulation of glutathione synthesis (Flora *et al*., 2005), induction of the major stress protein families (Lee *et al*., 2005; Madden *et al*., 2002, Sen *et al*., 2005), and altered regulation of signaling pathways involving nitric oxide synthetase (eNOS) (Tsou *et al*., 2005), and nuclear factor kappa-B and activator protein-1 (Felix *et al*., 2005). These various cellular responses to arsenical-induced oxidative stress are discussed later in greater detail.

5.6.3.3 Antioxidant Cellular Defense Systems

5.6.3.3.1 Glutathione A major intracellular antioxidant is reduced glutathione (GSH), which exists in many cell types at millimolar concentrations (Reed, 2001). Increased production of this small SH-rich molecule may occur by induction of glutathione synthetase in response to ROS mediated oxidative stress (Reed, 2001). Glutathione depletion studies using L-butathionine sulfoximine (BSO) have produced increased sensitivity among arsenic-exposed cells exposed to arsenic (III) *in vitro* (Maiti and Chatterjee, 2001; Shimizu *et al*., 1998), indicating the importance of this intracellular antioxidant molecule in mediating arsenical toxicity in target cell populations.

5.6.3.4 Stress Proteins/Heat Shock Proteins (HSPs 25/27, 32, 60, 70, 90)

Arsenicals are known to induce a number of the major stress protein families presumably as a consequence of ROS formation with attendant oxidation of protein side chains and the onset of proteotoxicity. Cells of similar nature vary in their a susceptibility to arsenicals in part because of the ability to produce stress proteins as demonstrated by Madden *et al*. (2002), who showed that rat and human kidney cell lines varied in their sensitivity as a function of 60-, 70-, and 90-kDa stress protein expression patterns. For both cell lines, toxicity occurred once the capacity to produce the stress proteins was exceeded at elevated dose levels. Similar results have been reported for hsp70 in lung cells (Han *et al*., 2005; Lau *et al*., 2004) and in human epidermal cells (Lee *et al*., 2005). Similar responses have been observed in female rat urothelial cells after *in vivo* exposure to DMA for 28 days (Sen *et al*., 2005) and the human UROtsa cell line exposed to arsenite (Rossi *et al*., 2002). Arsenite induction of hsp70 has been consistently observed by a number of investigators in a variety of cell types, including hepatoma cell lines (Farzaneh *et al*., 2005; Gottschlag *et al*., 2006), human MCF-7 breast cancer cells (Barnes *et al*., 2002), human lung fibroblasts (HEL cells) or human leukemia cells (THP-1 cells) (Rossner *et al*., 2003), rat oligodendrocytes (Goldbaum *et al*., 2001), and chick embryos (Papacanstantinou *et al*., 2003).

In addition to the major stress protein families, the formation of ROS also induces the SH-rich protein metallothionein (MT), which also has antioxidant properties (Gottschlag *et al*., 2006; Liu *et al*., 2001). It should also be noted that studies by Shimizu *et al*. (1998) reported that zinc induction of MT exerted no discernible protective effects on arsenical toxicity, so the potential mechanisms of protection against arsenical toxicity may be complex and system dependent. The lower molecular mass hsps 25(rat)/27(human) have also been reported to be induced by arsenite exposure in mouse kidney podocyte cultures (Eichler *et al*., 2005; 2006) seem to also play major roles in roles in the regulation of apoptosis via the extrinsic caspase 8 pathway (Eichler *et al*., 2006). It is interesting to note that arsenic (III) induction of the stress protein response for hsp 27 and hsp 70 has been shown to be regulated by reducing agents such as dithioerythritol (Kato *et al*., 1997) and by reduced levels of glutathione (GSH) in arsenic-exposed cells (Ito *et al*., 1998), indicating the strong linkage between arsenic-induced oxidative stress and stress protein induction patterns. In addition, arsenite exposure of cells has also been well documented to induce hsp32 (heme-oxygenase-1), which is the rate-limiting enzyme in the heme degradative pathway (Gong *et al*., 2002; Lee *et al*., 2005) and plays a major role in the recycling of heme iron.

5.6.3.5 Altered Cell Signaling Pathways

Arsenic-induced alterations of the blood vasculature leading to the development of "Blackfoot disease" are common findings among persons exposed to arsenic in drinking water. Studies by Tsou *et al*. (2005) reported down-regulation of nitric oxide synthetase (eNOS), supporting the hypothesis that alteration of eNOS function may play an important role in mediating this

clinically important outcome. Studies by others (Wu *et al*., 1999; 2002) demonstrated a number of alterations in cell signaling pathways, including the MAP kinases and extracellular regulated kinase (ERK) and Ras via the epidermal growth factor (EGF) receptor pathway in a human airway epithelial cell line (BEAS-2B). Similar alterations in the c-jun/AP-1 and NFkappaB signal transduction systems with concomitant alterations in the expression of a number of the major stress protein families (hsp32 and hsp 72) were observed by Wijeweera *et al*. (2001) in precision-cut rat lung slices.

5.6.3.6 Mechanisms of Cell Death

5.6.3.6.1 Apoptosis Arsenite at low *in vitro* dose levels has been demonstrated to produce apoptosis in rat thymocytes (Bustamente *et al*., 1997). Subsequent studies (Tian *et al*., 2005) demonstrated that As_2O_3 induces opening of the mitochondrial membrane permeability transition pore with attendant release of cytochrome *c* and intramitochondrial calcium stores. Other investigators (Zhang *et al*., 2003; Zheng *et al*., 2005) have shown that As_2O_3 also induces Bax activation in hematopoietic cells secondary to ROS formation. In agreement with the roles of ROS in mediating arsenical induction of apoptosis, administration of antioxidants such as ascorbic acid and alpha-tocopherol have been shown (Ramanathan *et al*., 2005) to reduce TNF-alpha levels and activation of the caspase cascade system. The value of arsenicals in mediating apoptosis has been explored in relation to the treatment of various cancers such as acute megakaryocytic leukemia (Lam *et al*., 2005) or promyelocytic leukemia (Zheng *et al*., 2005), where As_2O_3 has been shown to activate a number of central factors in the apoptotic pathway. Similar delays in recovery of leukocytopenia and neutropenia during acute promyelocytic leukemia have been reported after treatment of patients with As_2O_3 relative to treatment with all-trans retinoic acid (Shinjo *et al*., 2005). On the other hand, long-term, low-level exposure of cultured human keratinocytes to arsenite has been shown (Pi *et al*., 2005) to produce a generalized resistance to induction of apoptosis, which may provide a role for arsenic as a cocarcinogen with UV light in skin cancer as discussed later. A number of other investigators have reported similar arsenic-induced alterations of apoptosis regulation in other cell types such as neuronal (Jou *et al*., 2004; Namgung and Xia, 2000) and lymphoma cell lines (Muscarella and Bloom, 2002) and in developing mouse embryos (Liu *et al*., 2003). Interestingly, the heat shock protein 70 (hsp70) discussed earlier has been reported to protect against arsenic-induced apoptosis (Mosser *et al*., 2000) at a step in apoptotic pathway after cytochrome *c* release and before caspase 3 activation (Li *et al*., 2000).

5.6.3.7 Necrosis

As with many toxic metals, cellular necrosis is usually observed at high dose levels after administration of arsenicals (Shen *et al*., 2006) such that there is strong dose dependency as to whether apoptosis or necrosis is observed after exposure to this agent.

5.6.3.8 Cancer

In recent years, there have been many detailed reports on the diagnosis, molecular markers, and possible underlying mechanisms of arsenic-induced cancers in various organ systems. As with the mechanisms of cell injury and apoptosis discussed previously, the generation of oxidative stress from mitochondrial production of ROS seems to be a central feature for initiation of cancers (NRC, 1999). Yu *et al*. (2006) reviewed the arsenic-induced skin cancers and the probable combined roles of arsenic exposure and UVB radiation in producing skin lesions. Kuo *et al*. (1997) examined the possible roles of overexpression of the p53 gene in skin cancer and concluded that overexpression of this gene was not a prerequisite for development of skin cancer. Dong (2002) noted increased phosphorylation of extracellular signal-regulated kinases in cell lines and that arsenic did not induce p53 transactivation. Waalkes *et al*. (2004) reported altered estrogen signaling in male mice with hepatocellular carcinoma produced by *in utero* exposure to arsenic. They concluded that overexpression of estrogen receptor-alpha (ER-alpha) through probable hypomethylation of the promoter region of this gene may play a role in hepatocarcinogenesis in these mice. Subsequent studies by Cui *et al*. (2006) have demonstrated that arsenic trioxide does inhibit DNA methyl transferase and activates methylation-inhibited genes in human HepG2 liver cancer cells. Hour *et al*., (2006) reported differential expression of various molecular markers in surgical samples of uroepithelial cells from arsenic- exposed and nonarsenic-exposed patients. They found decreased GSH content in cells from the arsenic-exposed patients and increased expression of *bcl-2* and *c-Fos*. Shen *et al*. (2006) examined the effects of three methylated arsenicals (MMA [V], DMA [V], and TMAO) on the urinary bladder epithelium of both male and female rats exposed to these substances for 13 weeks. They noted both metabolic differences between males and females with regard to further metabolism and concluded that DMA (V) is more toxic to the bladder epithelium of female rats, which are also more susceptible to bladder carcinogenesis from this agent.

Mizoi *et al*. (2005) reported that oxidative stress caused by DMA(III) generated as a reduction product of DMA (V) seemed to play a central role in lung and skin tumors in mice exposed to DMA (V) as judged by

the increased formation of 8-oxo-2'deoxyguanosine (8-OHdG) DNA adducts. It is also important to note the increased presence of 8-OHdG adducts has been reported in urine samples of persons living in Inner Mongolia (Fujino *et al*., 2005) and Cambodia (Kubota *et al*., 2006) exposed to arsenic in contaminated drinking water, suggesting that these adducts may be potentially useful biomarkers for long-term risk for arsenic-induced cancers such as Bowen's disease (Matsui *et al*., 1999). It is important to note that the stress protein response discussed previously may play an important regulatory role in mediating carcinogenic or cocarcinogenic effects of arsenic by means of interactions with a number of proto-oncogenes and apoptotic caspase system (Khalil *et al*., 2006; Stanhill *et al*., 2006; Yaglom *et al*., 2003).

6 BIOLOGICAL MONITORING

In a review by Mandal and Suzuki (2002), they document many cases of human arsenic exposures around the world, as well as documenting sources of arsenic in the environment.

6.1 Organs

Median arsenic concentrations in organs of healthy people from Scotland who died in accidents ranged from 0.012 mg/kg in brain to 0.46 mg/kg in hair, all dry weight (Liebscher and Smith, 1968). Median concentrations in organs of people from Japan, who generally had consumed relatively high amounts of seafood, ranged from 0.02 mg/kg wet weight in the pancreas to 0.89 mg/kg in the nails (Kadowaki, 1960). The two first-mentioned authors used NAA on vacuum dried samples, and the third used polarography. Values for all organs analyzed in the two studies are given in Table 1.

Tissue analysis of organs taken from an individual after death from ingestion of 8 g of arsenic trioxide (approximately 3g of arsenic) showed a much higher concentration of arsenic in the liver (147 μg/g) than in the kidneys (27 μg/g) of muscle, heart, spleen, pancreas, lungs, or cerebellum (11–12 μg/g) (Benramdane, *et al*. 1999). Small amounts were also found in other parts of the brain (8 μg/g), skin (3 μg/g), and hemolyzed blood (0.4 μg/g).

Inorganic arsenic can easily pass through the placenta. High levels of arsenic were found in the liver, kidney, and brain during autopsy of an infant prematurely born to a young mother who had ingested inorganic arsenic at week 30 of gestation (Lugo *et al*., 1969).

Arsenic was detected in human breast milk at concentrations of 0.00013–0.00082 ppm (Somogyi and Beck, 1993). In a population of Andean women exposed to high concentrations (approximately 200 ppb) of inorganic arsenic in drinking water, concentrations of arsenic in breast milk ranged from about 0.0008–0.008 ppm (Concha *et al*., 1998a).

It is not known to what extent concentrations in human organs represent inorganic arsenic or organic arsenic originating in the diet. Concentrations of 0.014–0.21 mg As/kg wet weight have been observed in the lungs of smelter workers who had retired 2–19 years before death, whereas unexposed controls had 0.001–0.018 mg/kg, indicating a long retention time of arsenic in the lungs after inhalation, which may occur in certain occupations (Brune *et al*., 1980).

6.2 Urine

Most arsenic that is absorbed from the lungs of the gastrointestinal tract is excreted in the urine within 1–2 days. Therefore, measurement of urinary arsenic levels

TABLE 1 Arsenic Concentrations in Human Organs and Tissues in Scotland and Japan

	Arsenic concentration (mg/kg)		
	Scotland (dry wt.)		Japan (wet wt.)
Tissue/organ	Median	Range	Median
Adrenal	0.029	0.002–0.293	
Aorta	0.031	0.003–0.570	
Whole blood	0.038	0.001–0.920	
Bone	0.057	0.010–0.240	0.118(femur)
Bone			0.074(rib)
Brain	0.013	0.001–0.036	0.034
Hair	0.460	0.020–8.17	0.174
Heart	0.024	0.002–0.078	0.041
Intestine, large			0.025
Intestine, small			0.022
Kidney	0.033	0.002–0.363	0.041
Liver	0.028	0.005–0.246	0.042
Lung	0.082	0.006–0.514	0.047
Muscle	0.063	0.012–0.431	0.029
Nail	0.300	0.020–2.90	0.892
Ovary	0.037	0.013–0.260	
Pancreas	0.045	0.005–0.410	0.020
Prostate	0.046	0.010–0.090	
Skin	0.090	0.009–0.590	0.064
Spleen	0.020	0.001–0.132	0.021
Stomach	0.037	0.003–0.104	0.022
Teeth	0.050	0.003–0.635	0.078
Thymus	0.015	0.003–0.332	
Thyroid	0.042	0.001–0.314	
Uterus	0.031	0.010–0.188	0.036

(Compiled from Liebscher and Smith, 1968; and Kadowaki, 1960, respectively).

is generally accepted as the most reliable indicator of recent arsenic exposure, and this approach has proved useful in identifying above-average exposures in populations living near industrial point sources of arsenic (Polissar *et al.,* 1990). Normal levels of arsenic in the urine of people with no known high exposure are apparently in the range of 5–50 µg As/L (Baker *et al*., 1977; Bencko and Symon, 1977; Braman and Foreback, 1973; Buchet *et al*., 1980; Smith *et al*., 1977). Ingestion of seafood may, however, increase the concentrations to more than 1 mg As/L (Buchet *et al*., 1980; Norin and Vahter, 1981; Pinto *et al*., 1976; Schrenk and Schreibeis, 1958).

In a study of As_2O_3 exposure in a smelter, Pinto *et al*. (1976) found an average urinary arsenic concentration of 0.053 mg/L among 200 workers who had not been exposed to arsenic. The average arsenic concentration in urine of 24 men who had been exposed to a mean air concentration of 53 µg/m^3 (range, 3–295 µg/m^3) in the same factory ranged from 0.038–0.539 mg/L, with an overall average of 0.174 mg/L. They found a correlation between airborne arsenic concentrations and arsenic in urine, but the values showed a wide scatter.

Watrous and McCaughey (1945) determined urinary levels of arsenic among workers in a factory producing arsphenamines from arsanilic acid. The mean arsenic level was 0.44 mg/L with a range of 0.04–3.8 mg/L.

Calderon *et al*. (1999) found a correlation between the log of the mean total urinary arsenic concentration/creatinine (TAs/c, µg/mg) in people living in areas with arsenic-contaminated drinking water sources and the log of the inorganic arsenic concentration in the drinking water (lnAs, µg/L).

Arsenic concentration in urine may be used as an index of exposure, but a number of factors such as a diet containing mainly seafood and the time between exposure and urine sampling have to be considered. Because "fish arsenic" (arsenobetaine) is essentially nontoxic, total urinary arsenic content may overestimate exposures to arsenic species that are of health concern. Experience has shown that total urinary arsenic may lead to overestimation of exposure to inorganic arsenic even if the subjects are requested to refrain from eating seafood (Pinto *et al*., 1976). Dietary habits should be examined thoroughly when the total urinary arsenic concentration is to be used as an index of exposure. Analytical differentiation of the different forms of arsenic in the urine is an appropriate way of determining how much and what form of arsenic has been absorbed (Buchet *et al*., 1980; Norin and Vahter, 1981). The sum of urinary excretion of inorganic arsenic and its monomethylated and dimethylated metabolites is a good indicator of exposure to inorganic arsenic when there is some fish consumption. Buchet *et al*. (2003) determined such values by means of hydride generation combined with AAS or AAF and reported a mean value of approximately 7 µg/g creatinine and a 95% cut off of 18 µg/g creatinine in a group from the general population of Belgium. In a population group from China not known to be exposed to inorganic arsenic, there was a mean of approximately 25 µg/g creatinine and a 95% cut off of 50 µg/g creatinine (Buchet *et al*., 2003).

6.3 Blood

By using NAA, Brune *et al*. (1966) found a mean concentration of 0.004 mg/kg in whole blood of normal people and 0.035 mg/kg in uremic patients. Heydorn (1970) reported a mean arsenic concentration in whole blood of 0.022 mg/L in Taiwanese subjects. He also used NAA.

In three studies of As-exposed populations, Vahter *et al*. (1995), Concha *et al*. (1998a), and Concha *et al*. (1998b) found the average blood total As to be 7.6, 9, and 10 µg/L, respectively. In one of these studies, the range of blood As concentration of an unexposed population was 1–2 µg/L (Concha *et al*., 1998b). By use of HPLC-ICP-MS, Suzuki *et al*. (2002) and Mandal *et al*. (2004) were able to separate and quantify the As species in the blood of an As-exposed population. They found AsB, DMA, MMA, and inorganic As in the blood samples by speciating the blood. They also found the total As concentration of 10.1 µg/L As in blood from an As-exposed individual. Mandal *et al*. (2004) also separated the blood into red blood cells (RBCs) and plasma and determined the fraction of the various species in these blood components.

As stated in Section 5.2, the clearance of arsenic from blood is very rapid. The time lapse between exposure and sampling will, therefore, be of importance if the blood levels are being related to the exposure. Furthermore, intake of seafood may greatly influence the blood arsenic levels. For these reasons, blood arsenic cannot be regarded as a useful indicator of exposure.

A review article by Taylor *et al*. (2004) tabulates both the technologies used for the determination of As in blood, hair, nails, and urine and summarizes the basic findings for the different biological matrices.

6.4 Hair

Attempts have been made to correlate normal concentrations of arsenic in hair to exposure to inorganic arsenic. Smith (1964) found that 80% of 1000 people tested had a concentration below 1 mg As/kg in hair, with an average of 0.81 mg/kg and a median of 0.51 mg/kg. Liebscher and Smith (1968) gave

0.02–8.17 mg/kg as the range for arsenic concentrations in hair, based on an examination of 1250 people without known exposure to high levels of this element. Linsheng *et al*. (2002), Mandal *et al*. (2003), and Uchino *et al*. (2005) examined correlations between hair As and As intake from water. They all found that there was a correlation, but the results differed by a factor of 2–3 in the level of As in the hair versus the level of As in the water. This could be due to many factors that include the mode of washing the hair before analysis (external contamination), analytical methods (both ICP-MS), or digestion techniques. Linsheng *et al*. (2002) looked at the average hair As as a function of the extent of arsenism in the patients and found a range of 2– 4.5 μg/g As, depending on the extent of hyperkeratosis, depigmentation, and pigmentation. Mandal *et al*. (2003) found an average concentration of 4.5 μg/g As in the hair, with a range of 0.7–16.2 μg/g. Mandal *et al*. (2003) also speciated the hair by HPLC-ICP-MS and found As(III), As(V), MMA(V), and DMA(V) in the hair. The difficulty with the interpretation of the results is the variation in the speciation results depending on the technique in which the As was extracted from the hair. The average percent recovery of the species versus the total As analysis value was 57%. This is common in these types of analysis in which the laboratory is extracting analytes form solid matrices. Raab *et al*. (2005) speciated As in the hair of a exposed population and found As(III), As(V), MMA(V), and DMA(V). The study also found that the method of extraction varied with the relative species and the extraction efficiency of the specific species relative to the total As concentration. In all of these As speciation studies, the presence of arsenobetaine was not detected; therefore, the assumption is that it does not accumulate in hair.

The mean concentration of arsenic in hair has been found to reflect the degree of arsenic pollution in community air (Bencko and Symon, 1977; Bencko *et al*., 1971; Hammer *et al*., 1971). Concentrations of arsenic in hair were generally found to increase as the concentrations of arsenic in air increased. Hinwood *et al*. (2003) looked at the general types of environmental and personal As exposures and classified the population into exposure categories and determined the hair As concentration of these relative exposures. They classified the exposures into high water, high soil, and personal exposure. The greatest effect was for the high water exposures in which they found a mean of 5.5 μg/g As in the hair with a range of 1.2–20 μg/g.

In evaluating hair As from unexposed populations like the U.S. National Human Exposure Assessment Survey (NHEXAS), there is no correlation of hair As with food, drinking water, dust intake, or exposure (Pellizzari *et al*., 2006). The average hair As in this population was 0.17 μg/g As in the hair, with a range of 0.06–0.33 μg/g As (10–90 percentiles).

Arsenic trioxide (As_2O_3) has been used as a chemotherapeutic agent for some leukemia treatments. Shen *et al*. (1997) looked at the incorporation of As in hair while arsenic trioxide was being administered to several patients. They found that As was incorporated into the hair soon after the arsenic trioxide was administered to a level of 2.5–2.7 μg/g, about five to seven times greater than the baseline levels, and then the hair As decreased after the drug was no longer being administered. The literature on the medicinal uses of arsenical has been recently reviewed by Ratnaike (2003).

It should be noted that there are several caveats related to the analysis of hair As that must be taken into consideration when evaluating an individual or population suspected to have a chronic or acute As exposure or poisoning. It may not possible to distinguish between arsenic adsorbed onto hair from external contamination and arsenic incorporated into hair from the internal body burden. Hindmarsh (2002) has shown that one can measure As in hair well before the As could have been incorporated through hair growth. This could be due to As in the sweat or other mechanisms contaminating the hair. He has also shown that hair can uptake As directly from water containing As and that As is not removed by the normal washing procedures used in analytical methods. Therefore, one must use caution in using hair As as an indicator of absorbed arsenic, if the external contamination cannot be controlled in some way (Vahter *et al*., 1983).

7 EFFECTS

Inorganic arsenic has been recognized as a human poison since ancient times. The arsenic-related human toxicity is systemic, involving a number of organ systems. Acute, subacute, and chronic toxic effects of inorganic arsenic exposed through inhalation and ingestion have been reviewed periodically (ATSDR, 2005; Chen *et al*., 1997ab; IARC, 2004; 2006; NRC, 1999; WHO, 1981; 2001).

7.1 Lethality

It has been estimated that the acute lethal dose of ingested inorganic arsenic in humans is about 1–3 mg/kg/day, whereas inhalation and dermal exposures to inorganic arsenic have not been associated with acute lethality (ATSDR, 2005). Animals are not as sensitive to inorganic arsenic as humans, and this difference may be due to differences in gastrointestinal absorption and methylation capability (Vahter *et al*., 1994).

7.2 Acute and Subacute Effects

Both acute and subacute toxicity of arsenic are shown in Table 2. They involve many organ systems, including the gastrointestinal, dermal, nervous, renal, hepatic, hematological, cardiovascular, respiratory, and ophthalmic systems. The earliest and most common presentation of ingestion of large dose of arsenic is the acute gastrointestinal syndrome, which starts with metallic or garlic-like taste associated with dry mouth, burning lips, and dysphagia. The gastrointestinal syndrome, which is caused by paralysis of the capillary control in digestive tract, may lead to a decrease in blood volume and blood pressure, electrolyte imbalance, and shock. In acute arsenic poisoning, the fundamental lesion of endothelial cellular toxicity accounts for the predominant clinical features. Capillary damage leads to generalized vasodilation, transudation of plasma, and multiorgan failures. The sites of arsenic damage in the kidney include capillary, tubules, and glomeruli. Neuropathy is produced mainly by axonal degeneration, although myelin disruption is also present. Most studies on acute and subacute toxicity of inorganic arsenic do not have details of exposure dose and/or adequacy of sample size for the assessment of dose-response relationships (ATSDR, 2005; Chen *et al*., 1997a; Gorby, 1994; IARC, 2004; Morton and Dunnette, 1994; NRC, 1999; 2001; WHO, 1981; 2001).

TABLE 2 Acute and Subacute Toxicity of Arsenic

Organ system	Symptoms and sign
Gastrointestinal	Nausea, vomiting, thirst, anorexia, heartburn, abdominal pain, diarrhea with bloody stool
Dermal	Dermatitis, vesticulation, melanosis
Neural	Encephalopathy (hyperpyrexia, convulsion, tremor, coma, disorientation), neuritis, peripheral neuropathy (primarily sensory type, paresthesia, hyperesthesia, numbness of extremities, neuralgia, muscular cramp, and weakness)
Renal	Cortical necrosis, leukocyturia, glycosuria, hematuria, oliguria, uremia
Hepatic	Congestion, fatty infiltration, central necrosis, acute yellow atrophy, cholangitis, cholecystitis
Hematological	Anemia, thrombocytopenia, leukopenia, bone marrow suppression
Cardiovascular	Cardiac abnormality (ventricular fibrillation and atypical tachycardia), prolonged Q-T interval, abnormal T wave; congestive heart failure, hypotension
Respiratory	Irritation of nasal mucosa, pharynx, larynx and bronchi, pulmonary edema, tracheobronchitis, bronchial pneumonia, nasal septum performation
Ophthalmic	Conjunctivitis

Source: WHO, 1981, 2001; ATSDR, 2005; Gorby, 1994; Morton and Dunnette, 1994; Chen *et al*. 1994; NRC, 1999.

7.3 Chronic Noncardiovascular Effects

Chronic noncardiovascular effects of inorganic arsenic also involve multiorgan systems as shown in Table 3. The clinical appearance of these clinical manifestations of arsenic intoxication in humans is insidious in onset and depends on the magnitude of the dose and the time course of exposure. A multitude of effects might ensue from the interference of arsenic on the action of enzymes, essential cations, and transcriptional events in cells throughout the human body (ATSDR, 2005; Chen *et al.*, 1997a; Gorby, 1994; IARC, 2004; Morton and Dunnette, 1994; NRC, 1999; WHO, 1981; 2001).

7.3.1 Dermal Effects

The most distinct characteristic of arsenic toxicity is the classical cutaneous manifestations, including hyperpigmentation with depigmentation and palmoplantar hyperkeratosis. These have been consistently observed among those with occupational, environmental, and medicinal exposures to arsenic through inhalation and ingestion. The dermal effects were most commonly reported after ingestion of arsenic containing water in various regions of the world, including Argentina, Bangladesh, Chile, China, Japan, Mexico, Inner Mongolia, and Taiwan (IARC, 2004; NRC, 1999; WHO, 1981; 2001). The magnitude of arsenic dose and exposure duration needed to induce hyperpigmentation and hyperkeratosis has been investigated to a limited extent. A dose-response relationship between arsenic concentration in drinking water and prevalence of hyperpigmentation and hyperkeratosis was observed in a survey of 40,421 inhabitants in coastal areas of southwestern Taiwan (Tseng *et al*., 1968), and a survey of 7683 residents in West Bengal, India (Guha Mazumder *et al*., 1998). More recent studies in Inner Mongolia (Guo *et al*., 2001; 2006), Bangladesh (Ahsan *et al*., 2006), and West Bengal (Haque *et al*., 2003; Mukherjee *et al*., 2005) have reported similar dose-related increases in characteristic skin lesions in human populations consuming arsenic-contaminated drinking water.

7.3.2 Gastrointestinal Effects

Long-term low-dose exposure to ingested arsenic was reported to induce various gastrointestinal symptoms, including gastroenteritis, dyspepsia, nausea, diarrhea, anorexia, and abdominal discomfort in Japan, Chile, China, India, and Mexico. However, arsenic dose and exposure duration required to induce the gastrointestinal effect were well characterized (IARC, 2004).

TABLE 3 Chronic Noncardiovascular Toxicity of Arsenic

Organ system	Symptoms and signs
Dermal	Hyperpigmentation with depigmentation, facial edema, palmoplantar hyperkeratosis, desquamation
Gastrointestinal	Esophagitis, gastritis, colitis, abdominal discomfort, anorexia, malabsorption, weight loss
Neural	Hearing loss, mental retardation, encephalopathy, symmetrical peripheral polyneuropathy (sensimotor type resembling Landry-Guillain-Barre syndrome), electromyographic abnormalities
Hepatic	Cirrhosis, hepatomegaly, portal hypertension without cirrhosis, fatty degeneration
Hematological	Bone marrow hypoplasis, aplastic anemia, anemia, leukopenia, thrombocytopenia, impaired folate metabolism, karyorrhexis
Respiratory	Rhino-pharyngo-laryngitis, tracheobronchitis, pulmonary insufficiency (emphysematous lesions), chronic restrictive/obstructive diseases
Metabolic	Diabetes mellitus
Immunological	Decreased progression of lymphocytes from S-phase to M-phase of cell cycle
Ophthalmic	Lens opacity

Source: WHO, 1981, 2001, 2004; ATSDR, 2005; Gorby, 1994; Morton and Dunnette, 1994; Chen *et al*., 1997a; NRC, 1999; Chen *et al*., 2005.

7.3.3 Neural Effects

Peripheral polyneuropathy is often among sequelae of acute oral arsenic poisoning. However, the occurrence of the polyneuropathy is inconsistent in individuals chronically exposed to arsenic at low concentrations. Abnormal electromyographic findings suggestive mostly of sensory neuropathy were reported in Canada, India, Japan, and China. But the neuropathy was not reported among residents drinking arsenic-containing water in Taiwan, Argentina, and Chile (IARC, 2004; NRC, 1999). There was no significant association between nerve-conduction-velocity measurements and arsenic exposure in Alaska (Kreiss *et al*., 1983) and Utah (Southwick *et al*., 1983). Peripheral neuritis and cognitive and memory impairment have been reported in arsenic-exposed residents in Texas (Kilburn, 1997), whereas reduced intellectual function has been found to be associated with arsenic exposure from drinking water in Thailand (Siripitayakunkit *et al*., 1999) and Bangladesh (Wasserman *et al*., 2004). A cross-sectional study on neurobehavioral development in adolescents in northeastern Taiwan found a significant association with cumulative arsenic exposure for pattern memory and switching attention but not for continuous performance and symbol digit (Tsai *et al*., 2003).

7.3.4 Hepatic Effects

Chronic exposure to arsenic in medicines and drinking water was reported to induce liver cirrhosis, portal hypertension without cirrhosis, and fatty degeneration (IARC, 2004; Morton and Dunnette, 1994; NRC, 1999; WHO, 1981; 2001). Vineyard workers who ingested arsenic-contaminated wine substitute were found to have a high prevalence of cirrhosis (Luchtrath, 1983). The simultaneous use of excessive ethanol may also contribute to the occurrence of liver cirrhosis. Increased mortality from liver cirrhosis was also observed in two cohorts of arsenic-exposed copper smelter workers (Axelson *et al*., 1978; Welch *et al*., 1982). Hepatomegaly has also been reported in cases of chronic arsenic toxicity caused by drinking arsenic-contaminated water in India and China (Guha Mazumder *et al*., 2005; IARC, 2004).

7.3.5 Hematological Effects

Long-term exposure to arsenic has a general depressant effect on the hematopoietic system. The hematological changes included normochromatic normocytic anemia, megaloblastic anemia, erythrocyte karyorrhexis, granulocytopenia, thrombocytopenia, aplastic anemia, and myelodysplasia. However, no hematological abnormality was found to be associated with chronic arsenic exposure through ingestion in Alaska, Arizona, Michigan, and Utah (Morton and Dunnette, 1994; NRC, 1999).

7.3.6 Respiratory Effects

Chronic respiratory effects of arsenic have been reported primarily as a result of occupational exposure through inhalation of arsenic containing dust or fumes (Morton and Dunnette, 1994), as well as environmental exposure through ingestion of arsenic-containing drinking water (IARC, 2004; NRC, 1999). The respiratory effects included rhinitis, pharyngitis, laryngitis, tracheobronchitis, chronic cough, crepitation, shortness of breath, and chronic restrictive and/or obstructive lung diseases. More recent studies in West Bengal (Guha Mazumder *et al*., 2000, 2005; von Ehrenstein *et al*., 2005) and Chile (Adonis *et al*., 2005; Smith *et al*., 2006) have reported increased rates of both obstructive lung lesions, including bronchiectasis and lung cancer in persons exposed to arsenic through drinking water that were exacerbated by smoking. Adonis *et al*. (2005) reported that in men the estimated risk of lung cancer was correlated with the enzymes MspI and CYP 1A1 2A but not GSTM1.

7.3.7 Metabolic Effects

An increased prevalence of diabetes mellitus was observed among residents in the endemic area of

arseniasis in southwestern Taiwan, and there was a dose-response relationship between cumulative arsenic exposure and prevalence of diabetes mellitus (Lai *et al*., 1994). There was an excess mortality from diabetes mellitus among residents who lived in arseniasis-endemic area of southwestern Taiwan (Tsai *et al*., 1999). Both arsenic-exposed workers in copper smelter and glass-producing plants were reported to have an increased risk of diabetes mellitus (Rahman and Axelson, 1995; Rahman *et al.,* 1996). A dose-response relationship was reported between prevalence of diabetes mellitus and arsenic in drinking water in Bangladesh (Rahman *et al*., 1998; 1999) and between incidence of diabetes mellitus and arsenic in drinking water in southwestern Taiwan (Tseng *et al*., 2000; 2002). Glycosylated hemoglobin was significantly higher among arsenic-exposed workers than unexposed ones in Denmark (Jensen and Hansen, 1998). The previous epidemiological studies are consistent with prior experimental animal studies (Ghafghazi *et al*., 1980, Schiller *et al*., 1981) which demonstrated marked exacerbating interactive effects between arsenic exposure, altered insulin responsiveness, and urinary markers of diabetes in an alloxan-induced diabetes rodent model. More recent studies (Diaz-Vilasenor *et al*., 2006) have reported impaired insulin secretion and transcription in rat pancreatic cells treated *in vitro* with arsenite (AsIII) over a concentration range of 0.5–10 μmol/L As for 72 or 144 hours. They observed both altered cellular insulin secretion at 144 hours in response to glucose at the 5 μmol/L As concentration and decreased insulin mRNA expression at 72 hours at the 5 μmol/L As concentration. Possible underlying mechanisms for arsenic-induced diabetes have been recently reviewed by Tseng (2004). In addition to these suggestions, which are centered on altered molecular regulatory mechanisms, it should be noted that the metabolic impairments of carbohydrate metabolism at the level of mitochondrial respiration noted in Section 5.6 might represent an important basic aspect of altered glucose regulation by arsenic. This is reasonable, because mitochondria actively take up arsenic, thus concentrating this element at even low dose levels. The mitochondrion is also known to be the major intracellular source of reactive oxygen species (ROS) in cells. The specific inhibition of NAD-linked substrate respiration/loss of respiratory control by arsenic at low-dose levels has been shown to increase the formation of ROS, which will stimulate a number of the stress protein and intracellular signaling mechanisms on a secondary basis. The overall concept with regard to arsenic stimulation of the diabetic response is that, even at low-dose levels, arsenic inhibition of mitochondrial respiration will tax the finite capacity of hormonal/intracellular carbohydrate regulatory systems to compensate for this basic derangement of cellular metabolism. The ability of these regulatory systems to regulate carbohydrate metabolism would eventually be overwhelmed, resulting in the development and/or exacerbation of an arsenic-induced insulin-resistant diabetic state for susceptible individuals. This would be of particular concern under chronic exposure conditions.

7.3.8 Immunological Effects

The peripheral blood lymphocyte count of arsenic-exposed subjects was slightly increased relative to the unexposed controls, and the progression of lymphocytes from the S-phase to M-phase of cell cycle after phytohemagglutinin incubation was decreased (Gonsebatt *et al*., 1994). The retardation in cell replication of lymphocytes was much more striking in arsenic-induced skin cancer patients than matched controls (Hsu *et al*., 1997).

7.3.9 Ophthalmic Effects

Long-term exposure to arsenic was reported to be associated with an increased prevalence of lens opacity. There was a dose-response relationship between cumulative arsenic exposure and prevalence of posterior subcapsular opacity, but not with prevalence of nuclear opacity and cortical opacity, after adjustment for age, gender, diabetes mellitus, and sunlight exposure (See, 2000).

7.4 Chronic Cardiovascular Effects

Table 4 shows chronic cardiovascular effects of long-term exposure to arsenic through ingestion or inhalation. The cardiovascular effects included electromyographic abnormalities, especially QT prolongation and increased dispersion; peripheral, coronary, and cerebral artery diseases; carotid atherosclerosis; hypertension; and microcirculation abnormality (Chen *et al*., 1997a; IARC, 2004; NRC, 1999; WHO, 1981; 2001).

TABLE 4 Chronic Cardiovascular Toxicity of Arsenic

Organ system	Symptoms and signs
Heart	Arrythmias, pericarditis
Peripheral artery	Blackfoot disease (gangrene with spontaneous amputation), Rayuand's syndrome, acrocyanosis, intermittent
Coronary artery	Ischemic heart disease
Cerebral artery	Cerebral infarction
Atherosclerosis	Carotid atherosclerosis
Blood pressure	Hypertension
Microcirculation	Microcirculation abnormalities

Source: WHO, 1981, 2001, 2004; ATSDR, 2005; Chen and Lin, 1994; Engel *et al*., 1994; Huang, 1995; Tseng *et al*., 1995; Chen *et al*., 1997a; NRC, 1999; Chen *et al*., 2005.

7.4.1 Cardiac Effects

Characteristic arsenic-induced electromyographic abnormalities include decreased nerve conduction amplitude with little change in nerve conduction velocity. Pericarditis and arrhythmias have also been documented as arsenic-induced cardiac effects (Gorby, 1994; Morton and Dunnette, 1994). A dose-response relationship has been reported between cumulative arsenic exposure and heart rate–corrected increased QT dispersion and QT prolongation from the endemic area of arseniasis in southwestern Taiwan (Wang, 2003).

7.4.2 Peripheral Vascular Diseases

Blackfoot disease, a unique peripheral arterial disease characterized by the severe systemic arteriosclerosis as well as dry gangrene and spontaneous amputations of affected extremities and end stages, has been well documented as a characteristic vascular disease associated with long-term arsenic exposure (Chen *et al.*, 1988b; Tseng, 1977). Diagnostic criteria for Blackfoot disease include objective signs of ischemia (i.e., absence or diminution of arterial pulsations, pallor on elevation, or rubor on dependency of ischemic extremities) and various degrees of ischemic changes in the skin, as well as subjective symptoms of ischemia (i.e., intermittent claudication, pain at rest, and ischemic neuropathy). Not all patients are affected with black, mummified dry gangrene (Tseng *et al*., 1961). Extensive pathological study showed that 30% of Blackfoot disease patients had histological lesions compatible with thromboangiitis obliterans, and 70% showed changes of arteriosclerosis obliterans. Marked generalized atherosclerosis was observed in all autopsied cases of Blackfoot disease, and the fundamental vascular changes of the disease represent an unduly developed severe arteriosclerosis (Yeh and How, 1963).

The dose-response relationship between ingested inorganic arsenic and Blackfoot disease has been well documented in the endemic area of southwestern Taiwan, where residents had used high-arsenic artesian well water for more than 50 years (Tseng, 1997). Patients affected with Blackfoot disease have a high prevalence of arsenic-induced skin lesions, including hyperpigmentation, hyperkeratosis, and skin cancers (Tseng, 1997). They also have a high mortality from cancers of the lung, liver, bladder, kidney, and prostate, as well as ischemic heart disease (Chen *et al*., 1988b). In addition to the duration of consuming artesian well water and the arsenic-induced skin lesions, the development of Blackfoot disease is also associated with undernourishment and a family history of Blackfoot disease (Chen *et al*., 1988b).

In Blackfoot disease, overt peripheral vascular disease was not reported in other arsenic-exposed populations. However, comparable peripheral vascular disorders with varying degrees of severity, including Raynaud's syndrome, microangiopathies, vasospastic tendency, and acrocyanosis, have also been reported among workers who were exposed to inorganic arsenic through copper smelting (Lagerkvist *et al*., 1986), pesticide handling, wall painting, and wood burning; among vintners who had consumed arsenic-contaminated wine in Germany; among patients treated with arsenic-containing drugs; and among inhabitants exposed to high-arsenic drinking water in Poland, Chile, Mexico, Argentina, Japan, Bangladesh, and Xinjiang, China (Cebrian *et al*., 1994; Chen *et al*., 1997a; Engel *et al*., 1994; Hotta, 1989; IARC, 2004; NRC, 1999; Rahman *et al*., 1999; Wang and Huang, 1994; WHO, 1981; 2001). Lynn *et al*. (2000) reported that NADH oxidase activation is involved in oxidative DNA damage to human aorta vascular smooth muscle cells produced by As(III). Such an underlying mechanism is consistent with the broad spectrum of oxidative damage produced by arsenical exposure in a number of organ systems (NRC, 1999).

There was a decline in the incidence of Blackfoot disease after the implementation of a surface water supply system in the endemic area, and most newly developed cases were older residents who had consumed high-arsenic artesian well water (Wang *et al*., 1985). A survey has shown a dose-response relationship between cumulative arsenic exposure and subclinical peripheral vascular disorder detected by Doppler ultrasonography among seemingly normal subjects after cessation of drinking artesian well water in the endemic area of Blackfoot disease in Taiwan (Tseng *et al*., 1995a). In a recent ecological study, the prevalence of peripheral vascular disease among diabetic and nondiabetic residents was significantly higher in arseniasis-endemic than nonendemic areas in southwestern Taiwan (Wang *et al*., 2002; 2003).

7.4.3 Ischemic Heart Diseases

Both ingested and inhaled inorganic arsenic have been related to an increased mortality from cardiovascular disease, especially ischemic heart disease (Chen *et al*., 1997a; Engel *et al*., 1994; IARC, 2004; WHO, 1981; 2001). The mortality from cardiovascular disease was significantly higher among residents in endemic areas of Blackfoot disease than among the general population in Taiwan (Wu *et al*., 1989). A significant dose-response relationship between ingested arsenic level and risk of ischemic heart disease were observed

in cohort and case-control studies in southwestern Taiwan (Chen *et al*., 1996). In a recent ecological study, the prevalence of coronary artery disease among diabetic and nondiabetic residents was significantly higher in arseniasis-endemic than nonendemic areas in southwestern Taiwan (Wang *et al*., 2003). Myocardial infarction has been related to high-arsenic drinking water in several autopsy studies in Antofagasta, Chile (Chen *et al*., 1997a; Engel *et al*., 1994). But details of arsenic exposure and population at risk were not available for further evaluation of the dose-response relationship.

The association between long-term exposure to arsenic and increased mortality from cardiovascular disease has also been reported among copper-smelter worker in the United States and Sweden, among chimney sweeps in Sweden and Denmark, among glassblowers in Sweden, and among workers and neighboring residents of an arsenic refinery in Japan (Chen *et al*., 1997a; Engel *et al*., 1994; IARC, 2004; WHO, 1981; 2001).

A significant reverse dose-response relationship was observed between arsenic-induced ischemic heart disease and serum level of α- and β-carotene after adjustment for age, gender, body mass index, hypertension, as well as the ratio between total cholesterol and high-density lipoprotein cholesterol (Hsueh et *al*., 1998).

7.4.4 Stroke

A survey carried out in I-Lan County of northeastern Taiwan showed a significant dose-response relationship between arsenic concentration in drinking water and the prevalence of strokes, especially cerebral infarction (Chiou *et al*., 1997). The biological gradient of stroke by arsenic in drinking water remained statistically significant after adjustment for age, gender, body mass index, disease status of hypertension and diabetes mellitus, cigarette smoking, and alcohol consumption. In an ecological study in southwestern Taiwan, an increased mortality from stroke was observed for residents who lived in arseniasis-endemic area of southwestern Taiwan (Tsai *et al*., 1999). In a recent ecological study, the prevalence of coronary artery disease among diabetic and nondiabetic residents was significantly higher in arseniasis-endemic than nonendemic area in southwestern Taiwan (Wang *et al*., 2003).

7.4.5 Carotid Atherosclerosis

In a cross-sectional survey of residents in southwestern Taiwan, a dose-response relationship between prevalence of carotid atherosclerosis and long-term exposure to arsenic from drinking well water was observed (Wang *et al*., 2002). The biological gradients remained significant after adjustment for age, gender, hypertension, diabetes mellitus, cigarette smoking, alcohol consumption, waist-to-hip ratio, and serum levels of total cholesterol and low-density lipoprotein cholesterol.

7.4.6 Hypertension

A cross-sectional study in southwestern Taiwan has reported an increased prevalence of hypertension among residents in the arseniasis-endemic area than those in nonendemic area, as well as a dose-response relationship between ingested inorganic arsenic and prevalence of hypertension (Chen *et al*., 1995). The biological gradient remained significant after adjustment for age, gender, diabetes mellitus, proteinuria, body mass index, and serum triglyceride levels. Another study in Bangladesh also found a dose-response relationship between ingested arsenic and hypertension prevalence after adjustment for age, gender, and body mass index (Rahman *et al*., 1999b). Increased hypertension prevalence was also observed among patients affected with arsenic-induced skin lesions in the area where well water had a high arsenic concentration in Chile (Borgono *et al*., 1977; Zaldivar, 1980). Arsenic-exposed workers were reported to have a higher systolic blood pressure than unexposed workers in Denmark (Jensen and Hansen, 1998).

7.4.7 Microcirculation Abnormality

Based on the laser Doppler flowmetry, seemingly normal men living in villages where Blackfoot disease was hyperendemic were found to have poorer peripheral microcirculation than matched ones in nonendemic areas (Tseng *et al*., 1995b). However, the dose-response relationship between ingested inorganic arsenic and abnormality of peripheral microcirculation was not examined.

7.4.7.1 Microvascular Diseases

In a recent ecological study, the prevalence of microvascular diseases, including renal disease, retinopathy, and neurological disorders among diabetic and nondiabetic residents, was significantly higher in arseniasis-endemic than nonendemic area in southwestern Taiwan (Wang *et al*., 2003).

A significant ecological correlation between the arsenic content in drinking water and the mortality from cancer of the nasal cavity was reported recently (Chen and Wang, 1990). Although perforation of the nasal septum has been documented among smelter workers exposed to high levels of inorganic arsenic

(WHO, 1981), there were no other reports on the association between nasal cavity cancer risk and exposure to arsenic through inhalation or ingestion.

7.5 Carcinogenic Effects

Ingested and inhaled arsenic through occupational, environmental, and medicinal exposure is involved in the development of several cancers in humans without showing any organotropism as shown in Table 5 (Chen *et al*., 1997b; IARC, 2004; NRC, 1999; WHO, 1981; 2001). Ecological, case-control and cohort studies have shown a significant dose-response relationship between arsenic in drinking water and increased risk of cancers of the liver, nasal cavity, lung, skin, urinary bladder, kidney, and prostate in southwestern and northeastern Taiwan (Chen *et al*., 1988a, 1992; 2004; Chiou *et al*., 1995; 2001). Based on the sufficient evidence in humans that arsenic in drinking water causes cancers of the urinary bladder, lung, and skin, arsenic in drinking water has been classified as a group 1 carcinogen to humans (IARC, 2004).

7.5.1 Skin Cancer

There is clear evidence that long-term oral exposure to inorganic arsenic through drinking water, grape wine, or medication increases the risk of skin cancer (Chen *et al*., 1997b; IARC, 2004; NRC, 1999; WHO, 1981; 2001). Inhaled arsenic has also been documented to induce skin cancer among workers producing sheep-dip powder from sodium arsenite (Hill and Faning, 1948). The largest study of arsenic-induced skin cancer was carried out in southwestern Taiwan, and a dose-response relationship between the arsenic content in drinking water and the prevalence of skin cancer was observed (Tseng *et al*., 1968). In addition to long-term exposure to high-arsenic artesian well water, chronic liver disease and malnutritional status have recently been reported to be associated with the development of arsenic-induced skin cancer (Hsueh *et al*., 1995). The risk of skin cancer was significantly associated with the elevated serum arsenic level and decreased serum beta-carotene level in a dose-response relationship, but no significant differences in serum levels of selenium and zinc were observed between patients affected with arsenic-induced skin cancer and matched healthy controls (Hsueh *et al*., 1997). Arsenic methylation capability has been found to be associated with the development of skin cancer. Increased skin cancer was observed among those who had an elevated proportion of monomethylarsonic acid in total urinary metabolites of inorganic arsenic (Hsueh *et al*., 1997; Yu *et al*., 2000). An increased risk of arsenic-induced skin cancer was observed among those who had at least one null or variant genotypes of glutathione S-transferase M1, T1, or P1 (Tseng, 1999).

7.5.2 Lung Cancer

An increased risk of cancers of the lung and nasal cavity has been associated with long-term exposure to inorganic arsenic through inhalation and ingestion in a dose-response relationship (Chen *et al*., 1997b; IARC, 2004; NRC, 1999; Pinto *et al*., 1978; WHO, 1981; 2001). An excess of deaths caused by lung cancer has been observed among workers exposed to inorganic arsenic through inhalation in the production and use of

TABLE 5 Various Cancers with Increased Risks Caused by Arsenic Exposure Through Inhalation and Ingestion

	Ingestion			Inhalation	
Cancer site	Drinking water	Contaminated wine	Fowler's solution	Copper smelter	Pesticide factory
Skin	+	+	+		+
Lung	+	+	+	+	+
Urinary bladder	+	+	+	+	
Kidney	+		+	+	
Nasal cavity	+				
Larynx	+				
Prostate	+				
Breast	+				
Hepatic angiosarcoma	+	+	+	+	+
Hepatocellular carcinoma	+	+		+	
Gastrointestial tract	+	+		+	
Hematolymphatic system	+			+	+
Brain and nervous system				+	

pesticides, in gold mining, and in the smelting of nonferrous metals, especially copper. Individuals living within several kilometers of inorganic arsenic–emitting industries were also reported to have increased risk of lung cancer (Chen *et al*., 1997b; WHO, 1981; 2001). The dose-response relationship between inhaled inorganic arsenic and lung cancer risk was observed mainly in two large cohorts of copper-smelter workers in Anaconda, Montana (Brown and Chu, 1983; Higgins *et al*., 1982; Lee-Feldstein, 1983), and in Tacoma, Washington (Enterline and Marsh, 1982). The observed effects might be confounded by cigarette smoking and exposures to other chemicals. An interaction between inhaled inorganic arsenic and cigarette smoking for the risk of developing lung cancer has been reported (Jarup and Pershagen, 1991).

Significant association between ingested arsenic and lung cancer risk has been observed in patients treated with arsenic-containing medicine, in vintners exposed to arsenic pesticide-contaminated grape wine, and in persons exposed to inorganic arsenic from drinking water (Chen *et al*., 1997b; Guo, 2004; IARC, 2004; NRC, 1999; WHO, 1981; 2001). The dose-response relationship between ingested inorganic arsenic and lung cancer has been reported in cohort studies in Taiwan (Chiou *et al*., 1995) and in Japan (Tsuda *et al*., 1995). Both cigarette smoking and signs of arseniasis (i.e., skin cancer or Blackfoot disease) were reported to increase the risk of arsenic-induced lung cancer in these two studies. A synergistic effect on the development of lung cancer between cigarette smoking and ingested arsenic was observed in the endemic areas of arseniasis in southwestern and northeastern Taiwan (Chen *et al*., 2004).

These results are consistent with the positive interactive effects between benzo-a-pyrene and arsenic in producing lung cancers in hamsters after concomitant exposures to both agents (Pershagen *et al*., 1984).

7.5.3 Urothelial Cancer

Both inhaled and ingested inorganic arsenic have been well documented to induce cancers of the bladder and kidney, especially urothelial cancers (Chen *et al*., 1985; 1986; 1997b; IARC, 2004; NRC, 1999; WHO, 2001).

There have been several reports on the increased risk of bladder cancer among patients treated with Fowler's solution (Chen *et al*., 1997b). In a cohort study of patients treated with Fowler's solution, a significant association between the cumulative dose of arsenic intake and mortality from bladder cancer was observed (Cuzick *et al*., 1992). Moselle vintners who consumed arsenic pesticide–contaminated grape wine were found to have an increased mortality from bladder cancer (Luchtrath, 1983).

Ecological studies in Taiwan have well documented the dose-response relationship between ingested arsenic from drinking water and mortality from cancers of the urinary bladder and kidney in Taiwan (Chen and Wang, 1990; Chen *et al*., 1988a). A significant dose-response relationship between ingested inorganic arsenic from drinking water and risk of bladder cancer has been documented in cohort studies in Taiwan (Chiou *et al*., 1995; 2001), Japan (Tsuda *et al*., 1995), and, more recently, in Finland (Kurttio *et al*., 1999), Argentina (Bates *et al*., 2004), and the United States (Steinmaus *et al*., 2006). Those who have signs of arseniasis (i.e., skin cancer or Blackfoot disease) were found to have an increased risk of bladder cancer from drinking high-arsenic well water (Chiou *et al*., 1995; Tsuda *et al*., 1995). Cigarette smoking was not significantly associated with the development of arsenic-induced bladder cancer in Taiwan (Chiou *et al*., 1995); but a significant dose-response relationship between bladder cancer risk and arsenic exposure was observed only among cigarette smokers in the United States (Bates *et al*., 1995). The decreased ratio between urinary levels of dimethylarsinic acid (DMA) and monomethylarsonic acid (MMA) was associated with an increased risk of bladder cancer (Chen *et al*., 2003). Studies by Steinmaus *et al*. (2006) support the findings from Taiwan and demonstrated that persons with higher urinary levels of MMA were at greater risk for development of arsenic-induced cancers.

Inhaled inorganic arsenic has also been found to be associated with the risk of mortality from bladder cancer among copper smelter workers in the United States and Japan (Bates *et al*., 1992; Chen *et al*., 1997b) and among residents who lived near a plant roasting arsenopyrite and were exposed to inorganic arsenic through inhalation and ingestion in Japan (Hotta, 1989). There was no report examining the dose-response relationship between inhaled arsenic and bladder cancer.

Excess mortality from kidney cancer was observed among copper smelter workers and patients treated with Fowler's solution (Chen *et al*., 1997b). A dose-response relationship between ingested inorganic arsenic and kidney cancer mortality was observed among residents in the endemic area of arseniasis in Taiwan (Chen *et al*., 1988a, 1992; Chiou *et al*., 1995).

7.5.4 Liver Cancer

Both hepatic angiosarcoma and hepatocellular carcinoma have been associated with long-term exposure to ingested and inhaled arsenic (Bates *et al*., 1992; Chen *et al*., 1997b; Falk *et al*., 1981ab; IARC, 2004; NRC, 1999; WHO, 1981; 2001). Exposure was from ingestion of arsenic-contaminated wine, high-arsenic

drinking water and Fowler's solution; and from inhalation through copper smelting and production and application of arsenic-containing pesticides. Because hepatic angiosarcoma is a very rare disease, its association with exposures to inorganic arsenic does not seem likely to be a matter of chance.

Long-term exposure to inorganic arsenic through ingestion and inhalation has been associated with the development of hepatocellular carcinoma. The exposure of ingested arsenic was from high-arsenic artesian well water and arsenic-contaminated grape wine, and that of inhaled arsenic was from copper smelting (Chen *et al*., 1997b). Ecological studies have shown a dose-response relationship between ingested inorganic arsenic from drinking water and risk of hepatocellular carcinoma among residents in the endemic area of arseniasis in southwestern Taiwan (Chen and Wang, 1990; Chen *et al*., 1988a).

7.5.5 Other Internal Cancers

Inhaled and ingested inorganic arsenic have been associated with an increased risk of gastrointestinal cancers, hematolymphatic malignancies, and malignant neoplasms of the nervous system (Chen *et al*., 1997b; IARC, 2004; NRC, 1999; WHO, 1981; 2001). Excess mortality from cancers of the digestive tract has been observed among copper smelter workers, Moselle vintners, and residents in the endemic area of arseniasis in Taiwan. Workers in copper smelters and pesticide manufacturing plants were reported to have an increased mortality from malignant neoplasms of lymphatic and hematopoietic tissues. Copper smelter workers also had an increased mortality from malignant neoplasms of the brain and nervous system.

There were no reports on the association between exposure to ingested inorganic arsenic and mortality from malignant neoplasms of the hematolymphatic and nervous systems. Ingested inorganic arsenic through drinking high-arsenic artesian well water has been found to be significantly associated with mortality from prostate cancer in a dose-response relationship (Chen and Wang, 1990; Chen *et al*., 1988ab). There were no reports on the association between inhaled inorganic arsenic and prostate cancer.

A significant ecological correlation between the arsenic content in drinking water and the mortality from cancer of the nasal cavity was reported recently (Chen and Wang, 1990). Although perforation of the nasal septum has been documented among smelter workers exposed to high levels of inorganic arsenic (WHO, 1981), there were no other reports on the association between nasal cavity cancer risk and exposure to arsenic through inhalation or ingestion.

7.5.6 Lifetime Cancer Risk Induced by Arsenic

As shown in Table 6, the lifetime risk of developing cancers of the skin, lung, bladder, liver, and kidney have been estimated on the basis of Armitage–Doll multistage models. The lifetime risk of developing skin cancer from the ingestion of 1μg/kg/day inorganic arsenic was estimated according to the prevalence of skin cancer among residents in an endemic area of arseniasis and an unexposed control area (Tseng *et al*., 1968). The lifetime risk of developing lung cancer from 1μg/kg/day arsenic through inhalation was based on the data from copper smelter workers in Anaconda, Montana (Brown and Chu, 1983; Higgins *et al*., 1982; Lee-Feldstein, 1983), and in Tacoma, Washington (Enterline and Marsh, 1982).

The lifetime risks of developing cancer of the lung, liver, bladder, and kidney from 1μg/kg/day inorganic arsenic through ingestion were based on the mortality date of residents in the endemic area of arseniasis southwestern Taiwan (Chen *et al*., 1992). The lifetime risks of

TABLE 6 Lifetime Risk of Developing Cancers of Skin, Lung, Liver, Bladder, and Kidney Caused by 1μg/kg/day Inorganic Arsenic Through Ingestion and Inhalation

Cancer site	Exposure type	Study area	Reference	Lifetime risk (per 1000)	
				Male	Female
Skin	Ingestion	Taiwan	Tseng *et al*., 1968	3.0	2.1
Lung	Inhalation	Anacoda	Higgins *et al*., 1982	17.0	—
			Lee-Feldstein, 1983	9.8	—
			Brown and Chu, 1983	4.6	—
		Tacoma	Enterline and Marsh, 1980	24.0	—
	Ingestion	Taiwan	Chen *et al*., 1992	1.2	1.3
Bladder	Ingestion	Taiwan	Chen *et al*., 1992	1.2	1.7
Kidney	Ingestion	Taiwan	Chen *et al*., 1992	0.4	0.5
Liver	Ingestion	Taiwan	Chen *et al*., 1992	0.4	0.4

Source: US Environmental Protection Agency, 1984; Chen *et al*., 1992.

developing cancers of the skin, lung, liver, bladder, and kidney from ingested inorganic arsenic were within a sevenfold range of magnitude. The risks were practically identical for both men and women within a twofold range of magnitude, indicating no gender difference in arsenic-induced carcinogenic responses.

Epidemiological studies on arsenic-induced development and reproductive effects have been reviewed periodically (Chen *et al*., 1997b; NRC, 1999; WHO, 1981; 2001). Babies born to female employees of a copper smelter, who were exposed to inorganic arsenic through inhalation during pregnancy, were reported to have an increased incidence of congenital malformation, low birth weight, and spontaneous abortion (Nordstrom *et al*., 1979ab). Low birth weight was also observed among newborns in smelter areas than in nonsmelter areas (Tabacova *et al*., 1994).

An increased, but not statistically significant, risk of coarctation of the aorta was found to be associated with elevated arsenic level in drinking water (Zierler *et al*., 1988). Arsenic in drinking water has been reported to increase the mortality from congenital anomalies of the heart in females, and the mortality from congenital anomalies of the circulatory system for both sexes in the United States (Engel and Smith, 1994). Arsenic in drinking water was reported to be associated with an increased risk of spontaneous abortion in two studies (Aschengrau *et al*., 1989; Borzsonyi *et al*., 1992), and with a significantly increased risk of stillbirths in two studies (Borzsonyi *et al*., 1992; Ihrig *et al*., 1998). Arsenic in drinking water was also reported to be associated with an increased neonatal mortality in Chile (Hopenhayn-Rich *et al*., 1999). Similar findings of increased rates of spontaneous abortions, stillbirths, and infant mortality have been reported among populations consuming arsenic-contaminated well water in Bangladesh (Milton *et al*., 2005) and West Bengal (von Ehrenstein *et al*., 2006).

7.6 Experimental System Cancer Studies

No studies on cancer in humans after oral exposure to organic arsenicals have been located, but there are some animal studies on the carcinogenicity of organic arsenicals. In an early 2-year study of roxarsone (3-nitro-4-hydroxyphenylarsonic acid) toxicity in animals, no increase in tumor frequency was detected in dogs, rats, or mice given 1.5, 2.9, and 3.8 mg arsenic/kg/day, respectively (Prier *et al*., 1963). Lifetime studies of roxarsone at doses up to 1.4 mg arsenic/kg/day yielded no evidence of carcinogenicity in male or female mice or female rats, but a slight increase in pancreatic tumors was noted in male rats (NTP, 1980). Carcinogenesis studies with inorganic arsenic in hamsters (Inamasu *et al*., 1982; Pershagen *et al*., 1984) were also positive, particularly in combination with other organic carcinogens (see Section 7.7). Arnold *et al*. (2003) exposed male and female rats to 0, 50, 400, or 800 ppm MMA (monoarsonic acid) and male and female B6C3F1 mice to 0, 10, 50, 200, or 400 ppm MMA in the diet for 104 weeks; estimated average daily doses were up to 47.3 mg arsenic/kg/day for female rats and up to 48.5 mg arsenic/kg/day for female mice. No treatment-related neoplastic changes were seen in either sex of either species. A similar lack of carcinogenicity of MMA was reported by Shen *et al*. (2003a), who exposed male F344 rats to 0, 50, or 200 ppm of MMAA(V) in the drinking water for 104 weeks. Wei *et al*. (1999; 2002) exposed male F344 rats to 0, 12.5, 50, or 200 ppm DMAA (dimethylarsinic acid) for 104 weeks in the diet, the average daily doses were 0, 0.03, 0.14, or 0.53 mg arsenic/kg/day. Increases in the number of animals with bladder tumors were seen in the two highest dosed groups. No increases in tumor incidence were seen in other organs. Hayashi *et al*. (1998) reported that mice exposed to 400 ppm of DMA for 50 weeks, but not those exposed to 50 or 200 ppm, showed an increased incidence of papillary adenomas and an elevated number of average lung tumors per mouse. Dimethylarsinic acid (DMAA), which is a major methylated metabolite of inorganic arsenic, has been reported by Hayashi *et al*. (1998) to exhibit tumorigenicity and to stimulate tumor progression in mice after drinking water exposure at 400 ppm for up to 50 weeks. These data are in concert with epidemiological studies of humans who developed lung cancer from drinking water exposures noted above.

7.6.1 Developmental and Reproductive Effects

7.6.1.1 Teratogenic Effects

Until recently, there were very few reports on the teratogenicity of inorganic arsenic in humans. Congenital malformations were observed in children whose mothers worked, during pregnancy, at a Swedish copper smelter and were exposed to arsenic, other heavy metals, and sulfur dioxide. The observed incidence was five times greater than that in children born to other mothers from the same region (Nordstrom *et al*., 1979ab). No conclusion can be drawn as to whether arsenic is responsible for these malformations. Teratogenic effects have been shown to occur after a single administration of high doses (6–10 mg As/kg body weight) of sodium arsenate to pregnant golden hamsters (Ferm, 1977). The compound was given intravenously to the hamsters on the 8th day of gestation. Both the reabsorption and malformation rates in the fetus increased with increasing doses of the arsenate.

The teratogenic effects were characterized by anencephaly, renal agenesis, and rib malformations. Similar teratogenic effects have been induced in mice intraperitoneally injected with sodium arsenate at a dose of 11 mg As/kg body weight (Hood and Bishop, 1972) and in rats intraperitoneally injected with 5–12 mg As/kg body weight (Beaudoin, 1974). However, in all cases, the doses required to cause these effects resulted in significant maternal toxicity or even lethality. Recent studies in mice, rats, and rabbits found no evidence of developmental effects at exposure levels that did not produce maternal toxicity (Holson *et al*., 1999; 2000; Nemec *et al*., 1998; Stump *et al*., 1999).

7.6.2 Genotoxic Effects and Mutagenicity

There have been a large number of studies of the genotoxic effects of arsenic. The results are mixed, but in general, it seems that the inorganic arsenicals are either inactive or weak mutagens (Jacobson-Kram and Montalbano 1985) but are able to produce chromosomal effects (aberrations, sister chromatid exchange) in most systems. Increased incidence of chromosome abnormalities has been observed in lymphocytes of workers exposed to arsenic (Beckman *et al*., 1977) and patients therapeutically treated with arsenicals (Petres *et al*., 1977).

When cultured human lymphocytes were exposed to $NaAsO_2$ (trivalent) and Na_2HAsO_4 (pentavalent), significantly increased frequencies of chromosome aberrations have been found for trivalent, but not for pentavalent, arsenic (Nordenson *et al*., 1981). Sodium arsenite was found to be effective in increasing sister chromatid exchanges, but cultured lymphocytes obtained from 13 patients with Blackfoot disease were not different from those of healthy persons in terms of frequency of sister chromatid exchanges after exposure to sodium arsenite (Wen *et al*., 1981). Arsenical interference with normal DNA repair processes has also been noted (Fong *et al.*, 1980; Jung *et al*., 1969; 1971; Rossman *et al*., 1977), suggesting the induction of cellular genome damage.

Mutagenicity tests using *Salmonella, Escherichia coli* system, and Chinese hamster cell line V79 failed to produce point mutations with trivalent or pentavalent inorganic arsenic (Lofroth and Ames, 1978; Rossman *et al*., 1980). Arsenic trichloride, sodium arsenite, and arsenic pentoxide, however, gave positive results in *rec* assay in *Bacillus subtilis* (Kada *et al*., 1980; Nishioka, 1975).

Morphological transformation of Syrian hamster embryo cells derived from 13- to 14-day-old embryos occurred at concentrations of 2.5–5.0 µg/mL sodium arsenate using a cell culture method (DiPaolo and Casto, 1979).

The genotoxic effects of organic arsenicals such as DMA and roxarsone have been tested. They are shown to be able to cause mitotic arrest, chromosome aberrations, mutations and DNA strand breaks (ATSDR, 2005).

7.7 Interaction Between Arsenic and Other Compounds

Interaction between arsenic and selenium has been described by Levander (1977). He concluded that arsenic has a protective effect against the toxicity of selenium in several species. Likewise, selenium can decrease the effects of arsenic, including clastogenicity, cytotoxicity, and teratogenicity (Babich *et al*., 1989; Biswas *et al*., 1999; Holmberg and Ferm, 1969). The mechanism of this mutual inhibition of effects is not known, but may be related to the formation of a complex that is excreted more rapidly than either arsenic or selenium alone (Cikrt *et al*., 1988; Levander, 1977) or caused by selenium-induced changes in arsenic methylation (Styblo and Thomas 2001; Walton *et al*., 2003).

A combined exposure to arsenic and lead had additive effects on tissue respiration and functional changes in the CNS (Novakova, 1969). Mahaffey and Fowler (1977) first reported that rats given cadmium and arsenic (50 mg/kg) in food for 10 weeks showed lower serum alkaline phosphatase levels than those treated with either metal alone. Additive effects of dietary arsenic and lead (200 mg/kg) in coproporphyrin excretion were also subsequently noted (Fowler and Mahaffey, 1978, Mahaffey *et al*., 1981). More recent studies in rats using combined drinking water exposures to lead (25 mg Pb/L), cadmium (10 mg Cd/L), and arsenic (5.0 mgAs/L) for 30, 90, or 180 days at LOEL dose levels produced similar biochemical effects that showed variation with strong duration of exposure-dependence (Fowler *et al*., 2004). Please see Chapter 7 and review by Madden and Fowler (2000) on interactions among metals for a more complete discussion.

Arsenic has been shown to cause an increase in total plasma cholesterol in rats; coadministration of chromium (III) counteracts this effect (Aguilar *et al*., 1997).

The interaction between cigarette smoking, inhalation of arsenic, and the risk of lung cancer has not been extensively investigated. Smoking seemed to increase lung cancer risk synergistically in one study of smelter workers (Pershagen *et al*., 1981), although the data are not adequate to exclude a simple additive interaction (Thomas and Whittemore, 1988). Cigarette smoking has been shown to increase the occurrence of lung cancer in people with high levels of arsenic in the drinking water (Chiou *et al*., 1995; Tsuda *et al*., 1995).

Coexposure to ethanol and arsenic may exacerbate the toxic effects of arsenic. Simultaneous exposure of rats to ethanol (10% in drinking water) and arsenic for 6 weeks produced a significant increase in the concentration of arsenic in the kidney, a nonsignificant increase or arsenic in the liver, and a significant increase in the concentration of glutathione in the liver, compared with rats treated with either ethanol or arsenic alone (Flora *et al*., 1997ab). Histological damage to the liver, but not the kidneys, was increased in rats treated with both ethanol and arsenic compared with those receiving only arsenic.

Please see Chapter 7 on interactions in metal toxicology for a more detailed discussion.

8 DOSE-EFFECT AND DOSE-RESPONSE RELATIONSHIP IN ARSENIC POISONING

The dose-effect relationship in acute exposure to arsenic may be estimated from the 220 poisoning cases associated with an episode of arsenic contamination of soy sauce in Japan reported by Mizuta *et al*. (1956). The soy sauce was contaminated with approximately 0.1 mg As/mL, probably as calcium arsenate. Arsenic intake in the cases was estimated by the researchers to be 3 mg/day (0.05 mg/kg/day, assuming 55-kg average body weight for the Asian population). The duration of exposure was 2–3 weeks in most cases. The primary symptoms were edema of the face and gastrointestinal and upper respiratory symptoms initially, followed by skin lesions and neuropathy in some patients. Other effects included mild anemia and leukopenia, mild degenerative liver lesions, and hepatic dysfunction, abnormal electrocardiogram, and ocular lesions. An acute oral guidance value of 0.005 mg/kg/day (ATSDR, 2005) may be derived based on the LOAEL of 0.05 mg/kg/day, by applying an uncertainty factor of 10 for extrapolation from a LOAEL to a LOAEL and 1 for intrahuman variability. This guidance value is supported by the case of a man and wife in upstate New York who experienced gastrointestinal symptoms (nausea, diarrhea, abdominal cramps) starting almost immediately after beginning intermittent consumption of arsenic-contaminated drinking water at an estimated dosed of 0.05 mg/kg/day (Franzblau and Lilis, 1989). The UF of 1 for intrahuman variability reflects the fact that the database includes persons of different ethnicities and age groups, including infants.

Hamamoto (1955) reported on Japanese infants who ingested milk contaminated with arsenic over a period of 33 days. The amount of arsenic ingested was about 1.3–3.6 mg/day. When a total of about 80 mg arsenic had been ingested, these victims developed symptoms of arsenic poisoning, including pigmentation, swelling of the liver, and anemia. A total of 12,131 cases of poisoning with 130 deaths were reported. Impairment of learning and of clinical functions such as hearing had been described among those who survived (Yamashita *et al*., 1972).

In areas in Cordoba, Argentina, with endemic arsenicism, the main symptoms described among the inhabitants are symmetrical palmar and plantar hyperkeratosis. Concentrations in drinking water have been reported to be 0.9–3.4 mg As/L (Wickstrom, 1972).

The dose-effect relationship in chronic exposure may be estimated from the report by Tseng *et al*. (1968) and Tseng (1977), who investigated the incidence of Blackfoot disease and dermal lesions in a large number of poor farmers (both males and females) exposed to high levels of arsenic in well water in Taiwan. A control group consisting of over 7,500 people was identified. The incidence of dermal lesions increased with dose, but individual doses were not provided. The incidence data were provided based on stratification of the exposure population into low (<300 μg/L), medium (300–600 μg/L), or high (>600 μg/L) exposure levels according to arsenic concentration in the well water. Doses were calculated from group mean arsenic concentrations in well water, assuming the intake parameters described by Abernathy *et al*. (1989). The arithmetic mean concentration of arsenic in well water was converted to exposure doses assuming a water intake of 4.5 L/day, a body weight of 55 kg, and an estimation of arsenic intake of 0.003 mg/As/kg/day from food. The control, low, medium, and high exposure levels corresponded to doses of 0.0008, 0.014, 0.038, 0.065 mg/kg/day, respectively. A clear dose-response relationship was observed for characteristic skin lesions: 0.0008 mg/kg/day was identified as the NOAEL, at 0.014 mg/kg/day hyperpigmentation and keratosis of the skin were observed, and at 0.038–0.065 mg/kg/day increased incidence of dermal lesions was observed. A chronic health guidance value of 0.0003 μg/kg/day may be derived from the NOAEL of 0.0008 mg/kg/day, an UF of 3 was applied in consideration of the fact that most of the control population was less than 20 years of age and the incidence of skin lesions increased as a function of age, and because the estimates of water intake and dietary arsenic intake are highly uncertain (ATSDR, 2005). Schoof *et al*. (1998) estimated that dietary intakes of arsenic from rice and yams might have been 15–211 μg/day (mean, 61 μg/day), based on arsenic analyses of foods collected in Taiwan in 1993–1995. Use of the 50-μg/day estimate would result in an approximate doubling of the NOAEL (0.0016 mg/kg/day). This health

guidance value is supported by a number of well-conducted epidemiological studies that identify reliable NOAELs and LOAELs for dermal effects. Southwick *et al*. (1981) identified a NOAEL of 0.006–0.007 mg/kg/day for dermal lesions in several small populations in Utah. Harrington *et al*. (1978) identified a NOAEL of 0.003 mg/kg/day for dermal effects in a small population in Alaska. Guha Mazumder *et al*. (1988) identified a NOAEL of 0.009 mg/kg/day and a LOAEL of 0.006 mg/kg/day for pigmentation changes and hyperkeratosis in a small population in India. Haque *et al*. (2003) identified a LOAEL of 0.002 mg/kg/day for hyperpigmentation and hyperkeratosis in a case-control study in India. Cebrian *et al*. (1983) identified a NOAEL of 0.0004 mg/kg/day and a LOAEL of 0.022 mg/kg/day in two regions in Mexico. Borgono and Greiber (1972) and Zaldivar (1974) identified a LOAEL of 0.02 mg/kg/day for abnormal skin pigmentation in patients in Chile, and Borgono *et al*. (1977) identified a LOAEL of 0.01 mg/kg/day for the same effects in school children in Chile. Valentine *et al*. (1985) reported a NOAEL of 0.02 mg/kg/day for dermal effects in several small populations in California. Collectively, these studies indicate that the threshold dose for hyperpigmentation and hyperkeratosis is approximately 0.002 mg/kg/day.

Fierz (1965) discussed the dose-response relationship between skin cancer and the total dose of Fowler's solution ingested as medication. He reviewed 262 patients who had been treated for weeks or for several years with Fowler's solution for various chronic dermatoses. Of the solution, 10–2600 mL had been administered to the patients. Among those patients who developed late effects from this treatment, 40% had hyperkeratosis and 8% had skin cancer. A clear relationship between the increase in the incidence of both hyperkeratosis and cancer and the increased dose of arsenic was indicated (Fierz, 1965).

Dose-response relationships between skin cancer and arsenic have been discussed by Tseng *et al*. (1968) and Tseng (1977) for Taiwanese populations exposed to arsenic-contaminated well water. Of a total population of 40,421, arsenical skin cancer was found in 428 persons (10.6/1000). There were no patients younger than 20 years of age. The minimal amount of arsenic in well water necessary to induce skin cancer was estimated at less than 0.29 mg/L. The WHO Task Group on Environmental Health Criteria for arsenic (WHO, 1981) concluded, based on this study, that the lifetime risk for skin cancer is approximately 25% per mg As/L drinking water. Based on the Taiwan skin cancer data using the multistage model, the U.S. EPA has calculated a cancer unit risk for drinking water of 5E-5 per (μg/L) (EPA, 2005).

There is increasingly convincing evidence that long-term exposure to arsenic can result in the development of bladder cancer (Bates *et al*., 2004; Chen *et al*., 1992; Chiou *et al*., 1995, 2001; Cuzick *et al*., 1992; Guo, 2000), with transitional cell cancers being the most prevalent. Chiou *et al*. (1995) reported a dose-response relationship between long-term arsenic exposure from drinking artesian well water and the incidence of lung cancer, bladder cancer, and cancers of all sites combined (after adjustment for age, gender, and cigarette smoking) in four townships in Taiwan exposed to inorganic arsenic in drinking water (0–1.14 mg/L). In a later follow-up study of the same cohort, the increase in bladder cancer was found to be statistically significant only in subjects exposed for 40 years or longer (Chiou *et al*., 2001). Cuzick *et al*. (1992) evaluated a cohort treated with Fowler's solution (potassium arsenite) in Lancashire, England, during the period 1945–1969 and followed through 1991; the cohort of 478 patients showed a significant excess of bladder cancer, but no excess for other causes of death. Of a subcohort of 142 patients examined for signs of arsenicism around 1970, all 11 subsequent cancer deaths occurred in those with signs of arsenicism ($P = 0.0009$). Hopenhaya-Rich *et al*. (1996) investigated bladder cancer mortality for the years 1986–1991 in 26 counties of Cordoba, Argentina, and reported that bladder cancer SMRs were consistently higher in counties with documented arsenic exposure; a later case-control study by the same authors (Bates *et al*., 2004) did not report statistically significant increases in bladder cancers resulting from arsenic exposure, except in individuals exposed for 50 years or longs. Guo (2000) reported significantly increased rate differences for bladder cancer in men and women in Taiwan exposed to 0.64 mg arsenic/L in the drinking water, but not at lower exposure levels.

A study of arsenic-exposed individuals in northern Chile reported significantly increased odd ratios for lung cancer among subjects with >30 μg As/L of drinking water (Ferreccio *et al*., 2000), although when adjusted for socioeconomic status, smoking, and other factors, the increase was only significant at 60 μg/L or greater. Guo (2004) reported significantly increased rates differences (RD) for lung cancer for Taiwanese men and women exposed to 0.64 mg arsenic/L or greater, with those older than 50 years of age being particularly at risk. Nakadaira *et al*. (2002) suggested that even comparatively short exposure durations (less than 5 years) might be sufficient for the development of arsenic-induced lung cancer.

The National Academic Sciences (NRC, 1999; 2001) has analyzed the Taiwan data and estimated excess lifetime risk of bladder and lung cancer with various arsenic levels in drinking water: at a concentration of

arsenic in drinking water of 3 µg/L, the lifetime risk estimates for bladder and lung cancer combined are approximately 4 and 10 per 10,000 using the Taiwan or U.S. background rates of these cancers, respectively. The U.S. EPA has not yet calculated a unit risk value or slope factor for arsenic-induced internal tumors.

Some data on dose-response relationships for respiratory cancer have been given by Pinto *et al*. (1977). They found an almost linear increase in respiratory cancer with an increasing "exposure index." From the data, it can be estimated that exposure to airborne arsenic of about 50 µg/m^3 for more than 25 years might increase the risk for developing respiratory cancer nearly threefold.

The data must be interpreted with caution because of the possible influence-complicating factors. As for workers engaged in production of insecticides such as lead arsenate, calcium arsenate, copper acetoarsenite, and magnesium arsenite, a positive dose-response relationship between the degree of arsenic exposure and lung cancer was indicated (Blejer and Wager, 1976; Ott *et al*., 1974). The ratio of observed to expected respiratory cancer deaths ranged from 0.6 in the lowest exposure category to 7.0 in the highest. However, this dose-response relationship in regard to lung cancer should be reevaluated after epidemiological adjustment of the smoking histories of the workers.

Jarup *et al*. (1989) reported significantly increased lung cancer mortality (SMR = 372, 95% confidence interval [CI] = 304–450) based on 106 lung cancer deaths in a cohort of 3916 male workers employed for at least 3 months between 1928 and 1967 at the Ronskar smelter and followed for mortality through 1981. Workers were separated into low, medium, and high arsenic exposure groups, with mean time-weighted average exposure estimates of 0.05, 0.2, and 0.4 mg/m^3, respectively. Lung cancer mortality was significantly increased in all three exposure groups in a concentration-related fashion (SMR = 201, 353, and 480, respectively). A nested case-control analysis of 102 lung cancer cases and 190 controls from the cohort showed that lung cancer risk increased with increasing arsenic exposure in nonsmokers, light smokers, and heavy smokers (Jarup and Pershagen, 1991).

Enterline and Marsh (1982) reported a significant increase in respiratory cancer mortality (SMR = 189.4) based on 104 observed respiratory cancer deaths and only 54.9 expected over the years 1941–1976 in a cohort of 2802 male workers employed for at least 1 year between 1940 and 1964 at the ASARCO smelter. Enterline *et al*. (1987) reanalyzed these data using improved exposure estimates that incorporated historical measurements of arsenic in the ambient and personal breathing zone of workers. Respiratory cancer mortality was significantly increased in a concentration-related fashion in the low (SMR = 213.0), medium (SMR = 312.1), and high (SMR = 340.9) arsenic exposure groups, which had mean estimated time-weighted average arsenic exposure of 0.213, 0.564, and 1.487 mg/m^3, respectively.

Respiratory cancer mortality was significantly increased (SMR = 285) on the basis of 302 observed respiratory cancer deaths between 1938 and 1977 in a cohort of 8045 white male workers employed for at least 1 year at the Anaconda smelter (Lee-Feldstein, 1986). Analysis of a subset of the Anaconda cohort (n = 1800, including all 277 employees with heavy arsenic exposure) that included information on smoking and other occupational exposures showed that lung cancer mortality increased with increasing time-weighted average arsenic exposure, with a small nonsignificant increase in the low group (SMR = 138) exposed to 0.05 mg/m^3 and significant increases in the medium (SMR = 303), high (SMR = 375), and very high (SMR = 704) groups exposed to 0.3, 2.75, and 5.0 mg/m^3, respectively. Lubin *et al*. (2000) reweighed the exposure concentration on the basis of duration and time of exposure and reevaluated the effects of exposure. Relative risks for respiratory cancer increased with increasing duration. SMRs were significantly elevated after exposure to 0.58 mg/m^3 (SMR = 3.01, 95% CI = 2.0–4.6) or 11.3 mg/m^3 (SMR = 3.68; 95% CI = 2.1–6.4) for 10 or more years, and after exposure to 0.29 mg/m^3 (SMR = 1.86, 95% CI = 1.2–2.9) for 25 or more years.

On the basis of the dose-response relationships between arsenic exposure and excess lung cancer mortality in workers at the Anaconda smelter and the ASARCO smelter, the U.S. EPA (2005) has derived a unit risk estimate (the excess of lung cancer associated with lifetime exposure to 1 µg inhaled inorganic arsenic/m^3) of 4.3×10^{-3} per (µg/m^3).

9 DIAGNOSIS, TREATMENT, AND PROGNOSIS

When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice. There are several medical toxicology texts that provide specific information about clinical treatment after exposures to arsenic (Ellenhorn, 1997; Goldfrank *et al*., 1998; Tintinalli *et al*., 1996).

9.1 Acute Poisoning

9.1.1 Inhalation Diagnosis

Acute intoxication caused by inhalation of arsenic is unusual except in the case of arsine (see Section 10),

although respiratory symptoms may occur among workers suddenly exposed to high concentrations of arsenic in smelters. Gerhardson *et al*. (1988) reported a case of a smelter worker who inhaled a large quantity of arsenic-containing dust (80% As_2O_3) who died several hours later. Autopsy findings disclosed widespread hemorrhages in the respiratory tree and congestion of the major organs. Acute skin lesions such as contact and allergic dermatitis may be observed around the eyes and mouth, as well as on the face and neck. The diagnosis of such cases is based on the knowledge of a history of arsenic exposure. The measurement of arsenic in urine may be useful, provided the specimen is taken within a few days after exposure.

9.1.1.1 Treatment and Prognosis

Conservative treatment is usually applied to the skin lesions. Dimercaprol (BAL) may be given in cases of severe symptoms of the respiratory system or the skin. Chelation therapy is most effective when instituted within a few hours after exposure, and efficacy decreases as time after exposure increases (McFall *et al*., 1998; Peterson and Rumack, 1977). In general, chelating agents should be used with caution, because they may have serious side effects such as pain, fever, hypotension, and nephrotoxicity. Some water-soluble and less toxic analogs of BAL such as dimercaptosuccinic acid (DMSA), dimercaptopropyl phthalamadic acid (DMPA), and dimercaptopropane sulfonic acid (DMPS) are currently under investigation and may prove to be promising treatments for arsenic poisoning (Aposhian *et al*., 1997; Guha Mazumder, 1996; Kreppel *et al*., 1995). A recent article by Vantroyen *et al*. (2004) described a case of a massive arsenic trioxide overdose that was successfully treated by continuous gastric irrigation with sodium bicarbonate, forced diuresis, and administration of BAL and DMSA.

9.1.2 Ingestion Diagnosis

The determination of arsenic in urine, hair, and, in some cases, blood or stomach contents, is useful for diagnosis if exposure to arsenic cannot be readily identified.

9.1.2.1 Treatment and Prognosis

The treatment for acute arsenic poisoning by oral ingestion is referred to in standard medical textbooks (e.g., Haddad and Winchester's *Clinical Management of Poisoning and Drug Overdose*, 2nd edition (1990). Prognosis will depend on both dose and time span between the ingestion of arsenic and first treatment. In some cases where the patient has recovered from acute poisoning, dermatitis and peripheral neuritis may persist for a relatively long time (Le Quesne and McLeod, 1977).

9.2 Chronic Poisoning

9.2.1 Diagnosis

In the diagnosis of chronic arsenic poisoning, it is important to differentiate between senile pigmentation, senile leukodermia, and arsenical dermatoses, as well as between peripheral polyneuritis caused by arsenic poisoning and caused by other diseases. This is especially true for individuals exposed to arsenic by ingestion.

Concentrations of arsenic in biological tissues may be within the normal range at the time diagnosis is made. In such cases, a well-designed epidemiological study may be required if it is thought that a whole group of people might have been exposed to arsenic. White striae in the fingernails (Mees' lines) are also a useful clue in the diagnosis of arsenical polyneuritis, because urine and hair arsenic concentrations may be within normal limits despite the presence of this condition (Heyman *et al*., 1956).

Recognition of damage to the upper respiratory tract, specifically perforation of the nasal septum, may be quite helpful in the diagnosis of chronic arsenic poisoning caused by somewhat higher exposures to inorganic arsenic.

Liver damage, including cirrhosis and angiosarcoma, which may be seen in an arsenic-exposed individual, may require differentiation in terms of the cause-effect relationship. Among younger people exposed to inorganic arsenic–contaminated food and water, cardial and pulmonary symptoms are useful in diagnosis.

9.2.2 Treatment and Prognosis

BAL has been used for the treatment of chronic arsenic poisoning, particularly in cases of dermatosis. Pinto and McGill (1953) administered BAL to people with arsenic dermatitis. They succeeded in augmenting the excretion of arsenic into urine, but not in improving skin changes such as melanosis and keratosis. A randomized placebo trial of 2,3-DMSA as a therapy for chronic arsenosis caused by drinking contaminated water found no significant difference between patients treated with 2,3-DMSA and those treated with a placebo (Guha Mazumder *et al*., 1998a). Apart from the often-questionable use of BAL, the treatment of chronic arsenic poisoning should generally be conservative.

Skin changes and neuropathy may persist for a number of years. The skin changes seen in simple keratosis may progress into Bowen's disease, which will spread over the entire body in multiple forms.

10 ARSINE

Arsine (hydrogen arsenide, AsH_3) is a colorless, inflammable gas with a slight garlic odor. It is generated whenever nascent hydrogen is liberated in material containing arsenic. Because arsenic is present as an impurity in many metal ores, arsine may be generated in metal industries, nonferrous metal refineries, and in the manufacture of silicon steel, if the ores being processed accidentally come into contact with acid. Arsine may also be released when the hydrogen ion is formed by hydrolysis and in the reaction of moisture with arsenic-containing dross.

The toxicity and toxicological mechanisms of arsine are quite different from those of other inorganic or organic arsenic compounds. Arsine acts as a powerful hemolytic poison in cases of both acute and chronic exposure.

Henderson and Haggard (1943) observed that the lethal dose of arsine for humans was 250 mg/m^3 for 30 minutes, and that symptoms of poisoning occur after a few hours of exposure to 3–10 mg/m^3. Elkins (1959) reported a serious, nonfatal, acute case of arsine poisoning in a person who had worked in an atmosphere contaminated with an average of 0.5 mg arsine/m^3. Five workers, examined by Kipling and Fothergill (1964), developed typical arsine poisoning when working a plant where an acetylene-like odor was detected. This odor occurred during the slag-dissolving process that took place in a rotating drum. The concentration of arsine in this factory was 5 mg/m^3.

Morse and Setterlind (1950) studied two fatal cases of arsine poisoning in which aluminum had been used to extract arsenic. The concentration of arsine in this instance was 70–300 mg/m^3.

Arsine poisoning is characterized by nausea, abdominal colic, vomiting, backache, and shortness of breath, followed by dark blood urine and jaundice (Kipling and Fothergill, 1964). According to Bulmer *et al*. (1940), the following symptoms appeared after various lengths of exposure to arsine: shortness of breath on exertion, general malaise, nausea, poor appetite, palpitation on exertion, and headache. In some groups of workers, a tingling sensation in the feet, chills, garlic breath, changes in complexion, weakness, vomiting, and drowsiness were observed. Jaundice was also noted in a number of people. Some of the workers with the longest exposure had anemia. Arsenic in urine was almost always elevated and ranged from 0.04–4.3 mg/L. Even after the workers had left the arsine-contaminated area, arsenic in urine ranged from 0.04–0.1 mg/L. Peripheral neuritis was seen in arsine-exposed workers (Dudley, 1919). Liver disturbances may accompany arsine poisoning. In most fatal cases, renal failure caused by blockage of the renal tubules by hemoglobin casts seems to be the cause of death (Fowler and Weissberg, 1974). Residual functional impairment of the kidneys has been reported in one person after an acute episode of arsine poisoning (Muehrcke and Pirani, 1968).

The use of BAL for arsine poisoning has not had desirable results. Symptomatic therapy, which is generally used, is considered more reliable, particularly in the treatment of anuria. Blood transfusions may be necessary for severe anemia. Arsine toxicity and treatment have been reviewed extensively by Fowler and Weissberg (1974).

The mechanisms of arsine gas toxicity and more recent studies in experimental animal/*in vitro* model systems have also been studied over the last 20 years primarily because of possible health effects related to its use in the semiconductor industry.

Arsine is used in doping the silicon-based chips and in producing III-semiconductors, such as GaAs and InAs. Before the recent uses of arsine gas in the semiconductor industry, the main concerns regarding human health were related to its use as a war gas and from industrial accidents (Fowler and Weisberg, 1974). In the past, the chief clinical effects observed in persons with acute occupational arsine exposure were massive hemolysis followed by death from renal failure. Patients typically presented with decreased hematocrit values and red "port wine"–colored urine because of the presence of hemoglobin. Histopathological evaluations of postmortem kidney samples demonstrated the presence of renal tubular obstruction from hemoglobin casts that resulted in renal failure. With the development of renal dialysis, mortality from acute exposure has been greatly reduced in recent decades (Fowler and Weisberg, 1974).

10.1 Experimental Model Studies

Prolonged inhalation exposures of rodents to arsine at sublethal lower doses (Blair *et al*., 1990ab; Fowler *et al*., 1989; Hong *et al*., 1989; Morrissey *et al*., 1990; Rosenthal *et al*., 1989) have been shown to produce a regenerative anemia with immature erythrocytes and the presence of Howell–Jolley bodies in the peripheral blood. Splenomegaly and the characteristic arsenical-induced porphyrinuria (Woods and Fowler, 1978) dominated by increased excretion of uroporphyrins with lesser quantities of coproporphyrin (Fowler *et al*., 1989) are also major manifestations of arsine toxicity to the hematopoietic system. Other effects include alterations in immune system function that have been noted in animals exposed to arsine for prolonged periods of time (Rosenthal *et al*., 1989), indicating concomitant perturbation of this important biological defense

system. No negative reproductive and developmental outcomes in rodents exposed to arsine (Morrissey *et al*., 1990).

Studies conducted in cell culture systems exposed to arsine *in vitro* (Ayala-Fierro and Carter, 2000; Carter *et al*., 2003; Hatelid and Carter, 1997; Hatelid *et al*., 1996; Rael *et al*., 2000; Winski *et al*., 1997) have shown methemoglobin formation and ultrastructural effects on erythrocyte membrane structure with concomitant loss of ion regulation capabilities with attendant red cell lysis. Based on these *in vivo* and *in vitro* experimental studies, arsine is clearly a highly toxic chemical agent that exerts toxicity on a number of organ systems. Target cells in the hematopoietic, liver, kidney, and the immune systems are clearly sensitive to arsine toxicity after either short-term or more chronic exposures. The mechanisms by which this agent produces hemolysis seem to require oxygen and the internalization of arsine into the red cells with subsequent oxidation and inhibition of membrane ion regulatory mechanisms. Pharmacological factors such as dose and duration of exposure, as well as susceptibility factors such as age and nutritional status of those exposed, will also have an impact on prognosis.

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CHAPTER

20

Barium

AGNETA OSKARSSON AND ANDREW L. REEVES

ABSTRACT

The toxicity of barium compounds depends on their solubility. The free ion is readily absorbed from the lung or gastrointestinal tract, but barium sulfate remains essentially unabsorbed. After absorption, barium accumulates in the skeleton; an accumulation also takes place in the pigmented parts of the eye.

Acute or chronic exposure to barium salts results in a number of disorders, including renal intoxication, hypertension, and cardiac malfunction in experimental animals. A higher incidence of hypertension has been observed after occupational barium exposure, but no association was found between blood pressure and barium in drinking water up to 10 mg/L, corresponding to approximately 0.21 mg Ba/kg/day. The kidney seems to be the most sensitive target organ in rats and mice exposed repeatedly to barium chloride in drinking water. No evidence of carcinogenic activity of barium chloride was found in rats or mice after administration through drinking water.

Poisoning with soluble barium compounds has resulted from accidental or suicidal ingestion or when barium sulfate, used as an opaque contrast medium for X-ray studies of the gastrointestinal tract, has been contaminated with soluble barium. The Ba^{2+} ion is a muscle poison, causing gastrointestinal, cardiac and skeletomuscular stimulation followed by paralysis. In the Szechuan province of China, a subacute form of barium poisoning (pa-ping) was endemic because of use of contaminated table salt.

Barium seems to act as a potassium antagonist and calcium agonist. The mechanism of action is partly due to blocking of the K^+-channels of the Na–K pump in cell membranes, increasing the active inward transport and inhibiting the passive outward transport of potassium. Cases of barium poisoning are accompanied by severe hypokalemia, and potassium infusion is an effective antidote. If administered promptly, soluble sulfates are also an effective antidote, because they form a precipitate with barium that is not readily absorbed from the gastrointestinal tract or lung.

Inhalation of barium sulfate dust causes a benign pneumoconiosis ("baritosis") with conspicuous radiographic manifestations but no impairment of pulmonary function. The condition has been reproduced in rats.

Reviews on barium toxicology and metabolism were written by Schroeder *et al*. (1972), WHO (1990), ATSDR (1992), EPA (1999), and Dallas and Williams (2001).

1 PHYSICAL AND CHEMICAL PROPERTIES

With an atomic number of 56 and atomic weight of 137.3, barium is the heaviest of the stable alkaline earth metals (Group IIa of the Periodic Table). The free element is a silver-white soft metal melting at 725°C. It oxidizes readily in moist air and reacts with water or with dilute acids under evolution of hydrogen gas.

In its compounds, barium is a colorless divalent positive ion. The acetate, chloride, hydroxide, and nitrate are relatively water soluble (e.g., the chloride solubility in water is 375 g/L at 20°C), whereas the carbonate and sulfate have very low water solubility, 0.02 and 0.002 g/L at room temperature, respectively. The solubility in acids is higher, and 0.06 g/L of barium sulfate is soluble in 3% of hydrochloric acid. Volatile

compounds of barium (especially halide salts) stain the Bunsen flame a pale green color.

2 METHODS AND PROBLEMS OF ANALYSIS

Atomic absorption spectroscopy (AAS), inductively coupled plasma atomic emission spectrometry (ICP-AES), and ICP-mass spectroscopy (ICP-MS) are the most commonly used analytical methods for measuring low levels of barium and its compounds in air, water, geological and various biological materials. Flame AAS and graphite furnace AAS determine levels of barium in water and wastewater from 200 µg/L (ATSDR, 1992). By use of ICP-MS, detection limits has been reported for analysis of barium in urine of 1 µg/L (Komaromy-Hiller *et al*., 2000) and in water of 0.001 µg/L (Rosborg *et al*., 2003). Plamboeck *et al*. (2003) have developed a sensitive method for determination of barium in water, bone, and liver on the basis of flow injection analysis and flame atomic emission spectrometry (FIA-FAES) with a detection limit of 0.8 µg/L. Other analytical techniques include the less sensitive methods of X-ray fluorescence spectroscopy and neutron activation analysis and the less commonly used methods of scintillation spectroscopy, and spectrography (ATSDR, 1992). In general, analytical procedures measure total barium ion present and do not allow for speciation of barium compounds.

3 PRODUCTION AND USES

3.1 Production

Barium occurs chiefly as the mineral barite ($BaSO_4$). The total world production was approximately 6 million metric tons in 2002, with China producing 50% and India and Morocco being the other leading producers (http://www.indexmundi.com/en/commodities/minerals). In the United States, barite sales by domestic producers totaled about 550,000 tons in 2004 (US Geological Survey). For the manufacture of barium chemicals, barite is reduced to the much more reactive barium sulfide through high-temperature sintering with charcoal. Barium is also mined in small amounts as witherite ($BaCO_3$).

3.2 Uses

In recent years, approximately 85% of ground and crushed barite sold was used directly as weighting agent in oil- and gas-well drilling mud. Barium is also used in the manufacture of alloys, glass, cement, ceramics, electronics, roentgenography, pharmaceuticals, vinyl stabilizers, railroad flares, fireworks, fine chemicals, lubricating oil additives, permanent magnets, as well as in specialty arc welding, sugar refining, paper coating, and as pigment in ink and paint (lithophone).

Barium-containing household and consumer products constitute a minuscule part of barium consumption but have the greatest toxicological significance. They include rodenticides, insecticides, and depilatories. In addition, barium is used as a radio pacer both in composite fillings and in root canal fillings.

4 ENVIRONMENTAL LEVELS AND EXPOSURES

4.1 General Environment

4.1.1 Soil, Water, and Air

Barium constitutes approximately 0.04% of the earth's crust and is present in the environment in relatively high concentrations. Barium enters the environment through weathering of rocks and minerals and through anthropogenic releases, primarily industrial. Emissions may result from mining, refining, or processing of barium minerals and manufacture of barium products. Barium is released to the atmosphere during the burning of coal, fossil fuels, and waste. Barium is also discharged in wastewater from metallurgical and industrial processes. Deposition on soil may result from human activities, including the disposal of fly ash and primary and secondary sludge in landfills (WHO, 1990). Agricultural soils contain barium in the range of 10–5000 mg/kg, with an average abundance of about 500 mg/kg (ATSDR, 1992; WHO, 1990). Barium in soils is not expected to be very mobile because of the formation of water-insoluble salts and its inability to form soluble complexes with humic and fulvic materials. However, under acid conditions some of the water-insoluble barium compounds (e.g., barium sulfate) may become soluble and move into groundwater. The concentration in water is extremely variable and is related to the hardness of the water. Barium concentrations of 0.007–15 mg/L (average, 0.05) and 0.006 mg/L have been measured in fresh water and seawater, respectively (Schroeder *et al*., 1972). Drinking water levels in Sweden ranged from 0.001–0.123 mg Ba/L (Boström and Wester, 1967; Rosborg *et al*., 2003). Higher levels were found in acid than in alkaline well water (Rosborg *et al*., 2003). In some areas of the United States, concentrations up to 10 mg/L have been reported. In U.S. urban air, the average concentration of barium was 12 ng/m^3 (range, 0.2–28) (ATSDR, 1992).

4.1.2 Plants, Animals, and Dietary Intake

Increased barium concentrations have been detected in plants near waste disposal sites and in urban areas. Barium is present in gasoline. Monaci and Bargagli (1997) found a highly significant relationship between lead and barium in leaves from the evergreen oak, *Quercus ilex,* and suggested barium to be a valuable tracer for vehicle emissions. Llugany *et al*. (2000) studied barium toxicity in bush beans (*Phaseolus vulgaris)* and found that the presence of barium, even at concentrations that do not affect plant growth, had a depressing effect on the potassium concentrations in the leaves. In addition, barium interfered with the sulfate and calcium nutrition of the plant.

Despite relatively high concentrations in soils, only a limited amount of barium is taken up in plants, and there is a low transfer to animals. Thus, in most food the barium concentration is relatively low, <0.1 mg/kg and somewhat higher in cereal products with concentrations around 1 mg/kg. However, Brazil nut trees are known to accumulate soil barium and extremely high levels are found in Brazil nuts, 1500–3000 mg/kg (WHO, 1990). High levels are also found in pecan nuts and dry cocoa, with 6.7 and 12 mg/kg, respectively.

The daily dietary intake of barium has been estimated in several studies, ranging from 0.3–1.8 mg (WHO, 1990). In an American hospital diet, the average daily intake of barium was estimated as 0.4 mg, whereas in the diet of the general population it may be as high as 1.33 mg (Schroeder *et al*., 1972). Grummitt (1961) estimated that typical dietary barium intake originated 25% from milk, 25% from flour, 25% from potatoes, and 25% from miscellaneous high-barium foods consumed in minor quantities, especially nuts.

4.2 Working Environment

The industrial uses of soluble barium are such that hazardous conditions from atmospheric contaminations are uncommon. However, arc welding with barium-containing stick electrodes and flux-cored wires involves occupational exposure to soluble barium. Without appropriate preventive measures, the median barium concentration in the breathing zone was 2–4 mg/m^3 compared with 0.3 when preventive measures were taken (Zschiesche *et al*., 1992).

The OSHA Permissible Exposure Limit (PEL) for soluble barium salts is 0.5 mg/m^3 TWA (time weighted average). The figure has an "A4" notation (not classifiable as a human carcinogen). For barium sulfate dust, the PEL is 10 mg/m^3 as inhalable (total) particulate matter and 5 mg/m^3 for the respirable fraction.

5 KINETICS

5.1 Absorption

5.1.1 Inhalation

Soluble forms of barium are readily absorbed from all segments of the respiratory tract. Nasal absorption of $^{133}BaCl_2$ in hamsters was estimated at 60–80% of the dose at 4 hours after dosing (Cuddihy and Ozog, 1973), and alveolar absorption may be of similar magnitude. According to measurements at tracer levels, even $^{131}BaSO_4$ was found to be cleared from the lungs, with a biological half-time of 8–9 days, through absorption into the general circulation with subsequent urinary clearance (Morrow *et al*., 1964). This indicated some solubility of $BaSO_4$ in body fluids, possibly in colloidal form.

Clearance of various forms of barium after exposure was studied by Einbrodt *et al*. (1972) and by Cuddihy *et al*. (1974). Different compounds of barium were cleared from the lungs in proportion to their solubilities: in the case of $BaSO_4$, the clearance rate depended on the specific surface area of the inhaled particles and was lower for heat-treated than for untreated particles. Barium in fused montmorillonite clay had the lowest clearance rate.

5.1.2 Ingestion

The absorption of ingested barium depends on such factors as solubility of the salt, starvation, and age of the animal and presence of sulfate in the diet. The absorption of soluble barium chloride has been studied. In hamsters receiving $^{133}BaCl_2$ by intragastric intubation, absorption was 11–32% of the dose (Cuddihy and Ozog, 1973). Taylor *et al*. (1962) reported absorption efficiencies of barium chloride in rats varying from 7–85%, with the highest absorptions in fasted (20%) and in young (85% at 14–18 days of age) rats. Sutton *et al*. (1972) and Sutton and Shepherd (1973) found that addition of sodium alginate to the diet reduced intestinal absorption of barium in rat and man to 42–75% (average 64%) of control.

Barium sulfate can be used as X-ray contrast material for gastrointestinal examinations, because during the relatively brief period of passage through the alimentary canal, $BaSO_4$ remains essentially unabsorbed. However, McCauley and Washington (1983) reported similar absorption rates with barium sulfate as with barium chloride after administration to rats by way of gastric intubation of a low dose of barium, 10 µg/kg body weight. The peak in blood concentration after administration of the sulfate was 85% of the peak after administration of the chloride. Evidently, hydrochloric

acid in the stomach can solubilize small quantities of barium sulfate. Similarly, statistically significant increases in blood and urine levels of barium were found in humans ingesting 58–400 g of barium sulfate in X-ray contrast materials (Clavel *et al*., 1987).

5.1.3 Parenteral Administration

Thomas *et al*. (1973) measured the *in vivo* solubility of four barium salts after intramuscular deposition. Chloride and carbonate were about equally diffusible and left the injection site very rapidly. The sulfate showed a biological half-time of 26 days, whereas a fused aluminosilicate clay had a biological half-time of 1400 days.

5.2 Transport and Distribution

All alkaline earth metals have a tendency to accumulate in the skeleton, and approximately 91% of the body burden is in the bone (WHO, 1990). For circulating barium, the degree of osseous uptake was found to be 1.5–5 times that of calcium or strontium. Ellsässer *et al*. (1969) found this preference for barium to be due to uptake on bone surfaces rather than to diffuse uptake. The Ba/Sr bone uptake ratio varies with age. Domanski *et al*. (1980) found 1.9–2.2 in young and 1.5–1.9 in adult rabbits. After intravenous injection of $^{133}Ba^{2+}$ into beagle dogs, specific activity was highest in the sternum, followed by sacrum and coccyx, vertebrae, ribs, humerus, and femur. In the skull, mandible, radius, ulna, and tibia-fibula, the specific activity was less than whole-body specific activity.

For most soft tissues, barium accumulation after intravenous injection is low and in proportion to their calcium content. Exceptions are the submaxillary gland, which was found to concentrate barium from serum in preference to the other alkaline earth metals (Bligh and Taylor, 1962), the heart (McCauley and Washington 1983), and the eye, in which extremely high concentrations of barium are sometimes found (Sowden and Pirie, 1958). The pigmented parts of the eye (iris, sclera, and especially the choroid) accumulate the highest levels of circulating barium, with concentrations reaching the 0.1 g/kg (wet weight) level. High concentrations of barium were also found in melanoma cells (Kaufmann, 1980).

After inhalation exposure of rats to a $BaSO_4$ aerosol at 40 mg/m^3, 5 hours daily for 2 months, lymphatic transport was slight. The skeletal concentration of Ba was 0.8–1.5 mg/g dry substance (10–100 times the pulmonary concentration). Skeletal uptake decreased somewhat with advancing age (Einbrodt *et al*., 1972).

5.3 Excretion

In healthy humans with a steady-state level of barium (with virtually the entire intake occurring per os), approximately 91% of the total output was found in feces, 6% in sweat, and 3% in urine (Schroeder *et al*., 1972). Intravenously injected soluble Ba^{2+} in a healthy man was excreted through both feces and urine, in a ratio ranging from 3:1 (Bauer *et al*., 1956) to 9:1 (Harrison *et al*., 1966). Approximately 75% of the dose was cleared from the body in 3 days and an additional 10–20% during the following 7–42 days. The pattern of total excretion fitted a three-component exponential function with biological half-times of 3.6, 34.2, and 1033 days, respectively (Rundo, 1967). The whole-body retention for the first few days after injection was recalculated by Erre *et al*. (1980). In beagle dogs given a single dose of $^{133}BaCl_2$ orally, total excretion in 300 days was 98.3–99.3% of the dose (Cuddihy and Griffith, 1972). Of $BaSO_4$ injected intratracheally, about 5% of the initial dose was recovered in the urine during the first 10 days after administration (Cember *et al*., 1961).

Barium excretion at 3–6 hours after intravenous administration of a soluble salt was also measured in saliva and seminal fluid of a healthy man, yielding values of 0.22–0.33% and 0.81% of the dose, respectively (Harrison *et al*. 1967). In lactating cows, excretion in milk during the first 8 days after dosing was 0.6% of the dose after oral and 10% of the dose after intravenous administration (Garner *et al*., 1960).

6 BIOLOGICAL MONITORING

There are no well-established biomarkers of exposure or effects for barium at present. Schroeder *et al*. (1972) published normal levels for barium in various organs of unexposed persons. Barium concentration in the skeleton of a 70-kg American adult was estimated at 2 mg/kg. Other organs with measurable levels included the eye (330 µg/kg), lungs (160 µg/kg), connective tissue (125 µg/kg), skin (50 µg/kg), and adipose tissue (36 µg/kg). In other internal organs, barium concentrations were slight. Among various parts of the eye, choroid had the highest barium level, reaching 600 mg/kg in the cow and 10 mg/kg in man (Sowden and Pirie, 1958).

Most or all barium in blood is in the plasma fraction (Schroeder *et al*., 1972). Serum reference values of barium have been reported, varying from 1 µg/L (Cornelis *et al*., 1994) to 60 µg/L and higher with increasing age, especially in women (Rahil-Khazen *et al*., 2000). A temporary increase occurs after barium absorption, but the excess disappears from the bloodstream practically completely 24 hours after cessation of

absorption. Exposure to soluble barium compounds during welding resulted in median plasma levels up to 24.7 µg/L (Zschiesche *et al*., 1992). In a case of suicidal poisoning with barium chloride, barium levels at autopsy were 9.9 mg/L in blood and 6.3 mg/L in urine (Jourdan *et al*., 2001). Urinary levels of barium were determined by ICP-MS in 1437 samples collected from US patients (Komaromy-Hiller *et al*., 2000). The mean level was 3.5 µg/L (SD, 2.2) and the calculated representative ranges 1.0–7.0 µg/L (95% confidence interval after exclusion of the top 10% of the results), which was somewhat higher than the reference values, 0.25–5.7 µg/L from a control population, as reported by Minoia *et al*. (1990). Exposure to soluble barium compounds during welding resulted in median urine levels up to 101.7 µg/L (Zschiesche *et al*., 1992).

Yoshinaga *et al*. (1995) determined the concentration of barium in rib samples after autopsy of 35 elderly Japanese people. The median barium concentration, determined by ICP-MS, was 1.3 µg/g dry bone (range, 0.41–3.20). The bone concentration was not correlated with age. Whether bone levels reflect barium exposure has not been evaluated. In the same study an association was reported between the presence of cerebrovascular damage and barium concentration in rib (see Section 7.1.2).

Rosborg *et al*. (2003) determined 34 elements in hair in females from acid and alkaline regions in southern Sweden. The median hair concentration of barium was 1057 mg/kg (range, 10–7150). The concentrations of barium were significantly higher in hair collected in acid compared with alkaline regions ($P < 0.001$). No correlation was found between barium levels in drinking water and in hair. As for bone, the relevance for hair as a biological indicator for barium exposure has not been evaluated.

7 EFFECTS AND DOSE-RESPONSE RELATIONSHIPS

7.1 Humans

7.1.1 Acute Effects

Radiopaque barium sulfate is used worldwide to provide contrast for diagnostic radiographic examinations in the gastrointestinal mucosa. Because of the very low solubility in water and lipid, barium sulfate is normally not absorbed. During a recent incident in Brazil, 44 persons had suspected barium intoxication, nine of them died, after ingesting barium-containing contrast material (CDC, 2003). The most common symptoms were nausea, abdominal pain, diarrhea, and vomiting; cardiac arrhythmias and agitation were also reported. Primary ingredients in the implicated lot were found not to be of pharmaceutical grade. The concentration of soluble barium was on average 7.19 g/L, and levels up to 123.7 g/L were found. The most frequent solution dosage was 150 mL. The median interval between administration and symptom onset was 1.0 hour (range, 0.1–5 hours), and all deaths occurred within 24 hours after exposure.

Accidental or suicidal poisonings with barium-containing household and medicinal products have been reported. The compounds included the nitrate (Lydtin *et al*., 1965), sulfide (Downs *et al*., 1995; Gould *et al*., 1973; Jobba and Rengei, 1971), carbonate (Johnson and VanTassell, 1991; Lewi *et al*., 1964; Maretic' *et al*., 1957; Morton, 1945), and chloride (Jourdan *et al*., 2001). In the Chinese province of Szechuan, an endemic condition resembling familial periodic paralysis (*pa-ping*) has been described, which eventually turned out to be food poisoning from the very high proportion of barium, up to 26%) in the table salt mined there (Allen, 1943).

Barium is toxic to tissue, causing first stimulation and then paralysis. The symptoms usually begin with the gastrointestinal muscles, and acute barium poisoning manifests itself rapidly after ingestion of a toxic dose, with nausea, vomiting, colic, and diarrhea. Skeletomuscular and cardiac symptoms follow with myocardial and general muscular stimulation and tingling of the extremities. Severe cases of poisoning progress to loss of tendon reflexes, heart fibrillation, and general muscular paralysis, including the respiratory muscles, leading to death. Electrocardiographic tracings of barium-induced cardiomyopathies were given by Habicht *et al*. (1970) and Sassine (1970).

Threshold of a toxic dose in adult humans is approximately 0.2–0.5 g Ba; lethal in untreated cases is 3–4 g Ba (LD_{50} about 66 mg/kg). These figures apply to the portion absorbed from the gut. Lower lethal doses have also been reported, 0.8–0.9 g or approximately 11 mg/kg. Prompt administration of a soluble sulfate (e.g., Glauber's salt) causes precipitation of barium sulfate in the alimentary tract and thus stops intestinal absorption.

7.1.2 Chronic Effects

7.1.2.1 Inhalation

Inhalation of barium sulfate or barite ore causes a pulmonary reaction, with mobilization of polymorphonuclear leukocytes and macrophages and characteristic radiographic changes with dense, discrete, small opacities distributed throughout the lung fields ("baritosis"). However, the shadows seem to be due to the radiopacity of barium sulfate itself rather than

to any tissue lesions, and the condition is symptomless with no abnormality of pulmonary function (Lévi-Vallensi *et al*., 1966; Wende, 1956).

In a medical evaluation of barium workers by NIOSH (1982), a significantly higher incidence of elevated blood pressure was found in the barium-exposed group (7 of 12, 58%) than in a control group (5 of 25, 20%). However, the results should be interpreted cautiously, because of the small number of persons investigated, a mixed exposure to several chemicals, and the blood pressure being measured only once. Health effects were studied in 18 welders using barium-containing stick electrodes, exposed to 0.1–22.7 mg Ba/m^3 for 4 hours per day during a week (Zshiesche *et al*., 1992). No health symptoms related to exposure or any significant differences in ECG or pulse rate were observed. Plasma concentrations of sodium, magnesium, and calcium were not related to the barium exposure. A transient decrease in plasma potassium levels was noticed in some of the welders but was not attributed to the barium exposure by the authors.

7.1.2.2 Ingestion

Groundwater may locally have elevated concentrations of barium (>1 mg/L), and human health effects after barium exposure from drinking water have been investigated. In a retrospective epidemiological study, Brenniman and Levy (1984) compared mortality in a population living in Illinois with elevated barium levels in municipal drinking water (2–10 mg/L) with a control population with low barium levels (<0.2 mg/L). Significantly elevated mortality rates for cardiovascular diseases (combined) and heart diseases (arteriosclerosis) were found in the high barium communities. However, the study did not control for several important confounders, and it is not possible to conclude a causal relationship between cardiovascular disease and barium exposure. In a morbidity study, the same authors compared two communities, one with 0.1 and one with 7.3 mg Ba/L in drinking water (Brenniman *et al*., 1981). No significant differences in systolic or diastolic blood pressure, rates of hypertension, heart disease, stroke, or kidney disease were found between the two communities. The higher dose, 7.3 mg/L, which was not associated with any adverse health effects, corresponds to 0.21 mg Ba/kg body weight/day, assuming water ingestion of 2 L/day and 70-kg body weight.

Wones *et al*. (1990) administered barium chloride in the drinking water (daily consumption 1.5 L) of 11 healthy male volunteers and studied the effects on cardiovascular risk factors. The experiment lasted for 10 weeks, starting with 2 weeks with no added barium, followed by two periods of 4 weeks with 5 and 10 mg/L barium, respectively, in the drinking water. The corresponding doses were 0.11 and 0.21 mg/kg/day. There were no changes in blood pressures, plasma cholesterol, or lipoprotein levels, serum potassium, or urine catecholamine levels, and no arrhythmias related to barium exposure. A small, but statistically significant, increase in serum calcium was found, when normalized to albumin, but considered not clinically important.

The concentration of barium in ribs was used as an indicator of long-term exposure in a study of 35 elderly Japanese people (Yoshinaga *et al*., 1995). An association was found between barium levels in the rib and cerebrovascular damage (presence of infarction or hemorrhage in the cerebrum at autopsy). Significantly higher barium concentrations in the rib were found in cases with cerebrovascular damage than in cases without (1.52+1.6 vs 1.00+1.6 µg/g dry bone; geometric mean +SD). Because of the small sample size and questions about the grouping of subjects as positive or negative, no firm conclusions should be drawn on this study, and further studies are needed.

The barium ion is a physiological antagonist of potassium, and it seems that the symptoms of barium poisoning are attributable to Ba^{2+}-induced hypokalemia. The effect is probably due to a transfer of potassium from extracellular to intracellular compartments rather than to urinary or gastrointestinal losses (Roza and Berman, 1971). This imbalance between cells and intercellular fluids results from blockage by barium of the K^+-channel of the Na–K pump in the cell membranes (Yellen, 1987). Serum potassium in acute cases of barium poisoning may decrease from the normal range of 3.5–5.0 mEq/L to 1.5–3.0 mEq/L, and the symptoms and signs are promptly relieved by intravenous infusion of K^+ (Berning, 1975; Diengott *et al*., 1964).

7.2 Animals

7.2.1 Inhalation

Baritosis from inhalation of $BaSO_4$ dust was successfully reproduced in rats (Holusa *et al*., 1973). The lack of fibrotic reaction was confirmed, and response was confined to accumulation of alveolar macrophages and a reversible hyperplasia of the bronchial epithelium.

7.2.2 Ingestion

Median lethal doses (in mg/kg) for ingested barium chloride were reported as 90 in dogs, 170 in rabbits, 300–500 in mice and rats, and 800–1200 in horses. For the less soluble barium carbonate, the figures were 35–56 in cats, 104–139 in guinea pigs, 200 in mice, 418–557 in rabbits, 623–800 in chickens, and 1480–1500 in rats (NTP, 1994; Schroeder *et al*., 1972; Syed and Hosain, 1972). The wide

variability of these values from species to species was perhaps due to different degrees of sulfate precipitation in the alimentary canal before absorption, depending on dietary and gastrointestinal physiology factors. The approximate LD_{50} of $BaCl_2$ after intraperitoneal administration to rats was 24 mg/kg (Maretic' *et al*., 1957) and after intracerebroventricular administration to mice, 2.4 mg/kg (Segreti *et al*., 1979).

Toxicology and carcinogenicity studies were performed in rats and mice administered barium chloride dihydrate in drinking water in concentrations up to 4000 mg/L for 15 days, 13 weeks, and 2 years (NTP, 1994). No evidence of carcinogenic activity was found in rats or mice. There were no clear barium-related neurobehavioral effects or reproductive toxicity. Results from the general toxicity studies are briefly presented in the following. After 13 weeks at the highest dose, 200 mg/kg body weight to males and 180 mg/kg body weight to females, the body weights were significantly lower (13% in males and 8% in females) compared with controls. Increased kidney weight was observed in female rats at doses >65 mg/kg body weight, and in males at the highest dose. At the highest dose level, kidney lesions, characterized by renal tubule dilatation in the outer stripe of the outer medulla and cortex occurred in 3 of 10 males and 3 of 10 females. In the 2-year study, the highest doses, 60 mg/kg for males and 75 mg/kg for females, resulted in decreased body weights compared with controls (5% in males and 11% in females), a significant increase in kidney weight in females, but no kidney lesions. At 2 years, females in all dose groups had lower liver weights than controls. Cardiovascular studies in the rats did not reveal any effects on heart rate, systolic blood pressure, or electrocardiogram.

The NTP study in mice demonstrated greater liver weights after 15 days at the highest dose, corresponding to 70 and 85 mg/kg body weight in males and females, respectively. At 13 weeks with the highest dose, 450 mg/kg for males and 495 mg/kg for females, 6 of 10 male and 7 of 10 females died. Multifocal and diffuse nephropathy characterized by tubule dilatation, regeneration, and atrophy occurred in 10 of 10 males and 9 of 10 females at the highest dose. In the 2-year study, survival was significantly lower in the highest dose groups, 160 mg/kg and 200 mg/kg in males and females, respectively, because of renal toxicity. The incidences of nephropathy were increased in male and female mice. Body weights were 8% lower in males and 12% lower in females, compared with controls.

Long-term feeding studies in rats, with the drinking water containing 10–250 mg Ba^{2+}/mL (as chloride), resulted in no measurable toxic effects (Tardiff *et al*., 1980). Perry *et al* (1989) reported increased systolic blood pressure by 6 mmHg in rats after 8 months of exposure to 0.82 mg Ba/kg body weight (as barium chloride) and by 12 mmHg after 1 month of exposure to 7.4 mg/kg body weight. It should be noted that the animals received a diet with a low content of trace elements, including calcium and potassium, which may have caused a higher sensitivity to cardiovascular effects of barium. An increased incidence of proteinuria in male rats was reported after 5 months of exposure to 5 mg/L of barium acetate in drinking water, corresponding to approximately 0.6 mg Ba/kg body weight and a diet characterized as "low metal" (Schroeder and Mitchener, 1975).

Chickens were found to tolerate 1 g Ba^{2+}/kg in their diet; 2 g/kg caused slight depression of growth; 4 g/kg caused substantial depression of growth but no increase in mortality; 8 g/kg was the approximate LD_{50} in 4 weeks; 16 g/kg killed chickens in 7 days and 32 g/kg in 5 days. The results were similar with barium hydroxide and acetate (Johnson *et al*., 1960).

The human symptoms and signs of acute barium intoxication were well reproduced in animal experiments. The cardiomyopathies in dogs and guinea pigs included ectopic ventricular contractions, ventricular tachycardia, and, finally, ventricular fibrillation. The noncardiac effects were salivation, diarrhea, hypertension, skeletal muscle paralysis, and respiratory paralysis (Schott and McArdle, 1974). Infusion of barium chloride into anesthetized dogs produced all of the preceding, plus a prompt and substantial hypokalemia. Potassium administration prevented or reversed all of these effects except the hypertension, which seemed to have a different etiology not connected to the Ba^{2+}-K^+ antagonism (Roza and Berman, 1971). The involvement of the renin-angiotensin system in mediating the hypertensive effects after long-term, low-level barium exposure has been discussed, but there is a lack of direct information (EPA, 1999). Intracerebroventricular injection of $BaCl_2$ in mice produced severe convulsions, preventable by pretreatment with atropine sulfate or naloxone hydrochloride (Segreti *et al*., 1979). This effect seems related to the blockage by barium of the potassium conductance of axons, a phenomenon well studied in squids (Armstrong and Taylor, 1980; Eaton and Brodwick, 1980). However, barium could exert a stimulant action of the adrenal medulla even in denervated cats, attributable to increase in the permeability of chromaffin cell membranes and resulting in release of catecholamines (Douglas and Rubin, 1964).

8 TREATMENT

Treatment of poisoning by soluble barium salts may be preventive or curative. Preventive treatment entails inhibition of absorption by administration of sodium

sulfate or sodium alginate (Sutton *et al*., 1972; Sutton and Shepherd, 1973). Curative treatment entails counteracting the paralytic effect of the Ba^{2+} ion on muscle by intravenous infusion of a potassium salt (Berning, 1975; Diengott *et al*., 1964).

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CHAPTER

21

Beryllium

MAREK JAKUBOWSKI AND CEZARY PALCZYNSKI

ABSTRACT

Beryllium is a strategic and critical material for many industries. It is widely used despite its relatively high cost, because for certain critical applications it performs better than alternatives. The beryllium industry produces three primary forms of beryllium. Copper beryllium alloy is the largest, followed by pure beryllium metal and beryllium oxide ceramics.

As a result of the increasing industrial use of beryllium, occupational exposure to the metal may be an important issue. Beryllium exposure levels decreased significantly compared with the mid-1960s. At present, the TWA concentrations are below the occupational exposure limits of $2\,\mu g/m^3$.

Exposures to beryllium are much more hazardous by the inhalation route than by the ingestion route. Beryllium and its compounds are poorly absorbed from the gastrointestinal tract. In general, inhalation exposure to beryllium compounds results in long-term storage of appreciable amounts of beryllium in lung tissue, particularly in pulmonary lymph nodes, and in the skeleton, which is the ultimate site of beryllium storage. Urinary beryllium concentrations are below the detection limits of $0.03\,\mu g/L$–$0.06\,\mu g/L$.

Exposure to beryllium compounds has caused dermatitis, acute pulmonary inflammation, and chronic beryllium disease (CBD). Exposure to soluble beryllium salts may cause skin reactions such as edematous, erythematous, and papulovesicular dermatitis. Those changes usually disappear after cessation of exposure. Granulomatous necrotic changes and ulcerations caused by skin penetration by insoluble beryllium salts were also observed. These pathological changes are based on delayed allergic hypersensitivity. Acute toxicity of beryllium at concentrations usually $>25\,\mu g/m^3$ is manifested by skin, eye, nose, and throat irritation, followed by upper and lower airway inflammation, pulmonary edema, and ($>100\,\mu g/m^3$) chemical pneumonitis.

Chronic beryllium disease (CBD, chronic pulmonary granulomatosis, berylliosis) is the most common health problem caused by exposure to beryllium. Chronic beryllium disease is a T-cell–mediated disorder. Beryllium, acting as a hapten, interacts with the antigen-presenting cells in the lungs. The beryllium blood lymphocyte proliferation test (BLPT) is used as a medical surveillance tool for assessment of persons at risk for clinical and subclinical chronic beryllium disease developing. The LOAEL for beryllium sensitization and CBD progression was suggested as $0.55\,\mu g\ Be/m^3$. However, recent reports suggest that sensitization and CBD were associated with beryllium air TWA levels exceeding $0.2\,\mu g/m^3$. These results suggest that to avoid sensitization and CBD, the present occupational exposure limits should be $<0.2\,\mu g/m^3$.

An excess in lung cancer was found in persons occupationally exposed to beryllium and was higher for individuals after recovery from acute beryllium pneumonitis than those with CBD. IARC (1993) classified beryllium as a group 1 carcinogen (sufficient evidence for carcinogenicity in humans). The US EPA air unit risk amounts to 2.4×10^{-3} per $\mu g/m^3$. In general, it seems that the lung cancer observations

related to occupational exposure to beryllium are linked to the higher exposure levels that were associated with acute beryllium pneumonitis and predominated before the 1950s.

1 PHYSICAL AND CHEMICAL PROPERTIES

Elemental beryllium, atomic number 4 in group IIA of the periodic table, is a hard, brittle, steel gray, lightweight metal, and has the unusually high melting point of 1278°. With an atomic weight of 9.0122, beryllium is one of the lightest elements.

Consistent with the high charge-to-radius ratio, beryllium has a strong tendency to form compounds with covalent bonds; even the compounds with the most electronegative elements (e.g., BeF_2) have a substantially covalent character. Although beryllium belongs to group IIA of the periodic table, it is chemically very similar to aluminium, which also has a high charge-to-radius ratio. The metal has very high specific heat, heat of fusion, sound conductance, and stiffness-to-weight ratio. In alloys, it confers on other metals improved resistance to fatigue, vibration, and shock. It also has an extreme hardness and resistance to corrosion, attributable to a thin film of beryllium oxide rapidly forming on the surface of bare metal on exposure to air.

The chloride, fluoride, nitrate, and sulfate are highly soluble in water. The metal, its carbonate hydroxide, phosphate, and acetate are sparingly soluble in water but dissolve in acids or bases. Beryllium oxide, prepared by calcinating the hydroxide between 500°and 1750°C, is sparingly soluble in water, whereas oxide calcinated above 1750°C is considered to be insoluble. There is ample evidence that all chemical reactivity, including toxicity, of beryllium oxide is inversely related to the temperature of firing (Reeves, 1986; Toxicological Profile, 2002).

2 METHODS AND PROBLEMS OF ANALYSIS

Neither the flame atomic spectroscopy (AAS) nor atomic emission spectroscopy (AES) used in the past have adequate sensitivity for measuring beryllium concentrations found in body fluids and tissues. Graphite furnace atomic absorption spectroscopy (GFAAS) with background correction (deuterium or Zeeman effect) and inductively coupled plasma atomic emission spectroscopy are now common analytical methods. To avoid sample contamination, stainless steel needles should be avoided for the collection of whole blood samples. Certain polyethylene sample collection tubes with added heparin as an anticoagulant may contaminate whole blood samples (Toxicological Profile, 2002).

The detection limit of the GFAAS methods is at 0.05 µg/L (Paschal and Bailey, 1986; Shan *et al*., 1989) or 0.06 µg/L of urine (Wegner *et al*., 2000). ICP-MS methods include those of Paschal *et al*. (1998), providing the detection limit of 0.1 µg/L or the recently published method by Apostoli and Schaller (2002), who adapted the method of Schramel *et al*. (1997). The detection limit of this method is 0.03 µg/L of urine, and the between-series imprecision for a concentration of 0.5 µg/L expressed as relative standard deviation is 9.5%. According to Apostoli and Schaller (2002), the "normal" urinary Be concentrations reported previously in literature were too high, mainly as a result of the poor specificity and sensitivity of the adopted analytical methods. For example, in the reports published after 1990, the mean urinary beryllium concentrations in nonoccupationally exposed persons amounted to 0.4 µg/L (Minoia *et al*., 1990) and 0.28 µg/L (Paschal *et al*., 1998). On the other hand, in the control groups investigated by Wagner *et al*. (2000) or Apostoli and Schaller (2002), all the results of determinations were below the detection limits of 0.06 µg/L or 0.03 µg/L, respectively.

The determination of beryllium in lung tissue was used to differentiate between chronic berylliosis and sarcoidosis. The analyses for beryllium in the autopsied lung tissues were performed with GFAAS after digestion of lung tissue with the mixture of nitric acid and perchloric acid (Verma *et al*., 2003) or atmospheric thin-window energy-dispersive X-ray analysis (ATWEDXA) (Buntor *et al*. , 2003).

3 PRODUCTION AND USES

3.1 Production

Metallic beryllium was isolated in 1828. However, beryllium was not used extensively until a 1920s' discovery that 2% addition of beryllium to copper produced an alloy six times stronger than the original material. Beryllium is mined primarily from naturally occurring silicates including beryl ($Al_2Be_3Si_6O_{18}$, 5% beryllium by weight) and bertrandite ($Be_4(OH)_2Si_2$, 15% beryllium by weight). The world resources of beryllium are estimated at approximately 80,000 tons (Taylor *et al*., 2003).

The extraction of beryllium begins with the raw materials (bertrandite ore and/or beryl ore). The extraction process for beryl ore involves melting, fritting, and grinding of the ore followed by reacting it with sulfuric acid to produce water-soluble sulfate.

The bertrandite ore is crushed into slurry and leached with sulfuric acid at temperatures near the boiling point. The sulfate solutions undergo a series of solvent extraction steps ultimately producing beryllium hydroxide. The hydroxide is the common input material for copper beryllium alloy, pure beryllium metal, and beryllium oxide ceramics manufacturing (Kolanz, 2001).

Copper-beryllium alloy is commercially the most important beryllium alloy. Copper-beryllium master alloy is manufactured by an arc-furnace method in which beryllium oxide is reduced by carbon in the presence of molten copper at 1800–2000°C. The resulting master alloy contains 4.0–4.25% beryllium by weight. Other copper-beryllium alloys are produced by melting the master alloy together with virgin copper, copper scrap, and possibly other metals (Toxicological Profile, 2002).

3.2 Uses

Beryllium is a strategic and critical material for many industries. It is widely used despite its relatively high cost, because for certain critical applications it performs better than the alternatives. Some of beryllium's advantages are that it is light, strong, less prone to expand and shrink, transparent to X- rays, and a better electrical and thermal conductor than the alternative materials.

The beryllium industry produces three primary forms of beryllium: copper beryllium alloy is the largest, followed by pure beryllium metal and beryllium oxide ceramics (Kolanz, 2001).

By far, most manufactured beryllium (about 75%) ends up in alloys, approximately 95% of which is copper alloy. Beryllium alloys with metals such as copper, nickel, or aluminium have high strength and hardness. They typically contain 0.15–2.0% beryllium. Copper-beryllium alloys are common in the electronics, automotive, defense, and aerospace industries. All the three primary forms of beryllium-containing materials are used as critical, high-reliability elements in products such as air bag sensors, fire extinguisher sprinkler heads, X-ray windows for mammography, medical laser bores, pacemakers, landing gear bearings, and weather satellites. Other applications include computers, oil exploration equipment, and dental appliances. High beryllium content products (40–100% beryllium) are used in the military for advanced electrotargeting and infrared countermeasure devices, missile, advanced surveillance satellites, and radar systems. Beryllium metal is also used in fusion reactors and in the construction of nuclear devices for defense applications because of its nuclear and mechanical properties. Beryllium ceramics, produced from high-purity beryllium oxide powder (99.5%), are used in laser and electronic applications and for high-speed integrated circuits. Most recently, the sports industry has benefited from the properties of beryllium in the construction of golf clubs and bicycles (Kolanz, 2001; Taylor *et al.*, 2003; Willis and Florig, 2002).

4 ENVIRONMENTAL LEVELS AND EXPOSURES

4.1 General Environment

4.1.1 Soil, Water, and Air

Beryllium is the 44th most abundant element in the earth's crust. The average beryllium concentration in the earth's crust is estimated at 2–5.0 mg/kg. Approximately 50 beryllium minerals occur in nature, and more than half of these minerals are silicates. The most common beryllium silicates are beryl, chrysoberyl, and phenacite. The common beryllium silicates are highly insoluble in aqueous solutions and are resistant to chemical weathering. Soil concentrations for beryllium are variable. Natural beryllium concentrations range from a mean 0.1–40 mg/kg at locations around the world. The average beryllium concentrations in soil amounted from <1–7 mg/kg in the Northeast United States, to 1.14 mg/kg in California and from 0.01–5.92 mg/kg in Florida (Toxicological Profile, 2002).

Beryllium is highly mobile in acidic, organic-rich continental river waters, whereas the estuarine-ocean mixing zone exhibits a significant scavenging effect (Kusakabe *et al.*, 1991). It is not known whether beryllium is permanently sequestered in estuarine sediments or is subsequently released and dispersed into the deep ocean. Deep ocean water concentrations of beryllium are fairly uniform worldwide. The beryllium content of ocean waters (10^{-4}–10^{-1} µg/L for the Pacific Ocean) is approximately three orders of magnitude less than that of river waters (approximately 0.1 µg/L) (Taylor *et al.*, 2003). The average concentrations of beryllium in bottled and tap water in the United States were <0.1 and 0.013 µg/L, respectively (Vaessen and Szteke, 2000). In the United States, the groundwater standard for beryllium (0.004 mg/L) is lower than for most other hazardous metals (United States Code, 2001).

Measurements at 100 locations in the United States indicated an average daily concentration of beryllium in the air <0.5 ng/m^3. Between 1982 and 1992, annual averages of beryllium in air ranged from 0.02–2 ng/m^3 in the urban area of Detroit, Michigan (Toxicological Profile, 2002). An emission standard for beryllium extraction operations of not greater than 10 g/24

hours is required unless an owner or operator requests approval to meet an ambient concentration limit in the vicinity of the stationary source of 0.01 μg/m^3 averaged over a 30-day period (United States Code, 2000). The ambient air concentrations of beryllium found near power stations in Castellon, Spain, ranged from "not detected" to 1.61 ng/m^3 (Boix *et al*., 2001), and around a beryllium processing facility near Navi Mumbai, India, they amounted to 0.48–0.42 ng/m^3. In the last case, the levels of beryllium during the monsoon season were comparatively lower and often were below the detection level (Thorat *et al*., 2000).

4.1.2 Plants, Animals, and Dietary Intake

The concentration of beryllium in plants is very low. Soluble forms of beryllium must be present for uptake to occur in plants. In the case of beans, barley, sunflowers, and tomato plants, more than 95% of ^{7}Be was found in roots, and very little was translocated to the foliage and fruits (Romney and Childress, 1965).

Beryllium content in various foods, as extracted from literature reports, has been reviewed recently (Toxicological Profile, 2002; Vaessen and Szteke, 2000). According to Toxicological Profile (2002), the median concentration of beryllium in the 38 foods, fruits, and fruit juices from around the world is 22.5 μg/kg fresh weight (excluding kidney beans), and the range of concentrations is <0.1–2200 μg/kg. The highest concentrations (in μg/kg fresh weight) were reported for kidney beans (2200), crisp bread (112), garden peas (109), parsley (77), and pears (65). The average concentration of beryllium in fruit and fruit juices is 13.0 μg/kg, and the concentrations range from "not detected" to 74.9 μg/kg.

However, Vaessen and Szteke (2000) postulate that most of the higher beryllium levels reported in literature result from errors in the method of determination and that, with a few exceptions, beryllium levels in food will be ranging from <1–approximately 20 μg/ kg fresh weight.

4.1.3 Estimates of Daily Exposure

The average adult is exposed to trace amounts of beryllium by air, drinking water, and food. If the average concentration of beryllium in air is assumed to be <0.03 ng Be/m^3, and if a normal adult inhales approximately 20 m^3 of air per day, then the inhalation exposure would be approximately 0.6 ng/Be/day. This value may be somewhat higher for persons living near the sources of beryllium emission.

For drinking water, it is reasonable to assume that in highly industrialized countries, the contribution in the overall beryllium intake is of the order of magnitude of about 0.4 μg/day (ATSDR, 1991) or 1 μg/day (Toxicological Profile, 2002). Thus, compared with food, the drinking water will contribute very little to the total intake of beryllium.

Reliable data on the contribution of food consumption to the daily intake of beryllium are lacking. The average daily intake of beryllium was determined to fall within the range of 5–100 μg (Toxicological Profile, 2002; Vaessen and Szteke, 2000). The outlying intake figure of 0.12 μg/day (ATSDR, 1991) is based on an arbitrary value for beryllium content in the diet sample of 0.1 μg/kg and a portion of 1200 g of food for an average adult person. It is apparent that the value of 0.1 μg/kg is a substantial underestimate of the beryllium content in food.

At present, there are no indications that beryllium levels in food and drinking water pose a serious health risk.

4.2 Working Environment

Beryllium is used in alloys with other metals (particularly Cu and Ni, and to a lesser extent Co, Cr, Fe, and Mg) for improving hardness and resistance to corrosion, wear, vibration, and collision. As a result of the increasing industrial use of beryllium, occupational exposure to the metal may be an important issue. Estimates of the number of exposed workers in the United States alone currently range from 200,000–800,000. An estimated 30,000 US workers are thought to have been exposed to beryllium in the 1970s (Infante and Newman, 2004).

The estimated daily weighted average beryllium exposure levels for some workers in a plant that extracted and produced beryllium metal were >50 μg/m^3 during the mid-1960s. In mid-1970s, the exposure levels were >30 μg/m^3. After 1977, this plant complied with the OSHA maximum TWA concentration of 2 μg/m^3 (Kriebel *et al*., 1988). At present, beryllium concentrations during different industrial processes tend to be below the current occupational exposure limit. The average personal sampling concentrations amounted to 1.04 μg/m^3 during beryllium production (Stange *et al*., 1996), to 0.09–0.72 μg/m^3 at the atomic weapons establishment (Johnson *et al*., 2001) and to 0.2–0.6 μg/m^3 at beryllium machining (Martyny *et al*., 2000). Thorat *et al*. (2003) studied the mass size distribution of beryllium aerosols generated in various operational areas of a typical extraction and processing plant. The total concentration of beryllium was well below the threshold limit value. The mean value of mass median aerodynamic diameter of beryllium particles ranged from 5.0–9.5 μm. The alveolar disposition was estimated to be 3–5% for nasal breathing and 9–13% for oral breathing.

According to Mc Cawley (2001), the particle number concentrations are higher in the areas where the historical estimate of risk showed a high risk of disease despite a relatively lower mass concentration. Furthermore, the particle number rather than particle mass may be more reflective of the target organ dose and thus may be a more appropriate measure of exposure for chronic beryllium disease.

In view of the number of fatal and severe cases of respiratory diseases in beryllium workers in the 1940s, the US Atomic Energy Commission established an occupational exposure limit of 2 µg/m^3 in 1949. This still widely accepted TWA limit was not based on any observed dose-response relationships or thresholds but on an analogy to toxic heavy metals with an adjustment for molecular weight (ACGIH, 2002; Wambach and Tuggle, 2000). To date, abundant evidence has been gathered for the poisonous properties of beryllium. According to OSHA (ACGIH 2005), the ceiling value of 5 µg/m^3 and the permissible maximum peak exposure limit of 25 µg/m^3 over a 30-minute period per 8-hour work shift is recommended to protect from acute beryllium disease. The ACGIH TLV-TWA amounts to 2 µg/m^3. It has been suggested that CBD and lung cancer seem to be associated with exposure regimens in which short, high-level exposure occurs. Therefore, the TLV-STEL of 10 µg/m^3 during any 15-minute of exposure is recommended. The proposed value amounts to 0.02 µg/m^3 TLV-TWA without STEL (ACGIH, 2005). Wambach and Tuggle (2000) concluded that an 8-hour exposure limit of 0.1 µg/m^3 would provide a better control of both long-term mean exposure levels and short-term levels than the current occupational exposure limits. This conclusion is in concordance with the results of investigations performed at a copper-beryllium alloy strip and wire finishing facility (Schuler *et al*., 2005). These authors postulated that beryllium sensitization and CBD were associated with exposure in an area where air beryllium levels frequently exceeded 0.2 µg/m^3 and not in areas where this level was rarely exceeded.

According to presently expressed views, one of the primary objectives of beryllium research is to provide scientific data to define scientifically based occupational exposure limits (Infante and Newman, 2004, Kolanz, 2001; Paustenbach *et al*., 2001; Wambach and Tuggle, 2000).

5 KINETICS

5.1 Absorption

5.1.1 Dermal Exposure

Absorption of beryllium through intact skin is considered to be negligible. Low dermal solubility and absorption for beryllium salts relates primarily to their low solubility at physiological pH levels. Only small amounts of beryllium were absorbed through the tail skin of rats after exposure to an aqueous solution of beryllium chloride. Beryllium has been demonstrated to bind to alkaline phosphatase and nucleic acids in guinea pig epidermis *in vitro* (Reeves, 1986; Toxicological Profile, 2002). In contrast to intact skin, beryllium loading on injured skin may allow a larger fraction to be absorbed into the body (Ivannikov *et al*., 1982).

5.1.2 Inhalation

There are no data on the deposition or absorption of inhaled beryllium in humans, but it can be expected that, as with other inhaled particles, the dose, particle size, and solubility of beryllium compounds are the important factors governing deposition and lung clearance (WHO, 1990). Absorption of beryllium deposited in the lung by inhalation or intratracheal injection depends on the physical and chemical state of the compound.

Fifty-five percent of the intratracheal dose of the soluble ^{7}Be citrate was eliminated through the kidneys within the first 24 hours after injection, but only 15% of the $^7BeSO_4$ dose was eliminated in urine at that time, because of *in situ* conversion of the strongly ionized $BeSO_4$ to a less soluble precipitate (Van Cleave and Kaylor, 1955). The relevance of the test material with respect to beryllium clearance from rat lung has been confirmed by the results of other studies. Reeves *et al*. (1967) and Reeves and Vorwald (1967) also observed a long retention of beryllium in rat lungs after exposure to $BeSO_4$ aerosol at 34 µg Be/m^3 for 72 weeks. An equilibrium concentration was reached in the lungs and tracheobronchial lymph nodes at about 36 weeks. After cessation of exposure, pulmonary beryllium was at first eliminated with a half-time of about 2 weeks, followed by a logarithmically decreasing clearance rate. In guinea pigs and rats exposed to more soluble beryllium nitrate at concentrations of 2–40 mg/m^3 for 16 hours, steady-state concentrations in the blood were reached after 8–12 hours of exposure (Stiefel *et al*., 1980). After intratracheal administration of 25 µg or 100 µg of BeCu alloy to mice, Be cleared from the lung with a half-time of 0.5 and 0.4 days within the first phase and 20 and 13 days within the second phase of elimination. After intratracheal instillation of 2 µg of Be metal, the $t_{1/2}$ of Be clearance from the lung amounted to 14–346 days (Benson *et al*., 2000). Sanders *et al*. (1975) and Rhoads and Sanders (1985) found alveolar half-times in rodents of about 6 and 13 months, respectively, after inhalation of high-fired (1000°C) BeO.

Beryllium is mainly inhaled in the form of particulate matter. Its bronchial clearance is mainly accomplished

through the mucociliary transport (important during the first day) and later through the uptake of beryllium by alveolar macrophages. Beryllium-coated particles were highly toxic to macrophages, indicating that elevated beryllium concentrations inhibit pulmonary clearance (WHO, 1990).

5.1.3 Ingestion

On the basis of animal studies, beryllium and its compounds are poorly absorbed from the gastrointestinal tract in animals. Estimates from animal studies, in which trace amounts of carrier-free ^{7}Be were administered as the chloride, show that absorption of ingested beryllium is very low, with values generally <1% (Deubner *et al*., 2001; WHO, 1990). Urinary excretion data for rats treated by gavage with radioactive beryllium chloride indicate that the cumulative excretion of beryllium in urine and feces was 0.11 and approximately 100%, respectively. Also in mice, dogs, and monkeys, the urinary output was 0.24, 0.38, and 3.71% of the total dose, respectively, whereas most of the tracer was excreted in the feces. (Furchner *et al*., 1973).

5.2 Transport, Distribution, and Excretion

In general, inhalation exposure to beryllium compounds results in a long-term deposition of appreciable amounts of beryllium in lung tissue, in pulmonary lymph nodes, and in the skeleton, which is the ultimate site of beryllium storage (WHO, 1990). Immediately after the rats were exposed through the nose to aerosol containing $BeSO_4$ (2.9 mL solution, 1 mg Be/mL) and $^{7}BeCl_2$ (0.1 mL, 0.1 µg/mL) for 3 hours, the percentage of total body activity in the tissue was 60% in the lung, 0.9% in the liver, 1.5% in the kidney, 0.1% in the spleen, 1.4% in the brain, 9.5% in the muscle, 13.5% in the skeleton, 5.0% in the blood, and 10 % in the excreta (Zorn *et al*., 1977). After 408 hours, the liver, spleen, heart, lung, and muscle concentrations were <0.0005%; the concentrations in the kidneys, skeleton, blood, and excreta were 0.0005, 6.8, 0.05, and 92%, respectively. Beryllium concentration in the bone increased until 96 hours after exposure and then decreased.

Transport and distribution of beryllium takes place through the blood. The distribution spectrum of beryllium tracers in blood of animals and humans show that about 60–70% of beryllium is bound to two serum proteins, prealbumins and γ-globulins for normal, ubiquitous air concentrations. After exposure of rats and guinea pigs to 2–40 mg Be/m^3 as beryllium nitrate, 70% of the total Be (10–100 ng/g serum) were detected in the prealbumin, only about 1% was found in the γ-globulin fraction (Stiefel *et al*., 1980).

Beryllium accumulates primarily in the liver, presumably because of an uptake by the Kupffer cells (Cheng, 1956). This uptake seems to be result of a conversion of soluble beryllium sulfate to beryllium phosphate aggregates that are then phagocytized by the reticuloendothelial system in the liver (Vacher and Stoner, 1968). Lindenschmidt *et al*. (1986) administered 6.0 mg/kg of $BeSO_4$ plus 20 µCi/200 g body wt of ^{7}Be ($^{7}BeCl_2$, carrier free) intravenously to rats. Two days after administration, the highest concentration of 337 nmol/g wet tissue was found in the liver, followed by spleen (249 nmol/g), bone (105 nmol Be per femur), kidney 48 (nmol/g), and lung (24 nmol/g). Two weeks after the dose of 3.1 mg/kg was administered, the highest concentration was found in the spleen (133 nmol/g), bone (46 nmol/femur), liver (45 nmol/g), kidneys (12.7 nmol/g), and small intestine (8.5 nmol/kg). This study confirmed earlier reports (Piotrowski and Szymanska,1976; Price and Joshi, 1983) that beryllium does not induce synthesis of or bind to metallothionein and indicated that beryllium is bound *in vivo* to ferritin in the liver. A 3-day pretreatment of rats with an iron salt significantly elevated liver levels of ferritin and significantly reduced the lethality from intravenous beryllium injection (Lindenschmidt *et al*., 1986).

Bencko *et al*. (1979) reported a slight placental permeability for soluble $^{7}BeCl_2$ (0.1 mg/kg body weight), intravenously administered to mice. The concentration of beryllium in the placenta and in the remaining organs of the females was one order of magnitude higher than those in the fetuses. The transfer of beryllium ingested in the form of $^{7}BeCl$ to the milk was low. Less than 0.002% of the administered dose was secreted in the milk of cows (Mullen *et al*., 1972).

Elimination of absorbed beryllium occurs mainly in the urine and only to a minor extent in the feces. Most beryllium taken up by the oral route passes through the gastrointestinal tract unabsorbed and is eliminated in the feces (WHO, 1990).

Rats injected intramuscularly with carrier-free ^{7}Be (57 ng/rat) were found to eliminate 15, 14.6, 24.4, and 44% of the dose in the urine at 1, 4, 16, and 64 days, respectively, versus 4.25, 4.17, 9.25, and 13.1% in the feces (Crowley *et al*., 1949). Furchner *et al*. (1973) determined urinary/fecal ratios of 3.21 in mice and 10.2 in rats, during the first 24 hours after intraperitoneal administration and 3.5 in mice, 21.34 in rats, and 48.61 in dogs after intravenous administration. Thereafter, the high urinary excretion rate declined rapidly and the amount lost in feces equalled that in urine. After intravenous administration of very small doses of carrier-free ^{7}Be to rats (0.09 ng/kg) and rabbits (0.04 ng/kg), the elimination was highest during the first day and amounted to 38.8% of the dose in rats and 28.8% in

rabbits (Scott *et al*., 1950). In animals receiving a higher dose of 0.15 μg/kg (rats) or 0.05 μg/kg (rabbits), urinary excretion amounted to 24.2% in rats and 14% in rabbits. This reduction with an increasing dose may be explained by the increasing immobilization of beryllium, because of its binding on proteins. The mechanism of urinary excretion is probably active tubular secretion, because most of the colloidally bound plasma beryllium does not pass the glomerulus in the kidney (Reeves, 1986).

True biliary excretion seems to play a minor role in total beryllium elimination (Cikrt and Bencko, 1975).

The nonionized soluble forms of beryllium, such as citrate, are cleared from the lung rapidly, in about 4 days. The ionized soluble forms become precipitated in lung tissue and behave like particulate matter. Their clearance consists of a fast phase and slow phase. The half-time of the fast phase is in the range of 1–60 days and the slow phase of 0.6–2.3 years (WHO, 1990). After intravenous injection of carrier-free $^{7}BeCl_2$, Furchner *et al*. (1973) calculated slow-phase biological half-lives in lungs of 1210, 890, 1770, and 1270 days in mice, rats, monkeys, and dogs, respectively.

6 LEVELS IN TISSUES AND BIOLOGICAL FLUIDS—BIOLOGICAL MONITORING

This section discusses levels in tissues and biological fluids and possibilities for exposure monitoring. Effect biomonitoring is discussed in Section 7.6 and biomarkers of susceptibility in Section 7.7.

The average concentrations of beryllium measured in human organs were as follows: 0.21 mg/kg in the lung, 0.08 mg/kg in the brain, 0.07 mg/kg in the heart, and 0.02 mg/kg in the bone. However, it was not clear whether these organ samples had been obtained at biopsy or autopsy or whether the subjects had been exposed occupationally or environmentally (Toxicological Profile, 2002).

According to Apostoli and Schaller (2002), the "normal" urinary Be concentrations reported earlier in the literature were too high, mainly as a result of the poor specificity and sensitivity of the adopted analytical methods. For example, mean urinary beryllium concentrations of persons not occupationally exposed amounted to 0.9 ng/g (Stiefel *et al*., 1980), 0.4 μg/L (Minoia *et al*., 1990), 0.28 μg/L (Paschal *et al*., 1998). On the other hand, in the control groups investigated by Wagner *et al*. (2000) or Apostoli and Schaller (2002), all results of determinations were below the detection limits of 0.06 μg/L or 0.03 μg/L, respectively.

The data for biological monitoring of exposure to beryllium are not available because of the small number of occupational activities involving exposure to this metal and the lack of appropriate analytical methods in the past. The mean values for urinary beryllium in nonexposed persons as reported previously amounted to 0.4 μg/L (Minoia *et al*., 1990) or 0.28 μg/L (Paschal *et al*., 1998). In the studies by Apostoli and Schaller (2001) or Wegner *et al*. (2000) in which beryllium analysis was carried out using the ICP-MS method, the detection limit was 0.03 μg/L or 0.06 μg/L, and beryllium concentrations in urine samples of not occupationally exposed persons were below these detection limits.

Wegner *et al*. (2000) investigated 57 gemstone cutters. For 27 cutters working in contact with beryls for 21 hours/week on average, beryllium could be detected in 17 preshift and 12 postshift urine specimens. The median for the preshift urine samples was 0.09 μg/L (<0.06–0.56 μg/L) and for the postshift urine samples <0.06 μg/L (<0.06–0.29 μg/L). No analysis of the correlation between external and internal exposure was carried out.

Apostoli and Schaller (2001) examined 65 metallurgic workers in two electric steel plants and two copper alloy foundries. Beryllium concentrations in urine varied from <0.03–0.54 μg/L. A significant correlation was found for the relationship between external and internal exposure. The urinary Be levels were in the range of 0.12–0.15 μg/L, whereas Be concentrations in respirable dust were below the recommended TLV-TWA of 0.2 μg/m^3.

However, further studies need to be conducted to fully understand the time-varying trends in urinary excretion and the relations between exposure control measures and urinary beryllium.

The determination of beryllium in the lung tissue was used to differentiate between chronic berylliosis and sarcoidosis. The analyses for beryllium in the autopsied lung tissues were performed by means of GFAAS after digestion of the lung tissue with a mixture of nitric acid and perchloric acid (Verma *et al*., 2003) or atmospheric thin-window energy-dispersive X-ray analysis (ATWEDXA) (Buntor *et al*., 2003).

7 EFFECTS AND DOSE-RESPONSE RELATIONSHIPS

Beryllium and its compounds are harmful to human health; both directly, because of their toxicity and indirectly, through antigen-specific stimulation of immunity mediated by the cells. After the hygienic standards for beryllium in workplace (2 μg/m^3 time-weighted average exposure per 8-hour workday) had been introduced, the direct toxic effects were practically eliminated.

Soluble compounds of beryllium (such as the sulfate and the fluoride) exert the acute and subacute effects, whereas insoluble alloys and intermetallics (the oxides and the ores) usually produce health effects after long-term exposure or longer deposition within the system. The chief site of vulnerability from beryllium is the lung; however, nonpulmonary locations of granulomatous lesions may exist in many other organs such as the liver, spleen, abdominal lymphatic nodes, kidney, bone marrow, or skin.

7.1 Local Effects

7.1.1 Skin Contact

7.1.1.1 Humans

Occupational exposure to soluble beryllium salts may cause skin reaction such as edematous, erythematous, and papulovesicular dermatitis, mainly on the exposed surfaces. Those changes usually disappear after cessation of exposure. Beryllium may also cause conjunctivitis in occupationally exposed workers, as well as act on mucous membranes of oral cavities producing gingivitis in persons with dental implants made from alloys including beryllium (Haberman *et al*., 1993). Granulomatous necrotic changes and ulcerations caused by skin penetration by insoluble beryllium salts were also observed. Skin changes like papular and granulomatic rash were found to accompany chronic beryllium disease in workers exposed to oxide beryllium dust.

The pathological changes mentioned previously are based on delayed allergic hypersensitivity, which was proved *in vitro* in lymphocyte-blast transformation test (Hanifin *et al*., 1970) and migration inhibiting test of macrophages (Henderson *et al*.,1972) and also *in vivo* using skin patch testing with beryllium sulfate (Curtis, 1951).

7.1.1.2 Animals

Skin allergy and beryllium skin granulomas (Dutra, 1951), similar to those observed in humans, were induced experimentally in pigs and guinea pigs (Alekseeva, 1965).

7.1.2 Inhalation

7.1.2.1 Humans

Acute toxicity of beryllium at concentrations usually >25 μg/m^3 is manifested by skin, eye, nose, and throat irritation, followed by upper and lower airway inflammation, pulmonary edema, and (>100 μg/m^3) chemical pneumonitis (Maier *et al*., 1998). Acute beryllium disease occurs mainly in the case of exposure to soluble beryllium salts, but it was also recorded after exposure to metallic dust, beryllium oxide, and hydroxide (Eisenbud *et al*., 1948). The pathogenesis of inflammatory changes is probably connected with the acidity of beryllium salts, and the clinical parameters—progression of inflammatory process, its intensity, and the duration of illness, depend on the dose-response relationship (Van Ordstrand *et al*., 1943). In high-level exposure, the clinical symptoms of pneumonitis appear within 72 hours after exposure, whereas radiological abnormalities occur within several weeks (Maier and Newman, 1998). Recovery lasts for several weeks or months; however, some fatal cases were also noted. The treatment consists in the cessation of exposure, bed rest, systemic glucocorticoids, and oxygen therapy. Although in practice the severe cases of acute beryllium disorder are rare, the milder forms should be considered in the differential diagnosis of respiratory diseases with infectious etiology (Mroz *et al*., 2001).

7.1.2.2 Animals

Acute chemical pneumonia after inhalation exposure to beryllium compounds was induced experimentally in different kinds of animals (Stokinger *et al*., 1950). The observed pathological changes were similar to those in humans. In animals exposed to beryllium sulfate at a concentration of 13 mg/m^3 for 1 hour, proliferative changes were noted after 3 weeks, mostly concerning the lung macrophages (mice), type II alveolar epithelial cells (rats), and interstitial cells and epithelium (mice and rats). Hyperplasia of type II alveolar epithelial cells was accompanied with the thickening of interstitium infiltrated with macrophages and neutrophils. The presence of alveolar macrophages with ragged cellular membranes was a visible feature. In another experiment, interstitial pneumonia without granulomas was noted in rats exposed to 4.05 μg/m^3 beryllium as beryllium sulfate (mass median diameter, 1.9 μm) for 1 hour (Sendelbach *et al*., 1989).

Hall *et al*. (1950) observed that insoluble beryllium compounds also provoked acute pneumonia. High-fired (1150–1450°C) BeO did not induce lung damage in most of experimental animals, besides mild phagocytic cell response typical for exposure to every kind of inhaled molecules, in comparison with low-fired (400°C) BeO, which almost at 10 times lower concentrations caused death in rats and lung damage in dogs.

Metallic beryllium can also produce lung injury, however, at higher concentrations than the beryllium salts do. It has been noted that 50-minute exposure to metallic beryllium dust at the dose of 0.8 mg/m^3 (MMAD, 1.4 μm) (initial lung burden of approximately 0.625 mg) resulted in necrotic hemorrhagic pneumonia and interstitial fibrosis as a consequence

of inflammatory changes in male Fischer 344/N rats. Granulomatous changes were not observed, just a few lymphocytes, and mostly neutrophils, were found in BALF (Haley *et al*., 1990).

Acute inhalation exposure to beryllium in rats and dogs causes macrocytic anemia with pulmonary changes (Stroud *et al*., 1951). The anemia spontaneously subsided in dogs after 3.5–4 months despite beryllium presence in the liver and bone (Stokinger *et al*., 1953).

7.2 Systemic Effects

7.2.1 Acute Effects

There are several studies regarding deaths after acute inhalation exposure to beryllium compounds. A 50-minute exposure to aerosol of beryllium metal at 0.8 mg Be/m^3 caused death of 20 of 74 rats at 12–15 days after exposure (Haley *et al*., 1990). All rats exposed to 2.59 mg Be/m^3 as beryllium sulfate for 14 days (2 hours/day) died by day 18 (Sendelbach and Witschi, 1987). All monkeys exposed to ≥13 mg beryllium/m^3 as beryllium hydrogen phosphate for 8–10 days (6 hours a day) died after 8–10 days of exposure. Two of four monkeys exposed to 0.184 mg Be/m^3 as beryllium fluoride died after 7–17 days of exposure (6 days) (Schepers, 1964).

Oral LD_{50} values in animals vary according to the compound. LD_{50} values for beryllium sulfate were 120 mg Be/kg in rats and 140 mg Be/kg in mice. The LD_{50} values for beryllium chloride in rats were 200 mg Be/kg, for beryllium fluoride in mice 18–20 mg Be/kg, and for beryliumoxyfluoride 18.3 mg Be/kg in rats (Toxicological Profile, 2002).

7.2.2 Chronic Beryllium Disease (CBD, Chronic Pulmonary Granulomatosis, Berylliosis)

7.2.2.1 Humans

Chronic beryllium disease (CBD, chronic pulmonary granulomatosis, berylliosis) is the most common health problem caused by exposure to beryllium. This disease was described for the first time by Hardy and Tabershaw (1946) in fluorescent-lamp workers exposed to beryllium fluoride in Massachusetts and originally named as "Salem sarcoid." Further investigations demonstrated that this illness was also seen in workers exposed to metallic beryllium, its alloys and oxides, more often in workers exposed to insoluble beryllium compounds, especially low-fired oxide (De Nardi *et al*. 1949; Hardy and Tepper, 1959; Sterner and Eisenbud, 1951). The people working in factories using beryllium for technological processes but who were not directly exposed (secretaries, security guards) (Kreiss *et al*., 1993), as well as the occupationally exposed inhabitants of the vicinity of beryllium plants, were also found to develop CBD (Eisenbud *et al*., 1949). It was observed that the compliance with the 2 μg/m^3 OSHA hygienic standard did not protect from CBD (Cullen *et al*., 1987; Shima, 1980). This finding implied the role of immunological hypersensitivity in CBD pathogenesis. The results of many investigations indicated that the level of exposure to insoluble respirable molecules containing beryllium played an important role in the prevalence of CBD—probably the long-term presence of beryllium deposits in the lung promotes sensitization (Kreiss *et al*., 1993; 1996; Martyny *et al*. 2000; Stange *et al*. 1996). CBD is a systemic disease revealing the granulomatous changes mostly in the lung. There are different levels of lymphocyte infiltrations in the interstitial lung tissue; the granulomas are located mainly in bronchioles. They can also be found in lymph nodes, liver, spleen, muscles, and skin.

Both the innate and the antigen-specific, cell-mediated immune responses play central roles in the development of chronic lung inflammation. Beryllium sensitization is a precursor to this process. An *in vitro* assay beryllium lymphocyte proliferation test (BeLPT) has become the mainstay clinical test in CBD diagnosis. Blood BeLPT is used as a screening tool to identify individuals with beryllium allergy without clinical or laboratory symptoms of CBD (Kreiss *et al*., 1993; Stange *et al*., 1996). Rom *et al*. (1983) found the rates of beryllium sensitization at 15.9% (1979) and 8.9% (1982) among the workers of a surface mine and process mill. Other authors of the screening studies assessed beryllium sensitization among exposed workers at 1.9% (Kreiss *et al*., 1997), 1.8% (Stange *et al*., 1996), and 4.54% (Stange *et al*., 2001). CBD was seen to develop from beryllium sensitization at a rate of 10%/year during a follow-up study (Newman, 1995).

The results of Henneberger *et al*. (2001) indicate that to provoke beryllium hypersensitivity, a short time of exposure is sufficient, but to develop the CBD a longer period of time is necessary.

Most frequently, CBD develops after a long period of time lasting 10–15 years (range, 1–30 years) (Mroz *et al*., 2001). At the beginning, the disease is manifested by effort dyspnea that intensifies progressively. Later on, such symptoms including cough, chest pain, arthralgia, tiredness, fever, night sweating, and weight loss appear. In severe cases, pulmonary hypertension and cor pulmonale can develop.

Physical examination may be normal or may reveal crackles or wheezes on lung auscultation. Pulmonary function tests may reveal a restrictive or obstructive pattern or a reduced diffusing capacity for carbon

monoxide. The earliest marker of physiological impairment is exercise tolerance test that may reveal subtle gas exchange abnormalities, including abnormal fall in PaO_2 or a widening of the A-a gradient with exercise, decreased oxygen consumption, or ventilatory limitations (Pappas and Newman, 1993). Chest X-ray abnormalities rarely occur before CBD is at an advanced stage. Advanced disease shows diffuse reticulonodular infiltrates or fibrosis. The thin-section CT scan is more sensitive than X-ray, but this can also be normal at the early stages of CBD (Daniloff *et al*., 1997). Bronchoalveolar lavage shows increased cellularity and lymphocytosis (predominantly CD4-positive T cells) (Newman *et al*., 1989). These findings correlate with disease severity (Newman *et al*., 1994). Bronchoalveolar lavage fluid (BALF) contains lung lymphocytes that proliferate when exposed to Be *in vitro* (BeLPT) (Mroz *et al*., 1991, Newman, 1996a).

Lung biopsy reveals interstitial mononuclear cell infiltrates and/or noncaseating granulomas and/or fibrosis (Newman *et al*., 1989; 1994). Nonceasing granulomas in lung tissue and sometimes in the skin are the pathological hallmark of CBD, but their absence does not rule out the disease (Mroz *et al*., 2001).

The results of a cross-sectional study performed by Kreiss *et al*. (1996) helped to define the lowest observed adverse effect level (LOAEL) of 0.55 μg/m^3 for beryllium sensitization and CBD progression. Recently, Schuler *et al*. (2005) reported on investigations performed at a copper-beryllium alloy strip and wire finishing facility. These authors concluded that sensitization and CBD were associated with an area where beryllium air levels routinely exceeded 0.2 μg/m^3 and not with areas where this level was rarely exceeded.

These results suggest that to avoid sensitization and CBD, the present occupational exposure limits should be <0.2 μg/m^3.

7.2.2.2 Animals

At present, no animal model exists that mimics the chronic and progressive course of CBD observed in humans. Many studies in dogs, monkeys, guinea pigs, and rats have reported granulomatous inflammation in the lung, but histopathological findings do not reveal CBD, the effects are transient, or are not consistently associated with Be-specific immune response (EPA, 1998; Finch *et al*., 1996). Policard (1950) reported nodular granulomata in the lungs of guinea pigs exposed to beryllium oxide, but the lesions regressed after the 40th day of exposure. The results of some studies (Huang, 1992; Nikula *et al*., 1997) suggest that mice may be an appropriate model. In mice presensitized with beryllium sulfate and then administered a single intratracheal dose, the influx of CD4$^+$ T cells into the lung, Be-sensitized T cells, interstitial inflammation, and formation of granulomas were reported. However, these findings were observed at 8 months and resolved by 10 months. Many similarities between the effects of 90-minute nose-only exposure to beryllium metal in mice and human CBD were also observed by Nikula *et al*. (1997).

7.2.3 Other Chronic Systemic Effects

7.2.3.1 Cardiovascular Effects

The data regarding cardiovascular effects of beryllium and its compounds in humans are limited. Severe cases of chronic beryllium disease can result in right ventricle hypertrophy. In a case study of 17 individuals exposed to beryllium in a plant that manufactured fluorescent lamps, autopsies revealed right atrial and ventricular hypertrophy (Hardy and Tabershaw, 1946). An increase in death rate caused by heart disease was found in workers at a beryllium manufacturing factory (Ward *et al*., 1992). The cardiac effects are rather not due to the direct toxicity to the heart but to a response to impaired lung function.

Heart enlargement was observed in monkeys after acute inhalation exposure to ≥13 mg Be/m^3 as beryllium hydrogen phosphate, 0.184 mg Be/m^3 as beryllium fluoride, or 0.198 mg Be/m^3 as beryllium sulfate (Schepers, 1964). Also decreased arterial oxygen tension was observed in dogs exposed to beryllium oxide (30 mg Be/m^3 for 15 days or 3.6 mg Be/m^3 for 40 days (Hall *et al*., 1950). These effects probably represent compensatory increases in cardiac musculature because of pulmonary fibrosis and the reduced ability of the lung to oxygenate blood. This conclusion can be supported by the results of dietary exposure to beryllium compounds implying that oral exposure is not likely to cause cardiac effects (Toxicological Profile, 2002).

7.2.3.2 Hematological Effects

Reports on the hematological effects of beryllium and its compounds in humans after inhalation exposure are scarce. No difference in white blood cell count, hematocrit, or differential white blood cell percentage was observed in patients with chronic beryllium disease (Johnson, 1983).

Acute exposure of experimental animals to beryllium and its compounds had little effect on hematological parameters. Inhalation exposures of intermediate duration caused anemia in several species. Dogs exposed to 30 mg/m^3 Be/m^3 as beryllium oxide for 15 days exhibited a moderate, progressive leukocytosis, whereas dogs exposed to 3.6 mgBe/m^3 for 40 days developed macrocytic anemia. Differential counting of the bone marrow smears indicated a decrease in erythroblasts

and an increase in normoblasts. Macrocytic anemia developed in rats and rabbits exposed to 0.43 mg Be/m^3 and dogs exposed to 0.04 mg Be/m^3 as beryllium sulfate for 100 days (Stokinger *et al*., 1950). Hematological effects were not observed in rats, hamsters, or monkeys exposed to 0.21 or 0.62 mg Be/m^3 as bertranite or beryl ore, respectively, for 6–23 months (Wagner *et al*., 1969).

No evidence of microscopic abnormalities of the bone marrow or spleen was observed in rats exposed orally to ≥31 mg Be/kg/day for 2 years (Morgareidge *et al*., 1976).

7.2.3.3 Endocrine Effects

Beryllium and its compounds were found to produce effects on the endocrine system. In a study of workers at a plant manufacturing fluorescent lamps, 1 of 17 workers exposed to beryllium who died from chronic beryllium disease had marked hyperemia and vacuolization in the histology of adrenal glands (Hardy and Tabershaw, 1946).

Histological examination of monkeys exposed to 13 mg Be/m^3 as beryllium hydrogen phosphate or 0.184 mg Be/m^3 as beryllium fluoride revealed marked hypoplasia and hypotrophy of the adrenal gland. The adrenal glands of monkeys exposed to 0.196 mgBe/m^3 as beryllium sulfate presented no abnormalities (Schepers, 1964).

No adverse effects were observed in the adrenal, thyroid, pituitary, or pancreas of dogs exposed to 12 mg/Be/kg/day as beryllium sulfate in the diet for 143–172 weeks (Morgareidge *et al*., 1976) or in rats exposed to ≥31 mg Be/kg/day as beryllium sulfate in the diet for 2 years (Morgareidge *et al*., 1975).

7.3 Carcinogenic Effects

7.3.1 Humans

A number of retrospective cohort mortality studies examining workers at beryllium facilities have been conducted in the United States. In general, the studies carried out before 1987 that associated beryllium exposure with lung cancer had a number of deficiencies: they were inadequately controlled for the confounding factors such as tobacco smoking, they included workers in beryllium industry who were not actually exposed to beryllium, the expected number of deaths from cancer was miscalculated, or inappropriate controls were used (Toxicological Profile, 2002). The more recent studies corrected some of the methodological faults of the previous studies. Ward *et al*. (1992) reported on a mortality study of 9225 male workers at seven beryllium processing plants and found a statistically significant increase in cancer risk, with a standardized mortality ratio (SMR) of 1.26 (95% CI, 1.12–1.42). Steenland and Ward *et al*. (1992) extended the former cohort mortality study of Beryllium Case Registry enrollees to include women and an additional 13 years of follow-up. A comparison of 689 patients with beryllium disease within the general U.S. population revealed a statistically significant increase in the risk of lung cancer, with 28 cancer deaths for SMR of 2.0 (95% CI, 1.33–2.89). An excess in lung cancer was found for both genders and was higher for individuals with acute beryllium disease than those with CBD. The results of these two studies indicate beryllium dose-response relationship for cancer. A follow-up study by Sanderson *et al*. (2001) comprised 142 lung cancer cases and 5 age- and race-matched controls for each case. The overall lung cancer mortality rate was 1.22 (95% CI, 1.03–1.43), and no significant relationship was found between the duration of employment and cancer risk. Several authors have criticized the conclusions of the Steenland and Ward's (1992) and Ward's (1992) studies mainly because of the low excess in cancer risk and inadequate adjustment for the smoking habit (Toxicological Profile, 2002).

On the basis of these investigations, the International Agency for Research on Cancer (IARC, 1993) classified beryllium as a group 1 carcinogen (sufficient evidence for carcinogenicity in humans). IARC (2001) noted that (1) the epidemiological data generally showed increases in observed lung cancers at most of the beryllium processing plants, (2) these increases were generally associated with high exposure levels that occurred before 1950, and (3) the highest risk of lung cancer occurred in individuals with acute beryllium disease. IARC (2001) also noted a number of limitations with the existing cancer database (poor exposure characterization, relatively low excess cancer risk). American Conference of Governmental Industrial Hygienists (ACGIH) and Deutsche Forschungsgemeinshaft (DFG) consider beryllium and its inorganic compounds as substances that cause cancer in man (groups TLV- A1 and MAK 1, respectively) (ACGIH, 2005). US EPA (2005) located beryllium and its compounds in group EPA-B1 (probable human carcinogen, limited evidence of carcinogenicity from epidemiological studies) and EPA-L (likely to produce cancer in humans). According to US EPA, although the results of the Ward *et al*. (1992) study are suggestive that occupational exposure to beryllium can result in an increase in lung cancer mortality, the interpretation of these findings is limited by a number of factors. These include lack of job history data, the limitations in the available smoking habit data, and a possibility that the workers were exposed to other potential carcinogens. However, regardless of the shortcomings, the results of follow-up mortality studies on the same

cohort are suggestive of a causal relationship between beryllium exposure and an increased risk of lung cancer. The air unit risk amounts to $2.4 \times 10^{-3}/\mu g/m^3$ (US EPA, 2005) that means that at the present TLV-TWA of $2.0\,\mu g/m^3$, the risk of additional lung cancer caused by exposure to beryllium or its compounds amounts to 6.43×10^{-4}. Recently, Levy *et al*. (2002) reanalyzed the data and conclusions of the study by Ward *et al*. (1992). This study has shown that there is no statistical association between beryllium exposure in these workers and lung cancer when using the more appropriate population cancer rates (local rates instead of the U.S. rates). Their findings indicate lower and generally not statistically significant standard mortality ratios that are not compatible with the interpretation of a likely causal association. According to the authors, there is, at best, an extremely fragile association when the data are corrected for smoking.

In general, it seems that the lung cancer issues related to occupational exposure to beryllium are linked to the higher exposure levels that were associated with acute beryllium pneumonitis and predominated before 1950 when beryllium levels were much higher than in the subsequent decades (Gordon and Bowser, 2003; IARC, 2001).

7.3.2 Animals

Gardner and Heslington (1946) discovered osteosarcomas of the long bones in seven rabbits that survived 7 or more months after the intravenous injection of zinc beryllium silicate ($ZnBeSiO_3$) used in the fluorescent light tube industry. Both zinc oxide and zinc silicate alone were inactive, whereas beryllium oxide was also carcinogenic. Guinea pigs and rats did not develop bone cancer after intravenous injections of both zinc beryllium silicate and beryllium oxide. Several investigators reproduced beryllium bone sarcoma in the rabbit with beryllium metal and various beryllium compounds. The incidence of tumors was consistently high in the rabbit studies, varying from 13–100%. The latent period varied from 5.5–24 months after the last injection of beryllium. The osteosarcomas developed in different bones, including the humerus, tibia, femur, ilium, ischial tuberosity, lumbar vertebra, scapula, and ribs. Frequently, two or more bones were affected in the same animal (WHO-IPCS, 1990).

Beryllium and some beryllium compounds were found to be carcinogenic in animals exposed through inhalation. Nickell-Brady *et al*. (1994) reported a 64% incidence of lung tumors in rats after a single exposure, nose only, to 410–980 mg/m^3 beryllium metal aerosol for 8 to 48 minutes. Lung tumors were at first observed 14 months after inhalation. Lung cancer rate, compared with controls, was increased also in rats exposed to 0.035 mg beryllium/m^3 as beryllium sulfate for 180 days (Schepers *et al*., 1957). Carcinogenic activity to the lung was confirmed for other beryllium compounds, including the fluoride, phosphate, silicate, and for dust of beryllium ore (Wagner *et al*., 1969), as well as for pulverized free metal, passivated metal, beryllium hydroxide, and beryllium-aluminium alloy (Groth *et al*., 1980).

Lung cancers were also found in rhesus monkeys (Schepers, 1964; Vorwald, 1968). Vorwald reported that monkeys, exposed to inhaled BeSO4 aerosol 38.8 µg Be/m^3 in a chamber, averaging 15 hours/week over 3 years, developed pulmonary tumors with adenomatous or epidermoid characteristics with a number of metastases to the lymph nodes and to other organs.

The animal data indicated that beryllium is carcinogenic to animals. However, many of the studies performed on animals have been criticized because of poor documentation, being conducted at single-dose levels, or failure to include controls (EPA, 1987).

7.4 Genotoxic Effects

The evidence for beryllium as a directly acting mutagen is weak and inconsistent. Most studies have found that beryllium chloride, beryllium nitrate, beryllium sulfate, and beryllium oxide did not induce gene mutations in bacterial assays with or without metabolic activation. In the case of beryllium sulfate, all mutagenic studies (Arlauskas and Baker, 1985; Ashby *et al*., 1990; Dunkel *et al*., 1984; Rosenkranz and Poirer, 1979; Simmon, 1979) were negative, with the exception of the results reported for the forward mutation assay in *Bacillus subtilis* (Kanematsu *et al*., 1980). Beryllium nitrate was negative in the Ames assay (Arlauskas, 1985; Kuroda *et al*., 1991) but positive in a *Bacillus subtilis* rec assay (Kuroda *et al*., 1991). Beryllium chloride was negative in a variety of studies (Kuroda *et al*., 1991; Ogawa *et al*., 1987; Rossman and Molina, 1986). Positive results were reported for *Bacillus subtilis* rec assay (Kuroda, 1991). Beryllium oxide was negative in the Ames assay and *Bacillus subtilis* rec assay (Kuroda *et al*., 1991).

Unlike the generally negative results in reverse mutation assays in prokaryotes, gene mutations were induced in whole mammalian cell cultures by the addition of either beryllium sulfate or beryllium chloride (Hsie *et al*., 1979; Miyaki *et al*., 1979).

According to one study, beryllium sulfate induced a 10-fold increase in chromosomal aberrations in human lymphocytes and produced a 21-fold increase in chromosomal aberrations in Syrian hamster cells (HEC) (Larramedy *et al*., 1981). However, other studies indicate that beryllium sulfate did not induce chromosomal aberrations in hamster

ovary cells (Brooks, 1989) and in hamster lung cells (Ashby *et al*., 1990). Larramedy *et al*. (1981) found increased sister chromatid exchange (SCE) in HEC cells and cultured human lymphocytes after treatment with beryllium sulfate. Dose-dependent increases in SCE were also observed with both beryllium chloride and beryllium nitrate–exposed V79 cells (Kuroda *et al*., 1991). On the other hand, no increase in SCE by beryllium sulfate treatment of human lymphocytes or the cultured macrophage cell line was found by Anderson (1983).

Soluble beryllium compounds seem to be weakly genotoxic (Toxicological Profile, 2002). In the occupational settings, workers are exposed predominantly to beryllium as inhalable particles, and adverse effects of beryllium depend on the chemical species and surface properties of particles. According to Gordon and Browser (2003), it would be useful to reexamine the mechanisms of the mutagenic activity of beryllium using relevant chemical forms of beryllium.

7.5 Mechanisms of Toxic Action

Chronic beryllium disease is a T-cell–mediated disorder. Beryllium, acting as a hapten, interacts with the antigen-presenting cells in the lungs. Beryllium peptide associated with major histocompatibility (MHC) class II molecule is recognized by the T-cell receptor with the help of $CD4^+$ molecules (Newman, 1996a; Saltini *et al*., 1989). This interaction triggers $CD4^+$ T lymphocytes activation and proliferation. Only certain HLA-DP molecules allow Be-antigen presentation to Be-specific $CD4^+$ T-cell clones. Some authors suggest that particular amino acid residues, for example at positions 55–56 and/or 69 of the DPß-chain, are required for Be-antigen presentation in most Be-sensitized and CBD individuals (Fontenot *et al*., 2001). Beryllium-specific $CD4^+$ T cells were found in the peripheral blood and in bronchoalveolar lavage fluid (BALF) from individuals with the disease (Maier and Newman, 1998; Mroz *et al*., 1991, Newman, 1996b). These cells proliferate in the BeLPT. Their accumulation in large numbers (up to 29% of the BALF cells) is associated with the development of granulomatous inflammation in the lung of CBD patients (Fontenot *et al*., 2002). Fontenot *et al*. reported that a number of individuals with CBD revealed an increase in the percentage of T-cell receptor (TCR) variable ß3 regions (Vß3) in BALF (Fontenot *et al*., 1998; 1999). These expansions are composed of oligoclonal populations of T cells with related complementary determining region 3 (CDR3) with invariant aspartic acid (D) at the 96^{th} position of the ß-chain and length of 7–8 amino acids in some individuals. These findings suggest that in CBD, the pathogenic T-cell population are $CD4^+$ T cells with CDR3 motif.

The T-cell–mediated process is orchestrated by cytokines. Tinkle *et al*. (1997) reported that Be-stimulated cells from BALF of CBD subjects produce high levels of IFNγ protein and mRNA with a transient increase in IL-2 protein and mRNA production. Be-stimulated BALF cells obtained from healthy donors did not produce these cytokines. Macrophages isolated from BALF of sarcoidosis and CBD patients had significantly elevated levels of TNFa mRNA (Bost *et al*., 1994). Be-stimulated CBD BALF cells also produce TNFa, IL-6 (Tinkle *et al*., 1997), and IL-10 (Tinkle *et al*., 1999). IL-10 inhibits antigen-induced T-cell proliferation in many inflammatory diseases, but it had no effect on Be-stimulated T-cell proliferation (Tinkle *et al*., 1999). Moreover, recombinant IL-4 did not down-regulate beryllium-stimulated cytokines in CBD (Maier *et al*., 2001). Be-stimulated $CD4^+$ T-cell proliferation and TNFα production is blocked by anti-HLA-DP monoclonal antibodies (Parsons *et al*., 2002). The data show that Be-stimulated $CD4^+$ T-cell proliferation and production of proinflammatory cytokines are mediated primarily by HLA-DP.

7.6 Biomarkers of Effect

The beryllium blood lymphocyte proliferation test (BLTP) is used as a medical surveillance tool for assessment of persons at risk for developing clinical and subclinical chronic beryllium disease (Kreiss, 1977; Newman *et al*., 1989; Rossman *et al*., 1988). It serves to detect sensitization to beryllium both in asymptomatic persons sensitized and those with CBD. It is commonly used not only in the diagnostics but also in workplace surveillance. It is also regarded as exposure marker for exposures from 8 weeks to 30 years before the testing; however, its negative result is not the evidence for the absence of beryllium exposure.

During the analytical procedure, lymphocytes are at first incubated in a mixture containing beryllium salt, usually $BeSO_4$. After incubation, ^{3}H-thymidine (3H-TdR) is added to the mixture. The amount of ^{3}H-TdR taken up by the lymphocytes reflects lymphocyte activation expressed as counts per minute (cpm). The test is replicated six times, using three concentrations of $BeSO_4$. The response is expressed as a stimulation index (SI) where

$$SI = \frac{\text{cmp of cultures with beryllium sulfate}}{\text{cmp of cultures without beryllium sulfate}}$$

In 1995, the participants of a workshop organized by the Beryllium Industry Scientific Advisory Committee (BISAC) and representatives of five laboratories

that perform BLTP developed a consensus standard to provide guidelines for test interpretation. This consensus standard established the criterion for an abnormal result as "at least two of six tests with SI of 3.0 or higher." If none of the six replicates exceeds 3.0, the result is interpreted as normal. Only one positive result is considered negative borderline (Deubner *et al*., 2001). Because of the inter- and intralaboratory variability, the criterion used to define beryllium sensitization requires two abnormal BLPTs (Kreiss *et al*., 1997).

The problems of laboratory variability have been reported. According to Schuler *et al*. (2005), of two laboratories performing analyses, one laboratory was twice as likely to have abnormal results and five times as likely to have reported borderline or interpretable results. Deubner *et al*. (2001) evaluated results of BLTP determinations performed by three laboratories that involved two simultaneous tests on a split specimen. The level of agreement was expressed as kappa statistics. Intralaboratory agreement was fair to moderate, with kappa values between 0.3 and 0.6. Interlaboratory values were poor to moderate (kappa values from 0.2–0.6). Also the positive predictive values (PPV) that compared BLTP results against the results of bronchoscopy were assessed. A single unconfirmed abnormal test had a PPV of 39% for CBD, a confirmed (on the basis of subsequent testing) abnormal test had a PPV of 45% for CBD, and a first-time double abnormal test had a PPV of 49% for CBD. According to Deubner *et al*. (2001), the BLPT does not meet the criteria for a screening test, but despite its limitations, it remains a useful disease surveillance tool. BLPT can, however, be used to identify specific groups of workers with a higher prevalence of sensitization or CBD and thereby assist in more focused exposure control and CBD prevention efforts within a workforce.

For the assessment of CBD progression, some nonspecific inflammation markers (cytokines) or basic fibroblast growth factor (bFGF) have been used (Inoue *et al*., 1996). The presence of bFGF in blood indicates the lung fibrosis process. Mucin-like protein (Kl-6), the lung damage marker, can be found at high concentrations in patients with CBD, contrary to sensitized or healthy individuals. Its level correlates positively with radiological changes and impairment of the lung function (Inoue *et al*., 1997). Serum angiotensin-converting enzyme (ACE) activity levels reflect the extent of granulomatous inflammation in CBD, and it seems that the ACE genotype might play a role in the immune response and in disease progression (Newman *et al*., 1992). However, Meier *et al*. (1999) reported no statistically significant association between ACE genotype and CBD.

The beryllium skin patch test can be used in persons with an inconclusive result of blood BLPT. However, it can probably induce sensitization, as well as aggravate the CBD process, and thus should be administered with caution (Cotes *et al*., 1983; Epstein, 1983; Stoeckle *et al*., 1969; Tepper, 1972).

7.7 Biomarkers of Susceptibility

Blood biomarkers of CBD susceptibility include class II major histocompatibility complex (MHC) genes: HLA-DP, HLA-DR. It was found that in Be-exposed individuals with the HLA-DPβ1-Glu69 genotype, the relative risk for CBD was eight times as high as for the individuals without this genotype and that the prevalence of the HLA-DPβ1-Glu69 genotype was 32.3% (Richeldi *et al*., 1997). The considerable propagation of those genes in the general population limits their usefulness for the screening.

According to Richeldi *et al*. (1997), if a preplacement genetic screening had been used, 5 of 6 cases would be prevented, but 36 additional workers would be denied employment. Calculations performed by Nicas and Lomax (1999) suggest that, assuming that the screening test is perfect, in an unscreened workforce of 1000 persons, the expected number of CBD cases is 50, and in the screened one 16. Screening allows avoidance of 34 cases of CDB in 1000 exposed. The expected number of persons who must be screened so that 1000 normal-susceptibility individuals would be obtained is 1429. Of 429 individuals who were refused, 87.1% would never have been affected. Taking into account the ethical and social problems, genetic testing for susceptibility using HLA markers does not seem immediately applicable to disease prevention (Richeldi *et al*., 1997).

8 DIAGNOSIS AND TREATMENT

CBD diagnosis requires the following main criteria: (1) demonstration of beryllium sensitization in blood and/or lung lymphocytes or by skin patch test, (2) granulomas and mononuclear cell infiltrates on lung biopsy or radiographic findings consistent with CBD.

The presence of clinical findings such as altered pulmonary function, radiographic abnormalities, and pulmonary signs and symptoms of CBD is an additional, not obligatory, diagnostic criterion (Mroz *et al*., 2001).

8.1 Treatment

Removal of an affected worker from exposure to beryllium is recommended, although no epidemiological

studies have examined whether removal from exposure brings changes to the clinical course. Clinical case series have shown the efficacy of systemic glucocorticosteroids (GCS) in improving the symptoms and/or pulmonary function. Other immunosuppressive agents such as methotrexate and cyclosporine may be used in individuals nonresponding to GCS or those who develop severe side effects of GCS (Mroz *et al*., 2001). Supportive therapy includes oxygen, bronchodilators, and immunization against respiratory pathogens (Maier and Newman, 1998).

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CHAPTER

22

Bismuth

BRUCE A. FOWLER AND MARY J. SEXTON

ABSTRACT

Bismuth compounds are considered to be poorly to moderately absorbed after inhalation or ingestion, but there are no quantitative data. Absorbed bismuth is distributed throughout the soft tissues and bone, the highest concentrations being found in the kidney and liver. Absorbed bismuth is excreted primarily through the urine. The biological half-time for whole-body retention is about 5 days, but intranuclear inclusions containing bismuth seem to remain for years in the kidneys of patients treated with bismuth compounds.

High-level exposure causes renal failure associated with degeneration and necrosis of the epithelium of the renal proximal tubules, fatty changes and necrosis of the liver, reversible dysfunction of the nervous system, skin eruptions, and pigmentation of the gums and intestine.

There are no reports on occupational exposures. For the general population, the total daily intake in food is approximately 5–20 µg, with much smaller amounts contributed by air and water. An important source of exposure for specific segments of the population in the past was the therapeutic use of bismuth compounds. The cosmetic use of bismuth compounds still continues to be fairly widespread. Bismuth subnitrate has been used to induce metallothionein in heart tissue and kidneys and to attenuate the toxicity of Adriamycin and cisplatin compounds, respectively.

Short reviews on the toxicology of bismuth have been published by Browning (1969), Filipova (1971), Arena (1974), and Fowler and Vouk (1986).

1 PHYSICAL AND CHEMICAL PROPERTIES

Bismuth (Bi): atomic weight, 208.98; atomic number, 83; density, 9.7 (20°C); melting point, 271.3°C; boiling point, 1560 ± 5°C; white crystalline metal with pinkish stain. Bismuth belongs to the Va group of the periodic system together with arsenic and antimony. Its conductance in the solid state is only 0.48 of the liquid conductance, and it has the lowest thermal conductivity of all metals. Bismuth forms compounds in oxidation states +3 and +5. Of technological and toxicological interest are bismuth oxide, bismuth sulfide, bismuth oxychloride, and salts of inorganic oxoacids (carbonate, nitrate, sulfate), and of organic acids (salicylate, triglycollate). Many of these salts have a basic form, such as basic nitrate or subnitrate. Bismuth forms trialkyls that are unstable in air but stable and insoluble in water (e.g., trimethylbismuth).

2 METHODS AND PROBLEMS OF ANALYSIS

Atomic absorption spectrophotometry is an adequate method for the determination of bismuth in biological and environmental samples. Its limit of detection at 223.1 nm (air-hydrogen flame) is approximately 0.4 µg/L, and amounts as low as 1.5 µg can be determined in 1 g of tissue with a relative standard deviation (SD) of 5%. Flameless atomic absorption with hydride generation of BiH_3 has been reported (Lee, 1982) to reduce the detection limit for seawater to 0.01 ng/L. Similar hydride generation systems combined with inductively

coupled argon plasma (ICAP)-atomic emission spectroscopy (Hahn *et al*., 1982; Wolnik *et al*., 1981) have reported detection limits for bismuth of 40 ng/mL with a relative SD of <6%. Biological samples should be wet washed with nitric, sulfuric, and perchloric acids (Delves *et al*., 1973; Hall and Farber, 1972; Kinser, 1966). Spectrophotometry with dithizone has a detection limit of about 0.01 mg/L, but lead interferes and has to be separated from bismuth (Pinta, 1970). Spark source mass spectrometry (SSMS) has been used for the determination of bismuth in human tissues; it has a limit of detection of 0.002 µg/kg wet weight (Hamilton *et al*., 1972; 1973). There is not enough information to evaluate the accuracy of these methods.

Studies using inductively coupled-mass spectrometry (ICP-MS) on human serum samples have reported a detection limit of 0.007 µg/L with relative standard deviations of 5.7–13.6% (Vanhoe *et al*., 1993).

3 PRODUCTION AND USES

3.1 Production

Bismuth occurs in native form and in minerals such as bismite (bismite oxide) and bismuthite (bismuth sulfide), which is usually associated with sulfide ores of lead and copper and tin dioxide. The production of metallic bismuth is linked to lead and copper refining. The current world production is approximately 3600 tons, the main producers being Australia, Japan, Peru, and Mexico (Carlin, 1982; USGS, 2004). Pyrometallurgical separation of calcium-magnesium-bismuth drosses from which associated metals such as copper, lead, and zinc are removed by suitable fluxes is a widely used technological process for the production of bismuth (Panel on Bismuth, 1970; Paone, 1970). As indicated in Figure 1, bismuth production has steadily risen since 1945, except for two relatively short periods, one of decrease followed by one of stabilized production (USGS, 2004). In 2002,the world production of bismuth was 4070 metric tons.

3.2 Uses

Approximately 64.5% of bismuth is consumed in the United States in low-melting alloys and metallurgical additives, including electronic and thermoelectric applications. The remainder is used for catalysts, pearlescent pigments in cosmetics, pharmaceuticals, and industrial chemicals. Bismuth compounds have been used as dusting powders, astringents, antiseptics, antacids, and radiopaque agents in X-ray diagnosis (now replaced by barium sulfate). Another obsolete use is in the treatment of syphilis, where bismuth compounds have been replaced by penicillin. Bismuth compounds that have been most widely used in therapy include bismuth potassium tartrate, basic carbonate, gallate, nitrate, salicylate, and bismuth magma (suspension of hydroxide and basic carbonate suspension) (Panel on Bismuth, 1970). More recently, bismuth has been incorporated into the production of nanotechnology for a variety of purposes (Gao *et al*., 2005; Wang *et al*., 2004; Zvonkov *et al*., 2000). Effects of the clinical uses of bismuth compounds are discussed in Section 7.1.2.1.

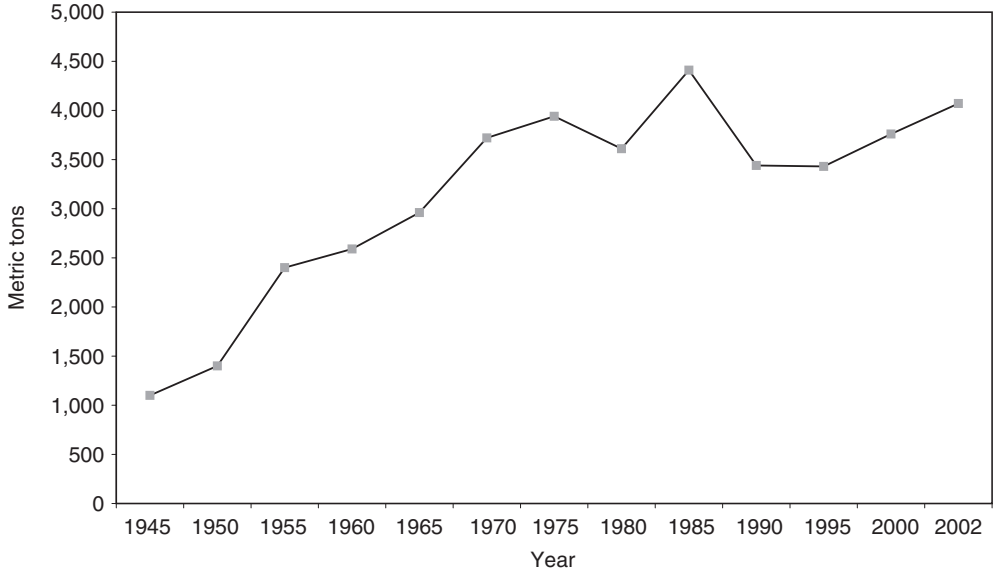

FIGURE 1 Bismuth world production (metric tons).

4 ENVIRONMENTAL LEVELS AND EXPOSURES

4.1 General Environment

4.1.1 Food

Using pooled samples of food representative of the main regions in the United Kingdom, Hamilton and Minski (1972; 1973) estimated the daily intake of bismuth as less than 5 µg (by SSMS). The concentrations in individual dietary samples were not reported. Studies (Hahn *et al*., 1982; Wolnik *et al*., 1981), which analyzed rice flour, wheat flour, spinach, and orchard leaves certified by the U.S. National Bureau of Standards, reported values of <0.08/µg/g. Similar values were also reported for corn, potatoes, and soybeans. According to Woolrich (1973), the daily intake from food and water is approximately 20 µg, but again data on concentrations of bismuth in specific food items were not given. This estimate agrees with the model value for daily balance of elements in the reference manual (ICRP, 1975). Jaysinghe *et al.* (2004) have recently reported the concentrations of bismuth in muscle and tissues of wildfowl with lead shot. They reported liver concentration in teal and mallards ranging from 0.05–0.09 µg Bi/g on a dry weight basis, respectively. Bismuth is a contaminant of lead shot, and consumption of shot by waterfowl is the apparent route of exposure to this element.

4.1.2 Ambient Air, Water, Soil, and Rocks

The concentrations of bismuth in urban air range from 1–66 ng/m^3, and in rural air from 0.1–0.6 ng/m^3 (Division of Atmospheric Surveillance, 1972). The concentration of bismuth in respirable fly-ash (ADD <5/µm*)* was found to be approximately 4–5 g/kg (Davison *et al*., 1974). The daily intake of bismuth through inhalation is estimated as <0.01–0.76 µg (ICRP, 1975; Woolrich, 1973).

Concentrations of bismuth in drinking water have not been reported. Seawater has been reported to contain approximately 0.2 µg Bi/L (Environmental Studies Board, 1972), but other studies (Lee, 1982) have reported much lower values in the range of 0.053–0.63 ng/L for ocean surface waters.

Bismuth levels in soil are approximately 1µg /kg, and in rocks they range from 0.1 µg/kg (coal) to 3 µg/kg (sandstone) (Bowen, 1966).

4.1.3 Pharmaceuticals and Cosmetics

Pharmaceuticals and cosmetics are still a source of more prolonged exposure to bismuth compounds for specific groups of the general population.

4.2 Working Environment

Exposure to bismuth and some of its compounds may occur in the production of metallic bismuth and in the manufacture of pharmaceuticals, cosmetics, and industrial chemicals, but no reports are available on such exposure.

5 METABOLISM

5.1 Absorption

Bismuth compounds are considered to be slightly to moderately absorbed through the respiratory and gastrointestinal tracts, depending on their solubility, but there are no quantitative data. Absorption through the skin is of interest in relation to the use of bismuth compounds in oil-based cosmetics, but again there is no quantitative information (Sollman and Seifter, 1939; Sollman *et al*., 1938). The gastrointestinal absorption of bismuth subnitrate has been reported (Chaleil and Allain, 1980) to be increased by concomitant administration of sulfhydryl compounds. Recent studies on the gastrointestinal absorption and tissue uptake of bismuth administered as a single oral dose of either ranitidine bismuth citrate or bismuth citrate to mice (Larsen *et al*., 2003) showed rapid uptake of Bi into cells of the gastrointestinal tract and kidneys within hours of exposure and that with the passage of time, Bi was found in a number of other organ systems weeks later. Subcellular distribution studies showed that the lysosomal compartment was the primary intracellular site of deposition.

5.2 Distribution

Four days after intramuscular injection of BiOCl or BiO(OH) to rats, 14.4% of the dose was found in the kidney, 6.6% in the liver, 1.5% in bone, 0.6% in muscle, and less than 0.1% in blood. Seventeen days after administration, only 0.6% remained in the kidney (Durbin, 1960). Two hours after intravenous injection of bismuth citrate and sodium bismuth thioglycollate to dogs and rabbits, approximately 3–5% of the dose was found in the kidney, 6–10% in the liver, and 0.4% in the lungs. Within 24 hours the relative concentration in the kidney increased to 7–120%, and in the liver it decreased 1–4%. Within 1 week, the concentration in the kidney and liver was reduced to 2.5%. After 4–5 weeks, the concentration in the liver was again higher (1%) compared with the kidney (0.45%) (Sollman and Seifter, 1942). Oral intubation of tripotassium dicitratobismuthate to rats for 14 months produced the highest tissue concentrations in the kidney (Lee *et al*., 1980). Similar results

have been reported by Wieriks *et al*. (1982), who found that the kidneys of both dogs and rats had the highest visceral concentrations of bismuth after 6 or 3 months, respectively. In the rat, the cecum also showed extensive bismuth accumulation. Pharmacokinetic studies in animals (Pieri and Wegmann, 1981) using ^{205}Bi-citrate have demonstrated a two-compartment model that also demonstrated high levels in the kidney and medulla of the brain. Bismuth has been found to bind to transferrin in serum but in a noncanonical manner or more open manner that confers a lower affinity than iron or indium for this protein, which may explain the relatively low efficiency of bismuth delivery to cells after administration of bismuth-containing pharmaceuticals (Miquel *et al*., 2004; Sun and Szeto, 2003; Zhang *et al*., 2004). Canena *et al*. (1998) studied the distribution of bismuth in rats treated with ranitidine bismuth citrate and bismuth subcitrate for 15 days with a twice per day oral gavage. Rats given bismuth subcitrate alone at a dose of 13.7 μg/kg showed marked uptake in a number of tissues with highest concentrations in the kidney. Rats given the ranitidine bismuth subcitrate at a dose of dose of 22.8 μg/kg showed markedly lower kidney bismuth concentrations and undetectable levels of bismuth in the brain. The intracellular binding of bismuth in the kidney has been studied (Piotrowski and Szymanska, 1976; Szymanska and Piotrowski, 1980; Szymanska and Zelazowski, 1979a) with respect to low molecular-weight bismuth-binding proteins that seemed to have some properties distinct from metallothionein. More recent studies (Boogaard *et al*., 1991; Kaji *et al*., 1994; Naganuma and Imura, 1994; Palmiter, 1994; Szymanska *et al*., 1993; Zindenberg-Cherr *et al*., 1989) have shown bismuth to be an effective inducer of metallothionein and to bind to this protein. This knowledge has been applied to protecting against the nephrotoxicity of anticancer drugs such as cisplatin (Imura *et al*., 1987; Kondo *et al*., 1991, 2004; Leussink *et al*., 2003) and Adriamycin (Satoh *et al*., 2000). These investigators observed, in tumor-bearing mice and patients with renal cell carcinoma, that orally administered bismuth was transported to normal tissues and not cancerous tissues. In addition, bismuth induction of metallothionein has been linked to the attenuation of the teratogenic effects of cadmium in mice (Naruse and Haysashi, 1989) and the adverse effects of gamma irradiation on the bone marrows of mice (Satoh *et al*., 1989).

The autopsy distribution of bismuth in 22 patients who received therapeutic intramuscular injections (mainly bismuth salicylate) was as follows (median values, μg/kg, wet weight): kidney. 33.3; liver. 6.8; spleen. 1.6; colon. 1.3; lung. 0.9; brain. 0.6; and blood. 0.5 (Sollman *et al*., 1938).

5.3 Excretion

Ingested bismuth is largely eliminated unabsorbed in feces. Model values for the daily balance of bismuth in a reference manual are dietary intake 20 μg; fecal elimination 18 μg; and urinary excretion 1.6 μg (Sollman *et al*., 1938).

Absorbed bismuth is mainly excreted in urine, although the biliary/fecal excretion of bismuth as reported by Pieri and Wegmann (1981). The rate of excretion of bismuth after intramuscular injection into rabbits of 13 different compounds was studied by Kolmer *et al*. (1939). Water-soluble compounds were excreted more rapidly than those suspended or dissolved in oil. Excretion in 4 days varied from 82.2% of the dose for aqueous solution of bismuth thioglycollate to 1.9% for oil suspension of bismuth oleate, but the excretion continued for at least 36 days. Durbin (1960) compared the excretion of elements of group V in rats after intramuscular injection of soluble compounds in oxidation state +3. The metabolism of radio bismuth closely resembled the metabolism of UO22+, suggesting that Bi(III) was oxygenated or in a "basic" form. The retention in the kidney was short, and by the 17th day after injection, 95% of the dose had been excreted.

The permeability of the placenta to bismuth was demonstrated by Leonard and Love (1928) after intramuscular injection of potassium bismuth tartrate and sodium potassium tartro-bismuthate into pregnant rabbits and cats.

5.4 Biological Half-Times

ICRP (1960) adopted the following model values for the biological half-times of bismuth in man: whole-body retention, 5 days; kidney, 6 days; liver, 15 days; spleen, 10 days; and bone, 13.3 days. Slikkerveer and deWolff (1989) reported that the elimination of bismuth from blood displayed multicompartmental kinetics in humans from 3.5 minutes to up to 17–22 years.

6 BIOLOGICAL MONITORING

The concentrations of bismuth in healthy human tissue in the United Kingdom have been reported by Hamilton *et al*. (1972; 1973) using SSMS. The highest mean concentration (400 μg/kg wet weight) was found in the kidney, followed by the bone (<200 μg/kg). The brain, lung, and lymph nodes contained bismuth in concentrations from 10–40 μg/kg. Concentrations in the range from 2–8 μg/kg were found in the testis,

blood, muscle, liver, and basal ganglia. Ninety-five percent of bismuth concentrations in normal lung and lymph node tissues (158 samples) were <40 µg/kg, dry weight using SSMS (Brown and Taylor, 1975). Warren *et al*. (1983) studied bismuth concentrations in brain, spinal cord, liver, and muscle from autopsy material of 15 patients with multiple sclerosis and 13 cases with no neurological disease. Concentrations ranged from undetectable to 13 µg/kg on a wet-weight basis with no discernible difference between the two groups. The liver generally showed higher concentrations of bismuth than the other tissues examined. Studies in Belgium (Vanhoe *et al*., 1993) on measured bismuth in human serum by ICP-MS showed a range of <0.007–0.067 µg/L in 19 healthy adults. Slikkerveer and de Wolff (1989) reported that the normal concentration of bismuth in blood is in the range of 1–15 µg/L. Bismuth-induced encephalopathy remains a clinical concern (Youngman and Harris, 2004). Serum concentrations of bismuth of 242 µg/L have been reported in patients with bismuth encephalopathy originally diagnosed with Alzheimer's disease (Summers, 1998). Even higher levels are found in those with renal dysfunction. A 68-year-old man who took a total of 864 mg bismuth daily for 2 months (twice the recommended dose) developed global cerebral dysfunction. His whole-blood bismuth concentrations rose to 880 µg/L (Playford *et al*., 1990).

7 EFFECTS AND DOSE-RESPONSE RELATIONSHIPS

Bismuth is not an essential element, either for man or for animals.

7.1 Local Effects and Dose-Response Relationships

7.1.1 Animals

Application of trimethyl- and triethylbismuth to the skin of rats and rabbits has been reported to produce intense inflammation and edema. Local necrosis at injection sites was also observed. Acute local effects of inhalation of trimethylbismuth by rats, cats, and dogs include pulmonary edema. Eye irritation is another local effect observed in inhalation exposures to alkylbismuth (Sollman and Seifter, 1939).

7.1.2 Humans

Application of trimethylbismuth to intact human skin produced no marked effects, but intense irritation was noted if the skin had been scratched. Irritations of the upper respiratory airways and of the eye were also observed (Sollman and Seifter, 1939). Another report (Krueger *et al*., 1976), however, has shown high concentrations of bismuth in the cerebral venous blood and cerebral spinal fluid of two patients using a bismuth-containing skin cream and suffering from an organic brain syndrome. After the use of bismuth subgallate as a colostomy deodorant, one patient developed paranoid ideation, memory impairment, and defective gustatory and olfactory function that improved after discontinuation of the compound. His 24-hour urine had reached 889 µg/day, and his whole-blood bismuth concentration was 67 µg/L (Friedland *et al*., 1993). The ingestion of colloidal bismuth can lead to acute renal failure. This was observed in a 2-year-old 2 days after he had taken 28 De-Nol tablets (colloidal bismuth subcitrate, 8.4 g) even though his physical examination was unremarkable when he was admitted to the hospital 6 hours after the ingestion (Islek *et al*., 2001).

Taking over-the-counter bismuth medications for gastric ulcers can have serious adverse side effects if high doses are chronically ingested. One woman was reported to have progressive dementia, dysarthria, and myoclonic jerks that were epileptic in nature. Although clinical improvement was achieved, chelation was stopped, because it seemed to be related to a deterioration in the patient's status (Teepker *et al*., 2002). Playford and coworkers (1990) reported a patient with chronic renal failure who developed global cerebral dysfunction with hallucinations, ataxia, and an abnormal EEG from overdosing on tripotassium dicitrato bismuthate. His functioning returned to normal after chelation.

7.1.2.1 Effects of the Clinical Use of Bismuth

Bismuth compounds have been used and evaluated extensively in the treatment of a large variety of gastric disorders, such as recurrent abdominal pain in children, bleeding and nonbleeding ulcers, and nonulcer dyspepsia (Chong, 1995; Kung, 1997, Laine *et al*. 2003; Moayyedi *et al*., 2005). Although longer duration of treatment seems more effective, even a short-term of 1 day results in reduction of symptoms (Kung *et al*., 1997; Lara, 2003). Moayyedi and colleagues (2005) conducted a meta-analysis of 17 randomized clinical trials and concluded that bismuth therapy was moderately effective in treating nonulcer dyspepsia. Treatments for children or adults that combine a bismuth compound with an antibiotic are more effective than either alone (Chong, 1995; Graham *et al*., 2005; O'Riordan *et al*., 1990). Bismuth compounds are routinely used with antibiotics to treat duodenal ulcers produced by *H. pylori* (De Francesco *et al*., 2001; Delaney, 1995; Fischbach *et al*., 2004; Hildebrand *et al*., 2001; Laine *et al*., 2003;

Sarosiek *et al*., 1989; Vakil and Cutler, 1999). Vondracek (1998) concluded that the antimicrobial and mucosal protective effects of bismuth contributed to the eradication of the *H. pylori* infection. In an assessment of the treatments of more than 50,000 patients throughout the world with *H. pylori*, Laheij *et al*. (1999) estimated effectiveness in approximately 80–85% of patients. Treatments were usually combinations of ranitidine bismuth citrate or proton-pump inhibitor (PPI) with two antibiotics. Sometimes treatments were a combination of a PPI or H2 receptor antagonist with bismuth subcitrate or tripotassium dicitrato bismuthate, metronidazole, and tetracycline. Because of expense, PPI-based treatments are less often used in developing countries (de Boer, 1999). The choice of the bismuth compound does not seem to be an important factor (Megraud, 2000).

On the basis of an analysis of five randomized clinical trials related to treatment of collagenous colitis, Chande and coauthors (2004) concluded that patients improved clinically and histologically but were not convinced that there was remission of the disease.

Bismuth has also been found to be useful for assistance in wound healing (Mai *et al*., 2003). Bismuth subgallate has been commonly used as an astringent and hemostatic in adenotonsillectomies in the form of bismuth subgallate-epinephrine paste. Conley and Ellison (1999) conducted a case series study that showed a reduction in posttonsillectomy hemorrhage when bismuth subgallate was applied to the surgical site. However, on the basis of a review of the literature, Hatton (2000) concluded that it was probably the epinephrine that was the hemostatic agent. Tramontina *et al*. (2002), though, did find that bismuth subgallate promoted wound healing in rats. Bismuth induction of metallothionein has been shown to reduce the cardiotoxicity of Adriamycin in mice (Satoh *et al*., 2000) and renal toxicity of cis-diammine dichloro-platinum (Kondo *et al*., 1993) in cancer patients and in mice (Kondo *et al*., 2004) treated with bismuth subnitrate. The tagging of antibodies to surface proteins on leukemia cells with the alpha-particle emitting 212Bi or 213Bi isotopes has shown promise in helping to kill these malignant cells (Kennel *et al*., 1999; Kolbert *et al*., 2001; Macklis *et al*., 1992; Vandenbulcke *et al*., 2003) apparently by a mechanism involving induction of apoptosis (Macklis *et al*., 1992; Vandenbulcke *et al*., 2003). This approach has also be used to study the *in vitro* toxicity of ^{113}Bi-labeled plasminogen activator inhibitor type 2 to human breast cancer cells (Ranson *et al*., 2002).

Shields incorporating bismuth have been used as protection against radiation during diagnostic imaging procedures, such as computed tomography, and shown to reduce radiation exposure to sensitive organ systems (Colombo *et al*., 2004; Fricke *et al*., 2003; Hopper *et al*., 1997; King *et al*., 2002).

7.2 Systemic Effects and Dose-Response Relationships

The main systemic effects of bismuth compounds both in man and in animals are exerted in the liver and kidney.

7.2.1 Animals

7.2.1.1 Liver

Cloudy swelling with nuclear degeneration and occasional small foci of necrosis in the liver were observed in rabbits after lethal injections of sodium and potassium tartro-bismuthate (intravenous, 10–30 µg/kg; intramuscular, 150–350 mg/kg) and bismuth trioxide (intramuscular, 450 and 500 µg/kg) (Lucke and Klander, 1923). After 6 months, peroral treatment of rats and rabbits with potassium bismuthate (2.5, 0.25, 0.025, and 0.05 µg/kg) and bismuth sulfate (5.0, 0.5, 0.05, and 0.025 µg/kg) produced dilatation of intertrabecular capillaries, vascular stasis, and marked dilatation and congestion of the vessels. The hepatic tissue contained large irregularly shaped foci of reticuloendothelial cells. The severity of these changes was closely related. The activity of succinic dehydrogenase in the liver and of cholinesterase in the serum and liver was reduced even at doses of 0.025 and 0.05 µg/kg. Hepatic excretory function was abnormal in rabbits (bromsulfthalein retention). There were no effects when animals were given 0.0005 µg/kg potassium bismuthate and 0.025 µg/kg bismuth sulfate (Seljankina *et al*., 1970). Woods and Fowler (1987) studied liver toxicity in rats after subcutaneous injections of bismuth subnitrate at doses of 0, 20, 40, and 80 µg/kg 16 hours after injection by electron microscopy. They reported swollen mitochondrial membranes in liver cells of rats given the bismuth injections that was most marked in animals receiving the two highest dose levels. The morphological changes were correlated with dose-related decreases in the mitochondrial heme pathway enzymes ALA-synthetase and heme synthetase (ferrochelatase), as well as the cytosolic enzyme ALA-dehydratase.

7.2.1.2 Kidney

Kidney damage was produced in rats by single intramuscular injections (0.03–1.5 g/kg) of 13 different bismuth compounds. Histological examination of 104 rats showed that 36 or 37 animals that died before 21 days had nephritis of varying degrees of severity as had 11 of the 67 surviving rats. The proximal tubules constituted the most markedly affected site of toxicity.

The least toxic compound was bismuth thioglycollate, doses of 0.04–0.080 g/kg producing severe nephritis (Kolmer *et al*., 1939). After subcutaneous injections (5 g of bismuth subnitrate each day for 3 days), degenerative changes and intranuclear and cytoplasmic inclusions appeared in renal proximal tubules of rabbits (Beaver and Burr, 1963a). These inclusion bodies are pathognomonic for bismuth exposure (Fowler and Goyer, 1975). Hemorrhages in the cortical and cerebral layer of the kidney and lymphohistiocytic infiltrations were found in rats after 6 months of peroral treatment with potassium bismuthate and bismuth sulfate (0.025–5.0 µg/kg) (Seljankina *et al*., 1970). Szymanska and Zelazowski (l979b) reported that administration of bismuth trichloride by subcutaneous injection at doses of 1.0, 2.0, and 3.0 µg Bi/kg every other day up to 2 weeks greatly increased renal concentrations of copper and concomitant levels of metallothionein. Rodilla *et al*. (1998) studied metallothionein induction in cultured human renal proximal tubule cells after exposure to bismuth subnitrate at time points up to 4 days. They found that bismuth induced metallothionein in these cells without production of toxicity at concentrations of up to 100 µmol/L. Leussink *et al*. (2000) studied the effects of a single oral overdose of bismuth subcitrate (3.0 mmol Bi/kg) at time points from 1– 48 hours. They observed necrosis in the S3 segment of the proximal tubule before damage in the S1/S2 segments. These data were correlated with bismuth concentrations in blood that were best described by a single compartment model with a peak 2 hours after treatment and an elimination half-life of 16 hours. Leussink *et al*. (2001) studied the reversibility of bismuth subcitrate overdoses in rats at dose levels of 0, 0.75, 2.5, and 3 mmol Bi/kg with measurement of a battery of standard clinical parameters for renal function. They found dose-related clinical changes, including increased proteinuria, glucosuria, and elevated plasma urea and creatinine levels. The kidney function of all but two of the animals that died returned to normal ranges by 10 days. The S3 segment of the proximal tubule was most markedly affected in terms of necrosis followed by the S1/S2 segments to a lesser degree. Leussink *et al*. (2002) conducted *in vivo* (rats) and *in vitro* (NRK-52E cells) follow-up studies into the mechanisms of cell death and concluded that necrosis and not apoptosis was the primary cell death pathway initiated by Bi exposure in both test systems. The interaction of Bi with the cell membrane was hypothesized to be a primary cause of cell death. Leussink *et al* (2003) studied the effects of 33 µmol/L Bi3+ in NRK52-E cells after 12 hours and found down-regulation of a number of genes and up-regulation of only glutathione S transferase subunit 3A by subtraction PCR.

7.2.1.3 Nervous System

Immediately after inhalation exposure to trimethyl bismuth (10–20 minutes, concentration not stated), cats and dogs showed ataxia, restlessness, and convulsive seizures. Between attacks, the animals were depressed. Within approximately 24 hours, they developed signs of severe encephalopathy (Sollman and Seifter, 1939). Disturbances in conditioned reflexes occurred in rats and rabbits treated with potassium bismuthate (ingestion, 2.5, 0.25, and 0.025 µg/kg) and bismuth sulfate (ingestion, 5.0 µg/kg) (Seljankina *et al*., 1970). Studies in mice (Ross *et al*., 1994) dosed with 2500 µg/kg intraperitoneally of bismuth subnitrate found neuropathological changes in relation to the tissue distribution of Bi 28 days after treatment. They found that Bi concentrations were highest in the olfactory lobe and hypothalamus followed by the septum and brain stem. The cerebral cortex and striatum had the lowest concentrations. Autometallography demonstrated that Bi concentrations were greatest near the fenestrated blood vessels. All treated mice had hydrocephalic changes but no discernible pathology by light microscopy. By electron microscopy, marked expansion of the extracellular space between the choroids plexus and the epithelial cells was noted, suggesting this interface may be a primary target for Bi neurotoxicity.

7.2.1.4 Other Systemic Effects

Blood pressure of dogs receiving hypodermic or intramuscular injections of trimethylbismuth (4 doses, 350 µg/kg body weight) sank progressively to shock level without significant change of heart rate, arrhythmia, or block. The animals were anesthetized with barbiturates (Sollman and Seifter, 1939). Intravenous injection of elemental bismuth (0.50 µg/kg) caused slight decrease in blood pressure and in amplitude and heart rate. Higher doses (1.8 µg/kg) resulted in heart block, and all fundamental functions of the heart were affected, including excitability, conductivity, and contractility (Mason, 1927). Ultrastructural/biochemical studies conducted on livers of rats dosed with 0, 20, 40, or 80 µg Bi/kg and examined 16 hours after treatment (Woods and Fowler, 1987) demonstrated mitochondrial swelling and marked alterations in the heme biosynthetic pathway enzymes ALA synthetase, ALA dehydratase, and heme synthetase (ferrochelatase). Recent studies on the testes (Pedersen *et al*., 2003) showed Leydig cell death in rats injected with bismuth subnitrate, and follow-up studies (Stoltenberg and Hutson, 2004) showed that bismuth subnitrate injection altered interactions between testicular macrophages and Leydig cells. Huston (2005) studied the *in vitro* effects of Bi over a concentration range of 1–100 µmol/L on the viability

of Leyding cells in relation to testicular macrophages. This investigator found no effect on the secretion of tumor necrosis factor (TNF-alpha) and concluded that bismuth does not directly affect Leydig cells but rather decreases serum testosterone concentrations by means of toxicity to testicular macrophages. Arata *et al*. (2002) studied the cytotoxic effects of triphenylbismuth relative to bismuth chloride and triphenyl tin chloride in rat thymocytes. They found that triphenyl bismuth at a concentration of 30 µmol/L increased the number of thymocytes positive for annexin V binding, suggesting an increase in apoptosis. At a 3 µmol/L concentration, they observed both a decrease in cellular glutathione and an increase in intracellular $Ca2^{+}$. Bismuth chloride did not significantly alter cellular viability over a 10–30 µmol/L concentration range.

7.2.2 Humans

Although bismuth compounds had been used for the treatment of syphilis and for other therapeutic uses, Beerman (1932) compiled only 22 fatalities resulting from such therapy. Delayed deaths were mainly attributable to the involvement of the gastrointestinal tract, the liver, or the kidney, or a combination of the two or all three of these systems. Deaths of 11 children occurred within 2–5 days after the use of suppositories containing bismuth salt of heptadiencarboxylic acid and were preceded by vomiting, drowsiness, abdominal pain, convulsions, and coma (Weinstein, 1947). There are no reports on the occupational exposure effects of bismuth (Filipova, 1971). No effects were observed in 13 normal volunteers receiving approximately 450 µg Bi daily (as "Bistrimate" [bismuth sodium triglycollamate]-$C_{24}H_{28}O_{25}N_4BiNa_7$) from a few days to over a year (Lehman and Fassett, 1947).

7.2.2.1 Liver

An instance of jaundice with liver damage was reported in a 6-year-old child who received 4–5 µg/kg body weight of bismuth thioglycollate in a single injection. On the basis of this and a number of fatal cases reported by other authors, Karelitz and Freedman (1951) concluded that soluble bismuth compounds are definitely hepatotoxic to man, producing fatty degeneration of the liver. This confirms the analysis of Beerman (1932) and Wolman (1940). Jaundice indicative of hepatitis occurred in 10.3% of 1032 syphilis patients treated with bismuth compounds between 1932 and 1942 (Kulcher and Reynolds, 1942).

7.2.2.2 Kidney

Acute renal failure can occur after oral or parenteral administration of bismuth compounds such as bismuth sodium triglycollate or thioglycollate, particularly in children (Boyette, 1946; Urizar and Vernier, 1966). The tubular epithelium is mainly affected with little change in the glomeruli. In 30 cases of bismuth nephropathy reviewed by Urizar and Vernier, the interval between medication and onset of symptoms and signs ranged from 6–7 weeks (mainly bismuth sodium thioglycollate, intramuscular doses 5–200 µg; oral, 1.5–19 g). Functional alterations in acute bismuth nephropathy include severe depression of glomerular filtration rate, renal plasma flow, and proximal tubular reabsorption, as indicated by glucosuria, phosphaturia, and aminoaciduria (Czerwinski and Ginn, 1964). Bismuth inclusions were found in the renal tubular epithelium of 12 of the 14 patients treated parenterally with bismuth compounds (Beaver and Burr, 1963b).

7.2.2.3 Neurological Effects

A neurological syndrome possibly associated with bismuth subgallate ingestion and characterized by confusion, tremulousness, clumsiness, myoclonic jerks, and gait disturbance was observed in four patients (Burns *et al*., 1974). Robertson (1974) also described similar neuropsychiatric symptoms and signs in four geriatric patients administered large doses of the same compound orally for several months. Emile *et al*. (1981) reported 59 cases of bismuth encephalopathy between 1972 and 1979 with serum bismuth concentrations between 900 and 2320 µg/L. More recent studies using silver enhanced staining of brain sections from six patients with bismuth toxicity showed that bismuth accumulated in both neurons and glial cells in various brain regions (Stoltenberg *et al*., 2001). The blood vessels of the cerebellum also showed intense staining. Tissue analyses using proton-induced X-ray emission analysis and atomic absorption spectroscopy confirmed the presence of bismuth in affected brain regions. By electron microscopy, bismuth was found in the lysosomes and along the vasculature basement membranes. Follow-up studies in rats using bismuth subnitrate injections (Stoltenberg *et al*., 2003) showed reduction in the numbers of A and B cells in the nerve roots but not the area of axonal cross-sections or myelinated nerve fibers in the ventral or dorsal root of the dorsal root ganglion. These types of neuronal effects are consistent with the observations of myoclonic encephalopathy reported in persons with chronic bismuth abuse (Teepker *et al*., 2002).

7.2.2.4 Skin and Mucosa

Pityriasis, rosea-like eruptions, and other skin manifestations such as the "erythema of the ninth day" syndrome (Milian's erythema) have been occasionally described as a result of therapy with bismuth

compounds (Dobes and Alden, 1949; Goldman and Clarke, 1939). Ulcerative stomatitis has been observed after bismuth therapy (Peters, 1942; Silverman, 1944). Bismuth pigmentation has been found in the colon, vagina, and the skin (Heyman, 1944).

7.2.2.5 Other Effects

Colitis, gastrointestinal bleeding, purpura, agranulocytosis, and aplastic anemia have also been reported resulting from administration of bismuth compounds (Arena, 1974). Emile *et al*. (1981) reported osteoarticular lesions in 8 of 59 patients with bismuth encephalopathy. The most common lesion was osteonecrosis of the humeral head.

7.3 Carcinogenicity, Teratogenicity, and Mutagenicity

There is no evidence of carcinogenicity or mutagenicity of bismuth compounds, although bismuth penetrates the placenta (Leonard and Love, 1928). No teratogenicity has been reported.

8 TREATMENT OF BISMUTH POISONING

According to Arena (1974), dimercaprol (BAL) yields good results if given early. Other methods include the administration of atropine and meperidine to relieve gastrointestinal discomfort. Caution is required in fluid administration during anuric and oliguric phases of nephrosis, but loss of fluid and electrolytes should be covered in the subsequent diuric phase (Karelitz and Freedman, 1951). Basinger *et al*. (1983) reported comparative studies on nine chelating agents with respect to reducing bismuth toxicity. Compounds with vicinal thiol groups were most effective, particularly if adjacent aromatic hydroxyl groups were also present. D-Penicillamine was the most effective compound approved for clinical use.

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CHAPTER

23

Cadmium

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ABSTRACT

Cadmium (Cd) is chemically similar to zinc; it occurs naturally with zinc and lead in sulfide ores. Elevated concentrations in air, water, and soil may occur close to industrial emission sources, particularly those of nonferrous mining and metal refining industries.

Cadmium metal has been used as an anticorrosive, electroplated onto steel, and Cd compounds are used as pigments, often in plastics. Cadmium and its compounds are also used in electric batteries, electronic components, and nuclear reactors. Because some of the applications of Cd can be performed by other less-toxic materials, the use of Cd has, therefore, been restricted by law in some countries.

The absorption of Cd compounds through the skin is negligible. Between 10 and 50% of inhaled Cd will be absorbed, with the degree of absorption being greater for smaller particles and fumes than for larger dust particles. Humans absorb 5–10% of ingested Cd. A low intake of calcium, zinc, or iron increases the degree of absorption; for example, in iron-deficient individuals, the gastrointestinal absorption rate may be as high as 20%.

Cadmium is transported in plasma when bound to metallothionein—a low-molecular-weight protein and/or to certain high-molecular-weight proteins. The accumulation of Cd occurs in many tissues, with particularly long half-lives (10–30 years) having been reported for Cd in muscle, kidney, and liver tissue. Cadmium stimulates metallothionein production in the same manner as other bivalent metals, such as zinc, copper, and mercury. Metallothionein-bound Cd in plasma is filtered through the renal glomeruli and reabsorbed in the tubuli, where the metal ion is released after lysosomal degradation of the protein. The unbound Cd stimulates the production of new metallothionein, which binds the Cd in the renal tubular cells. When not all of this new Cd is bound, toxic effects occur, possibly because of the interference of Cd with zinc-dependent enzymes and/or membrane function.

The average amount of Cd ingested in most European and North American countries is approximately 10–20 μg/day. The corresponding average urinary excretion is approximately 0.5–1.0 μg/day. Most of the Cd in blood is located in the cells. The average blood concentration is approximately 0.5–1.0 μg/L in nonsmokers; it is twice as high in smokers because of Cd absorption from cigarette smoke. Concentrations of 10–20 μg/kg are usually found in the kidney cortex of nonsmokers in European countries. Although the intake of Cd through food has been higher in Japan than in Europe, and the reported tissue levels are correspondingly higher, the food intake of Cd has decreased in Japan during the last few decades.

Ingestion of highly contaminated food or drink results in acute gastrointestinal effects with concomitant diarrhea and vomiting. Acute inhalation of Cd in air—for example, from soldering or welding fumes—may lead to severe chemical pneumonitis. Long-term exposure to low air levels may lead to chronic obstructive lung disease and possibly lung cancer. Long-term excessive exposure from the air or food leads to renal tubular dysfunction. The first sign of damage is a low-molecular-weight proteinuria. This condition is the critical effect of such exposure to Cd and is used in quantitative risk assessment.

Long-term exposure from food, often combined with other means of delivery, may also lead to disturbance

†Deceased

of calcium metabolism, osteoporosis, and osteomalacia, mainly among postmenopausal women. A disease exhibiting these features—called *Itai-Itai* disease—occurred in the 1950s in Cd-polluted areas of Japan; 124 cases were diagnosed up to 1970, and decreasing numbers of clinical cases have been diagnosed later, with 66 cases during the period between 1970 and 2006.

In animals exposed to Cd through injection, inhalation, or oral exposure, cancer may develop at the injection site, in the lungs and prostate, or in other organs. Although some epidemiological studies have found an increase in the rates of cancer of the lungs and prostate, other studies have not demonstrated such effects. Cadmium is classified as a human carcinogen (Group 1) by the International Agency for Research on Cancer.

Exposure to Cd in the air at concentrations of 5–10 μg/m^3 during a working life of 45 years may give rise to renal tubular dysfunction in a small proportion of exposed workers. At approximately 100 μg/m^3, signs of chronic obstructive lung disease may develop even after exposure for a shorter duration. After a lifetime of exposure from food at an average intake of approximately 200 μg/day, renal effects have been observed at age 50. There is considerable individual variation in the sensitivity of these renal effects. It has been suggested that such effects can be avoided if renal cortex levels are kept <50 μg/kg and urine levels <2.5 μg/g CR. Recent reports of low, but statistically significant, increases at even lower levels of urinary Cd are, however, noteworthy. Such increases are observed in the general population, particularly among people with diabetes.

There is no specific treatment for Cd poisoning. When there are signs of osteomalacia, large doses of vitamin D should be given. Because of the long half-life of Cd in the kidneys, which are the critical organs and the irreversibility of the critical effect, primary prevention is essential. Prevention can be assisted through environmental and biological monitoring.

The extensive literature on the toxicological and environmental aspects of Cd has been reviewed in detail by Friberg *et al*. (1974, 1985, 1986a), Tsuchiya (1978), Nriagu (1980, 1981), the WHO/IPCS (1992), the IARC (1993), Jarup *et al*. (1998c), the ATSDR (1999), Nordberg and Nordberg (2002), the EU (2003), Satarug and Moore (2004), and the WHO/FAO (2003, 2005).

1 PHYSICAL AND CHEMICAL PROPERTIES

Cadmium (Cd): CAS #7440-43-9; atomic weight, 112.4; atomic number, 48; density, 8.6 g/mL; melting point, 320.9°C; boiling point, 765°C; crystalline form: hexagonal, silver-white malleable metal; oxidation state, +2. The naturally occurring isotopes are 106 (1.22%), 108 (0.88%), 110 (12.9%), 111 (12.75%), 112 (24.07%), 113 (12.6%), 114 (28.86%), and 116 (7.5%) (Weast, 1986). Among its many radioactive isotopes, Cd 109, 111, and 115m are used in experimental studies. Some common Cd compounds are cadmium acetate, cadmium sulfide (yellow pigment), cadmium sulfoselenide, cadmium selenium sulfide (red pigment), cadmium stearate, cadmium oxide, cadmium carbonate, cadmium sulfate, and cadmium chloride. Of the many inorganic Cd compounds, several are quite soluble in water (e.g., cadmium acetate, chloride, and sulfate); cadmium oxide and sulfide are almost insoluble. Cadmium oxide and cadmium carbonate might, however, be soluble at gastric pH.

Cadmium readily complexes with some organic compounds (e.g., thiocarbamates); this property provides the basis for several analytical methods. Although some synthetic organometallic Cd compounds are known, they are not found in the general environment because they decompose rapidly.

2 METHODS AND PROBLEMS OF ANALYSIS

Particular care must be taken when collecting and preparing samples for Cd analysis. Because of the great variations of Cd concentrations in different materials, contamination from a high-level material to a low-level material may occur. On the other hand, liquid solutions may release Cd to their storage containers. Suitable precautions are reviewed in Chapter 2. The representativeness of samples collected is another major concern discussed in Chapter 2.

The most common methods for the final analysis of prepared and treated samples are atomic absorption spectrophotometry (AAS), atomic fluorescence (AAF), and inductively coupled plasma mass spectrometry (ICP-MS). For samples at higher concentrations, the traditional flame AAS method is adequate; for samples at low concentrations, however, the more-sensitive electrothermal atomization ETA-AAS, ETA-AAF, or ICP-MS methods are needed (Chapter 2). Interference from other elements must be taken into account; such interference may result from the analytical procedure itself (e.g., from the argon plasma used in ICP-MS). The use of recently invented techniques such as ICP-MS coupled to HPLC or FPLC makes it possible to analyze samples according to their isotopes and also the chemical nature of their Cd compounds (WHO/IPCS, 1992; Chapter 2). Other newly developed techniques can further improve the quality of the analysis. For example, in samples containing protein-bound Cd, the amino acid composition of the protein also can be determined.

The flame AAS method has a detection limit in pure water of 1–5 mg/L, whereas the more sensitive methods can detect levels more than 100-fold lower. In environmental or biological samples, the sensitivity is usually not as great because of matrix interference.

Destructive neutron activation analysis (NAA) is an accurate method that was developed early but not used widely for Cd determination, except as a means of quality control (Chapter 2).

The *in vivo* determination of Cd in liver using NAA was first described by Biggin *et al.* (1974). In Cd-exposed workers, it was possible (Harvey *et al.*, 1975) to demonstrate elevated liver levels of Cd. Since then, the sensitivity has increased; at present, approximately 15 µg Cd/g wet weight in kidney and 1.5 µg Cd/g wet weight in liver can be detected using portable equipment (Ellis *et al.*, 1981). The radiation dose from one measurement is low (gonad dose: <40 mrem; Vartsky *et al.*, 1977).

Cadmium in tissues can also be determined *in vivo* through the use of X-ray fluorescence (Nilsson *et al.*, 1995).

Another activation method, which, however, is not used *in vivo*, is proton-induced X-ray emission; this tool can be used to analyze several elements simultaneously and for microanalysis within histological structures of tissue (Hasselman *et al.*, 1977; Lindh *et al.*, 1980).

Electrochemical methods, such as differential pulse anodic stripping voltammetry, depend on complete destruction of all organic materials in the sample and transfer of the Cd ions into a noncontaminated electrolyte. It is, therefore, particularly suitable for water analysis (O'Laughlin *et al.*, 1976), but it has also been used for analyses of blood, urine, food, and tissues (Elinder and Lind, 1985).

In the past, a substantial amount of published data has been based on inadequate methods. Errors, with values 10–100 times too high, have been observed from both emission spectroscopy and AAS measurements (Friberg *et al.*, 1974; Lauwerys *et al.*, 1975; WHO, 1979), and there is a continuous need for adequate quality assurance (Nordberg, 1996a).

Detailed quality assurance programs for the analyses of Cd in the blood and kidney cortex have been organized by the WHO/UNEP and the EU (Friberg and Vahter, 1983; Jin *et al.*, 2002). These programs indicate that, despite poor initial agreement between laboratories, a joint effort to improve the accuracy of analyses can produce excellent results. All reports on Cd analysis in biological or environmental samples should be accompanied by sufficient quality control data (Chapter 2).

3 PRODUCTION AND USES

3.1 Production

Cadmium displays chemical similarity to that of zinc; it often occurs in zinc or lead ore in relatively high concentrations. The cadmium-to-zinc ratios in minerals and soils are within the range from 1:100–1:1000. Cadmium is obtained as a by-product of the refining of zinc and other metals, particularly copper and lead. There is no specific Cd ore worth mining solely for its Cd content. The world consumption of the metal has increased continuously during the past century to a global supply of 22,000 metric tons (International Cadmium Association, 2002), as indicated in Figure 1. Table 1 presents the global supply distribution among the various regions of the world.

Although Cd toxicity has been recognized for only a relatively short time, environmental pollution has taken place for several thousand years, ever since man started to produce metals from ores that happened to contain Cd.

3.2 Uses

Cadmium is used in a number of industrial processes, but for most of its applications, there are lower toxicity alternatives. In the 1970s, the Swedish government introduced legislation to limit the use of Cd, and such limitations have been developed more recently by many EU countries. Because of its ability to protect iron products from rusting, Cd is used to coat them through electroplating. Cadmium-plated parts for automobiles are more resistant to rust than are zinc-coated (galvanized) objects. In Sweden, the use of Cd for plating is not allowed, and most applications of Cd compounds as pigments and stabilizers in plastics are banned. Cadmium sulfide and cadmium sulfoselenide are used as color pigments in plastics and in various types of paints. Cadmium stearate is used as a stabilizer in plastics. Because of its ability to stiffen copper and increase its mechanical resistance at increased temperatures, Cd is used in copper-cadmium alloys, which are used in items such as automobile radiators. Cadmium may serve as an electrode component in alkaline batteries, and this application is one of the most important uses of Cd at present. Cadmium is also used in silver solders and welding electrodes. In the early 21st century, the global use of Cd was 77% for NiCd batteries, 11% for pigments, 8% for coatings, and 4% miscellaneous (International Cadmium Association, 2002). The United States now contributes less than 10% of the world's production and imports the

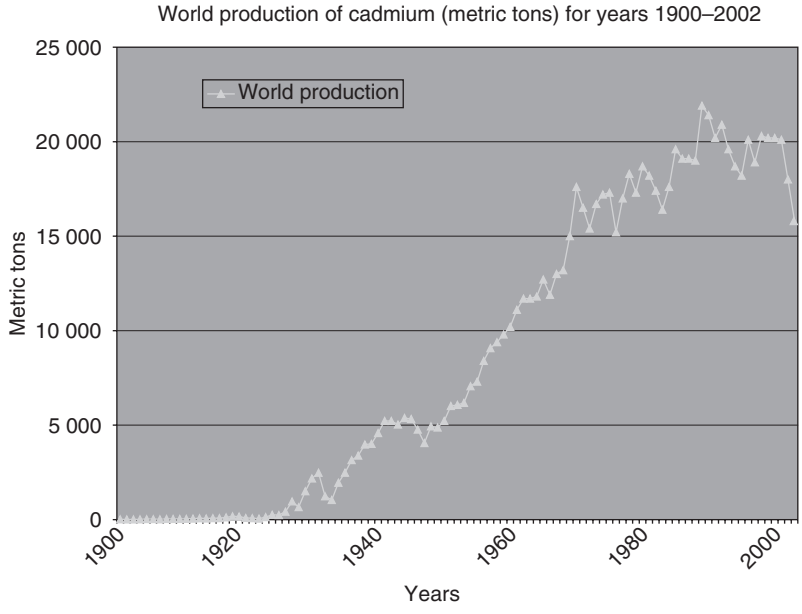

FIGURE 1 World production of cadimium (metric tons) for years 1900–2002

TABLE 1 Cadmium Global Supply 2002 (22,000 Metric Tons)

Asia	41%
America	16%
Europe	15%
Recycling and stockpile	25%
Australia	3%

Source: International Cadmium Association (2002).

metal from Canada, Australia, and Mexico (WHO/IPCS, 1992). In China and India, the use of NiCd batteries has increased recently.

4 ENVIRONMENTAL LEVELS AND EXPOSURES

4.1 General Environment

4.1.1 Food and Daily Intake

Extremely wide ranges of Cd concentrations have been reported in foodstuffs from various countries. Different results from different investigators analyzing the same types of food in the same area can be explained, in some cases, by the possibility of the use of inadequate analytical methods. Although such discrepancies were common in the past, they may still occur if adequate quality control is not used in sampling and chemical analysis.

Data from reliable analyses performed in several countries (Elinder, 1985a; WHO/FAO, 2003) indicate that most foodstuffs have Cd concentrations in the range 0.005–0.100 mg/kg (mean values, wet weight; see Table 2). Certain foods (e.g., kidney and oysters) may contain much higher concentrations. Cadmium concentrations in brown rice in Japanese noncontaminated areas are presently usually 0.01–0.2 (average, 0.06) mg/kg (the Ministry of Agriculture, Forestry and Fisheries, Japan, 2006).

In some contaminated areas of Japan, Cd concentration of rice of 0.1–4.2 mg Cd/kg have been reported (Tsuchiya, 1978). In contaminated areas in China, average concentrations of approximately 1.0–3.7 mg Cd/kg have been found in rice (Jin *et al*., 2002; Nordberg *et al*., 1997). Cui *et al*. (2004) reported high levels of Cd in vegetables sampled in 2002 in an area near Nanning, Guangxi Province, China, where cultivation of rice had been terminated because of contamination. In these countries, and in the UK and New Zealand, high concentrations in certain marine products have also been found (Elinder, 1985a; WHO/FAO, 2003).

A high intake in consumers of seafood and shellfish in New Zealand (Nordberg *et al*., 1986) has been

TABLE 2 Concentrations (mean values mg Cd/kg wet weight) in Various Foodstuffs

Food	Cd mg/kg wet weight	Food	Cd mg/kg wet weight
Potatoes	0.01–0.06	Beef kidney	0.2–1.3
Wheat grains	0.005–0.08	Beef meat	0.005–0.02
Rice Noncontaminated areas	0.008–0.13	Fish meat other than crab	0.004–0.1
Milk	0.00017–0.002	Spinach	0.043–0.15
Oysters	0.1–4.7	Carrots	0.016–0.030

Sources: Nordberg and Nordberg (1988; 2002); WHO (1992).

reported; it was also demonstrated that the chemical species of Cd was different in two species of oysters, with one binding protein being structurally similar to metallothionein. The Cd-binding proteins identified in various foodstuffs have been reviewed by Petering and Fowler (1986). The chemical species of Cd, particularly those forms bound to metallothionein-like proteins, are of importance (see Sections 5 and 7) for its uptake, distribution, and toxicity.

Estimations of daily Cd intake based on data of Cd concentrations in single foods and from total diet studies have been made in several countries. In "noncontaminated" areas, the average daily intake is usually in the range from 10–60 μg/day for a 70-kg person (WHO/FAO, 2003; Table 3). There was a tendency for values to be lower in Europe and North America than in Japan. In rural "uncontaminated" areas of Japan, the daily Cd intake in the 1960s was estimated to be 59–113 μg (Japanese Association of Public Health, 1970). Intake of Cd was studied by use of the double-basket technique (Ysart *et al*., 1999). Watanabe *et al*. (2000) found that the Cd intake of Japanese women from food was 37.5 μg in 1977–1981 and 25.5 μg in 1991–1997 (i.e., a decrease of 12 μg between the two periods). The contribution of Cd from rice was 11.7 μg/day, constituting 40% of the intake.

TABLE 3 Daily Intake in μg/kg/day

Australia	0.15	Italy	0.33
Belgium	0.1	Japan	0.34
Canada	0.22	The Netherlands	0.36
China	0.25	Norway	0.14
Czech Rep.	0.26	Portugal	0.26
Denmark	0.28	Spain	0.30
Finland	0.16	Sweden	0.13
France	0.22	UK	0.17
Germany	0.18	USA	0.14
Greece	0.84		

Source: JECFA (2003).

Food baskets containing 60 different foodstuffs, collected in Sweden in 1987, indicated a daily intake of 12 μg Cd (Becker and Kumpulainen, 1991). For example, the intake of one crab/year will contribute an increase (KEMI, 1995) in the average daily intake of Cd during one year of approximately 2 μg Cd (Jorhem and Sundstrom, 1993; Jorhem *et al*., 1984).

The average daily intake in Sweden of 12 μg/day is based on the following concentrations of Cd in foodstuffs: meat, fish, and fruit, 1–5; cereals, potatoes, other root fruits, 10–50; bran, 150; kidney and liver, 100–400 μg Cd/kg. These values for Sweden are in general agreement with the values listed in Table 3 for noncontaminated areas in the world.

Table 3 lists the daily intake in various countries in units of μg/kg body weight per day (WHO/FAO 2003). The 1994–1995 intake in a number of European countries was reported within the framework of the Scientific Cooperation Project (SCOOP, 1997). For Greece and Portugal, the daily intake of Cd was reported to be 50–60 μg, which is 70–80% of the value that JECFA (WHO and FAO, Joint Expert Committee on Food Additives) recommended as the highest tolerable daily intake. Belgium and Italy reported daily intakes (WHO/FAO, 2003) of 20–30 μg Cd/day, and the other countries reported intakes at or below 20 μg Cd/day. On the basis of limited data, SCOOP (2004) considered that intake levels in European countries were lower than 38% of the JECFA recommendation. A low daily intake in France was reported by Leblanc *et al*. (2005) based on determinations of Cd in food (82% of the Cd values were below the limit of quantification).

Legislation of the levels of Cd in food in EU countries has been in effect since 2002 (see Section 10.2.1).

The intake of Cd in contaminated areas of Dayu county, Jiangxi province, China (Cai *et al*., 1995), was reported to be an average of 313 μg/day in males and 299 μg/day in females, mainly arising from rice consumption. In a Cd-contaminated area near Wenzhou,

Zhejiang province, a daily intake of 1850 µg was estimated in 1995, derived mainly from high levels of Cd in rice (Nordberg *et al*., 1997). In this area, farmers have not been allowed to eat the contaminated rice from 1996 onward. In contaminated areas of Japan, daily intakes of up to 400 µg have been reported (Japanese Association of Public Health, 1970). The intake has decreased by twofold to threefold during the past two to three decades.

Measurement of Cd in feces provides a reasonably good estimate of the daily intake, because 90–95% of ingested Cd remains unabsorbed. Such studies agree with the ranges estimated previously for daily Cd intake (Kjellstrom *et al*., 1978; Tati *et al*., 1976; Wester, 1974). Daily Cd intake varies with age in a manner similar to daily energy intake (Kjellstrom *et al*., 1978). Even in "uncontaminated" areas of Japan, the intake values were higher than those in areas of Europe and North America, as estimated from fecal Cd (Iwao *et al*., 1981; Kjellstrom, 1979), but the difference is less prominent at present, as described in previous parts of this section. Kikuchi *et al*. (2003) studied 25 young nonsmoking Japanese females and found that the first day after eating a standardized diet corresponding to an average daily intake of 4.8 µg, fecal Cd amounted to 13.6 µg. After 5 days on the standardized diet, fecal Cd content approached the intake content. Determination of fecal Cd has also been used to study Cd intake in contaminated areas in Japan, where intakes have been found in the range of 150–250 µg Cd/day (Iwao *et al*., 1981). Hermanson and Brozowski (2005) estimated that the intake of Cd by an Inuit population group in the period 1970–1990, averaged 48 µg/person per day, based on determination of sewage sediments.

4.1.2 Water and Soil

In natural water, Cd is found mainly in bottom sediments and suspended particles; the concentration in the water phase is low. In samples of seawater from the coast of Japan, a Cd content of 5–9 ng/L was reported (unpublished report, Ministry of Environment, Japan, 2003). Cadmium concentrations in nonpolluted natural waters are usually considerably lower than 1 µg/L. In the Jinzu River system, which was heavily contaminated in the past, a level of 17–267 ng/L was reported by Kagaya *et al*. (2003). In the Asano River (flowing through Kanazawa city), levels of 60 ng/L or lower were detected, whereas in the Kakehashi River system, which is known to be contaminated by Cd, levels of 150–700 ng/L were detected in unfiltered samples, and 60–90% of these values in filtered samples (Kobayashi and Kizu, 2001).

Contamination of drinking water may occur as a result of Cd impurities in the zinc of the galvanized pipes or from Cd-containing solders in fittings, water heaters, water coolers, and taps. Leaking of Cd into ground water from dumped Cd oxide sludge may also be a route toward the contamination of drinking water.

Concentrations of up to 16 mg/L have been reported in fruit juices that were placed in contact with Cd-containing parts of vending machines (Nordberg, *et al*., 1973). Regular drinking water usually does not have concentrations of Cd exceeding 5 µg/L. The Cd concentration in drinking water from shallow wells in areas of Sweden where the soil had been acidified by acid precipitation contained concentrations of Cd (approaching 5 µg/L) that were higher than that from similar wells in nonacidified areas (Nordberg *et al*., 1985a).

Both waterborne and airborne Cd can cause an increased concentration of Cd in soil. In nonpolluted areas, the Cd concentration in soil is usually less than 1 mg/kg. In certain areas of Japan, where Cd pollution has been suspected, levels of between 1 and 69 mg/kg have been found in the topsoils of rice fields. The Cd associated with the epidemic *Itai-Itai* disease (see following) arose mainly from rice field soil contaminated by Cd-polluted irrigation water. The use of Cd-containing sewage sludge and superphosphate as agricultural fertilizers may also contaminate the soil. Sewage sludge may contain 2–1500 mg Cd/kg dry weight (Berglund *et al*., 1983). It has been demonstrated that the pH and concentrations of other minerals are of importance for the uptake of Cd into plants (Page *et al*., 1981). Both rice and wheat can take up considerable quantities of Cd from soil (Elinder, 1985a). It was recognized in the 1980s (Nordberg *et al*., 1985a) that acidification occurs in some types of arable soil as a result of various agricultural practices, including fertilization, with a small contribution from acid precipitation. Such acidification is still ongoing and gives rise to elevated levels of Cd in crops.

In Sweden, the levels of Cd in fertilizers have been regulated, and the use of Cd for plating and as a pigment has been restricted during the past 20 years. At present, there is almost a balance between the input and output of Cd in arable soils. A small increase is, however, still occurring (0.03–0.15% per year), depending on the region and type of farming. The uptake and accumulation of Cd in crops are influenced by factors such as the Cd concentration in the soil, the nature of the plant species and cultivar, the soil pH, and the organic matter content of the soil (Oskarsson *et al*., 2004). Cadmium uptake from the soil, through crops and feed, to the blood and kidneys of pigs has been studied, as well as the Cd contents in men and women living at those farms (Oskarsson *et al*., 2004). There was

an increased concentration, by a factor of three, from the pig feed to the pig kidneys. The major contribution of Cd to the pig feed was not, however, from the locally grown grain but rather from nonlocally produced mineral, protein, and vitamin additives of the feed.

Guidelines for drinking water quality issued by WHO have recently been revised from 5 to 3 µg/L (WHO 2004), see Section 10.2.2.

4.1.3 Ambient Air

Ambient air usually has a low Cd concentration. Cigarette smoking contributes to the indoor air concentration of Cd (see Section 4.1.4). Cadmium in ambient air occurs in particulate form. The exact chemical form has seldom been reported, but it is probable that cadmium oxide is an important constituent. In the 1970s and 1980s, the annual average Cd content of the air in the larger cities of the United States, Europe, and Tokyo ranged from 2–50 ng/m^3. In Stockholm, the weekly mean concentration of Cd has been reported to be approximately 5 ng/m^3, whereas that in rural areas is approximately 0.9 ng/m^3 (review by Nordberg and Nordberg, 2002). A review by WHO in the air quality criteria document indicates levels in Europe of 1–10 ng/m^3 in urban areas and 0.1–0.5 ng/m^3 in nonurban areas in the 1980s, with a downward trend since the 1970s (WHO, 2000). In remote areas, values 10–100 times lower have been reported (ATSDR, 1999; Duce *et al*., 1983; Elinder, 1985a). Higher values weekly means of 200–600 ng/m^3 have been recorded around certain Cd-emitting industries (ATSDR, 1999; Elinder, 1985a). Cadmium in air can also be measured indirectly by analyzing the Cd contents in mosses and leaves (Elinder, 1985a). In Sweden, the levels in moss samples have decreased substantially during the three past decades, particularly in urban areas and around point sources. Although Cd in moss reflects Cd deposition, it is not a direct indicator of human exposure (Hellstrom *et al*., 2004).

4.1.4 Tobacco

Smoking tobacco is an important route of exposure for the general population. Smoking one cigarette, generally containing 1–2 µg Cd, results in the inhalation of approximately 0.1–0.2 µg of the metal (Elinder, 1985a). Most inhaled Cd is in the respirable particulate phase (Szadkowski *et al*., 1969). The Cd concentration in cigarettes seems to be higher in countries where they are prepared from tobacco grown in the United States rather than in developing countries such as India (Elinder *et al*., 1983a). Someone who smokes two packs per day of ordinary cigarettes in Western countries may accumulate an additional body burden of approximately 15 mg Cd over a 20–year period (Lewis *et al*., 1972). One cigarette can contain up to 2 µg of Cd. An average of approximately 10% of the Cd (Jarup *et al*., 1998c; Nordberg *et al*., 1985b) is inhaled during smoking; assuming a 50% uptake of the inhaled Cd suggests a daily uptake of 2 µg Cd for 20 cigarettes.

Persons smoking cigarettes made from tobacco grown in Cd-contaminated areas may absorb considerable amounts of Cd through smoking. In a Cd-polluted area in China, it was calculated (Cai *et al*., 1995) that male farmers smoking locally grown tobacco had taken up more than 10 µg of Cd per day through tobacco smoke (inhaled amounts given later—uptake 50% of inhaled amount) and almost 20 µg per day from food (calculated on the basis of a daily intake from food of 380 µg/day and an uptake of 5% of the intake).

The higher Cd exposure among smokers is also reflected in their higher Cd concentrations in blood (Cai *et al*., 1998; Friberg and Vahter, 1983, Jarup *et al*., 1998c; Ulander and Axelson, 1974).

It has been reported (Cai *et al*., 1995) that Chinese farmers residing in a Cd-polluted area, where local tobacco was grown, inhaled between 28 and 34 µg of Cd/day in different time periods from smoking locally grown tobacco.

4.2 Working Environment

Most exposure to Cd compounds in the working environment occurs through inhalation of the workroom air, although oral exposure through contaminated food, drink, or cigarettes consumed at work may also be significant. Skin exposure is of minor importance because of the low degree of absorption through the skin.

High acute inhalation exposures may occur among people welding Cd-plated materials or using silver-cadmium solder. Such exposure is extremely dangerous (Beton *et al*., 1966; Townshend, 1982); see Section 7.1.1. If such operations are performed, fatal levels of exposure may occur. The exposure levels during cadmium-product manufacturing have been high in the past. In a Swedish cadmium-nickel battery factory (Friberg, 1950), the total Cd concentration in air in the 1940s was 1000–10,000 µg/m^3. The levels decreased to approximately 200 µg/m^3 in the 1950s and to 50 µg/m^3 in the 1960s (Kjellstrom *et al*., 1977a). In the late 1970s, the average concentration was approximately 5–10 µg/m^3 in the breathing zone, as measured from long-term studies (Adamsson *et al*., 1979).

In the platemaking department of a British battery factory, the average Cd concentration was determined to be 500 µg Cd/m^3 (Adams *et al*., 1969; Hassler, 1983); in the assembly department it was 100 µg Cd/m^3. In the production of copper-cadmium alloys, the exposure levels were 20–106/µg Cd/m^3 (Bonnell *et al*., 1959); they were 13–89/µg Cd/m^3 at positions close

to the furnace (King, 1955). Similar levels have been reported from a Japanese silver-cadmium alloy factory (130 μg Cd/m^3; Tsuchiya, 1967) and from Belgian electronics, battery, and Cd production factories (31–134 μg Cd/m^3; Lauwerys *et al*., 1974). The mean value of the Cd concentration in the air of workshops in China was variable (0.61–3.54 mg/m^3; Lu *et al*., 2001).

Reported exposure levels from the production of cadmium stearate PVC stabilizers (20–700 μg Cd/m^3; Suzuki *et al*., 1965) and Cd pigments (20–7000 ug Cd/m^3; Harada and Shibutanni, 1973) have varied considerably.

All these values refer to the total Cd concentrations in air. Measurements of Cd concentrations in separate particle-size fractions are rare; we know of only one systematic study (Hassler, 1983) in which Cd was monitored in the breathing zone of workers whose jobs involved long-term exposure.

Significant Cd exposure may also occur in lead, zinc, and cadmium smelters, in the electroplating industry, and in electric welding work using Cd-containing electrodes.

Smokers in a Cd-exposed workplace may contaminate their cigarettes or pipes with Cd and increase their Cd dose by up to approximately 20 μg per cigarette (Piscator *et al*., 1976).

With modern industrial hygiene technology, it is possible to reduce the occupational air Cd concentrations. This phenomenon has been demonstrated in a battery factory (Adamsson *et al*., 1979) and in a cadmium-zinc smelter (Ahlman and Koponen, 1980).

Exposure limits for Cd have been lowered in many countries; as a result, exposure has decreased (Table 4), but there is limited published literature documenting current levels.

NIOSH currently recommends maintaining the inhalation of Cd to as low a level as possible, and the US OSHA has an 8-hour PEL of 5 μg/m^3. The National Board of Occupational Health in Sweden has issued a hygienic limit value for occupational exposure from air of 5 μg/m^3 for respirable dust and 20 μg/m^3 for total dust (AFS, 2005). Cadmium is also listed as a carcinogenic compound. For Cd exposure in the working environment, a medical checkup is compulsory in Sweden with periodic determinations of blood Cd (cf. Section 6 of this chapter). The intention for the future is to limit exposure to all Cd compounds to either 1 or 5 μg/m^3. In the United States, the threshold limit values (ACGIH, 2006) for Cd are 10 μg/m^3 (8-hour TWA) and 2 μg/m^3 (8-hour TWA) for the respirable fraction.

5 TOXICOKINETICS

Data on absorption, retention, distribution, and excretion of Cd are fundamental for the evaluation of the risks to human health connected with Cd exposure. Although an abundance of such data is available, a few gaps in the present knowledge continue to hamper exact evaluations.

5.1 Absorption

5.1.1 Inhalation

Cadmium exposure through inhalation occurs in the form of an aerosol. General laws governing deposition of particulate matter in the human lung indicate that, depending on particle size, 10–50% of the inhaled particles will be deposited in the alveolar part of the lung and a major part of the remainder on the tracheobronchial mucosa. Poorly soluble particles deposited on the ciliated tracheobronchial mucosa will be transported to the pharynx and swallowed into the gastrointestinal tract (see Chapter 3). For finely dispersed (submicron) Cd aerosols, as is the case with exposure through smoking cigarettes, it can be calculated, on the basis of the Cd concentration in cigarette smoke and autopsy data from people who had smoked different quantities of cigarettes, that absorption of the inhaled amount is between 25 and 50% (Elinder *et al*., 1976; Friberg *et al*., 1974; Lewis *et al*., 1972). The large differences in blood Cd levels between smokers and nonsmokers (Friberg and Vahter, 1983; Elinder *et al*., 1983b) indicate that the respiratory absorption may be even greater. These observations are in accordance with recent general knowledge concerning pulmonary deposition and absorption of ultrafine particles (see Chapter 3). There are no industrial data that allow the exact calculation of absorption of inhaled Cd.

Animal data, both from single and chronic exposure studies, indicate a high absorption of Cd through the

TABLE 4 Occupational Exposure Limits

China	0.01 mg/m^3	Sweden (resp.)	0.005 mg/m^3
Japan	0.05 mg/m^3	USA (ACGIH)	
		total dust	0.01 mg/m^3
Sweden (total)	0.02 mg/m^3	USA (resp.)	0.002 mg/m^3

respiratory route: 7–40% (and possibly even more) of the Cd inhaled (Boisset *et al*., 1978; Friberg *et al*., 1974). Particles of larger size and particles with very low solubility will probably be in the lower part of this range, whereas particles with high solubility and smaller diameters will be in the upper part (Nordberg, *et al*., 1985b).

5.1.2 Ingestion

In several reports in which the fate of a single oral dose of radioactive Cd was followed in rats, mice, and monkeys, between 1 and 6% of the dose was taken up (review by Nordberg, *et al*., 1985b). The importance of the general diet composition, including a decreased uptake when the fiber content was increased, and similar effects of adequate zinc and iron intake compared with deficiency, has been demonstrated in animals (Andersen *et al*., 2004; Chaney *et al*., 2004). The absorption in humans is higher. Five volunteers given a single dose of radioactive Cd by mouth showed an average absorption of 6% (Rahola *et al*., 1972), with a range between 4.7 and 7.0%. In another similar study of 14 volunteers (McLellan *et al*., 1978), the average absorption, 2–6 weeks after dosing, was 4.6% (SD ± 4.0%); uptake was higher in women than in men. Vanderpool and Reeves (2001) gave 14 women a sunflower kernel preparation labeled with the stable isotope ^{113}Cd; the fractional absorption was calculated to be 10.6 ± 4.4% through repeated fecal determinations during a 21-day period.

Animal experiments have demonstrated that a low intake of iron, zinc, calcium, or protein may increase the degree of absorption considerably (Andersen *et al*., 2004; Chaney *et al*., 2004; Nordberg, *et al*., 1985b). Pretreatment of animals with Cd may also increase subsequent absorption (Nordberg, *et al*., 1985b). Cadmium bound to metallothionein may be taken up from the gastrointestinal tract partly in intact form and enter the circulation (Cherian *et al*., 1978). There is evidence (for a review, see Jarup *et al*., 1998c) that Cd is, to a large extent, bound to metallothionein in the gastrointestinal mucosa. Low maternal iron stores and a high intake of fiber (with a relatively high Cd content) increased the accumulation of Cd in the placenta of Swedish women (Moberg-Wing *et al*., 1992). The increased intake of Cd in food with higher dietary fiber content (or other dietary components not studied) may have counteracted the probable protective effect of the fiber (cf. the animal data described previously).

In humans, the absorption in persons (mainly women) with low body iron stores (serum ferritin values <20 µg/L) was on average four times higher than that in subjects with normal stores (Flanagan *et al*., 1978); more recent studies have confirmed these observations (for a review, see: Jarup *et al*., 1998c). The higher uptake in women with low iron stores may be explained by recent findings, in human enterocytes, of a close correlation between the expression of the divalent metal transporter 1 (DMT-1) and Cd absorption (Tallkvist *et al*., 2001). DMT-1 transports Cd and Fe in a competitive manner, and such competition most probably explains the interaction between Fe and Cd in gastrointestinal absorption (Zalups and Ahmad, 2003). Because low iron stores (low serum ferritin) are common among women of fertile age, it is reasonable, when considering data on entire populations, to assume a higher gastrointestinal absorption in women than in men. Choudhury *et al*. (2001) assumed an absorption of 5% in men and 10% in women by use of a toxicokinetics model; they found good agreement between the calculated urine values and those measured by the NHANES program in the United States (cf. Chapter 3). Thus, 5% for men and 10% for women seems to be the most reasonable estimate of average gastrointestinal absorption in Western populations. Higher uptake rates (i.e., 37 or 47% for the two different subgroups) were reported in a balance study in young female Japanese volunteers given an experimental diet for 20 days with only 50% of the recommended dietary allowance of iron. Serum iron and serum ferritin levels decreased during the study; more than 50% of the volunteers had serum ferritin levels <20 µg/L at the end of the study (Kikuchi *et al*., 2003).

5.2 Transport and Distribution

5.2.1 Systemic Transport

After absorption from the lungs or the gut, Cd is transported through the blood to other parts of the body. Cadmium in blood is found mainly in the blood cells (Friberg, 1952; Nordberg, G. *et al*., 1985b), where it is bound to a high-molecular-weight fraction and a low-molecular-weight fraction (Nordberg, *et al*., 1971b). Further studies (Garty *et al*., 1981; Nordberg, 1978) have demonstrated that the latter fraction is similar to metallothionein, a protein that also binds Cd in plasma (Nordberg, *et al*., 1971b) and plays an important role in the transport of Cd in animals and humans (Nordberg, 1972; Nordberg and Nordberg, 2000). Cadmium may also possibly be bound in small amounts to low-molecular-weight SH-rich compounds, such as glutathione and cysteine (Zalups and Ahmad, 2003), although evidence for such binding in the plasma of mammals is limited; the main transporting protein for Cd into the kidneys is most probably metallothionein (Nordberg and Nordberg, 2000).

Metallothionein has a molecular weight of approximately 6000–7000 g/mol. Up to 11% of its weight can consist of cadmium, zinc, or copper atoms bound by several sulfhydryl groups (Elinder and Nordberg,

1985; Kagi *et al*., 1984). This protein has been isolated from the livers and several other tissues of Cd-exposed animals.

The small size of the metallothionein molecule enables the protein to be filtered through the kidney glomerular membrane. Like other proteins in the primary urine, metallothionein is reabsorbed into proximal tubular cells. The transport of Cd bound to metallothionein from the blood to renal tubular cells is rapid and almost complete (Johnson and Foulkes, 1980; Nordberg and Nordberg, 1975). Cadmium not bound to metallothionein does not enter the kidneys to the same extent. A similar difference was observed in animals fed cadmium-metallothionein and cadmium chloride (Cherian *et al*., 1978). The former gave rise to a much higher accumulation of Cd in the renal cortex than the latter, most probably because Cd from the chloride binds to albumin (Nordberg, 1978).

Cadmium exposure induces the synthesis of metallothionein in a number of tissues (Elinder and Nordberg, 1985). During the first 12 hours after a high acute exposure to Cd (not bound to metallothionein), there will be an increase over time of Cd bound to metallothionein as a result of the increased production of the protein (Leber and Miya, 1976; Nordberg, *et al*., 1971a). Because the transport of Cd to the kidney depends on the metallothionein binding of Cd in plasma, the distribution of Cd within the body after acute exposure will be different from that after repeated exposures. Figure 2 presents a scheme, first presented by G. Nordberg (1984), describing the transport of Cd in the blood and its uptake into kidney tubules. A detailed description (Nordberg *et al*., 1985b) constituting the background for this scheme was also presented by WHO/IPCS (1992) and by Nordberg and Nordberg (2000). Immediately after uptake of Cd from the gastrointestinal tract or the lungs, the Cd is bound mainly to albumin and other larger proteins in blood plasma. There is, however, only limited information on the variation of binding with time, dose, and route of administration in animals, and none in humans. Available evidence indicates that there is a pattern with proportionally more plasma Cd in a low-molecular-weight form (probably mainly bound to metallothionein [MT]) when low doses of Cd are given through the oral route compared with that when large doses are given through injection. There is also a time dependence of plasma binding, with a larger proportion of plasma-Cd being bound to low-molecular-weight plasma proteins at longer time intervals after a single administration.

Cadmium bound to albumin is to a large extent taken up by the liver, where the complex is split, and Cd can cause toxicity to liver cells (at relatively high doses, particularly after injection). Cadmium also induces the

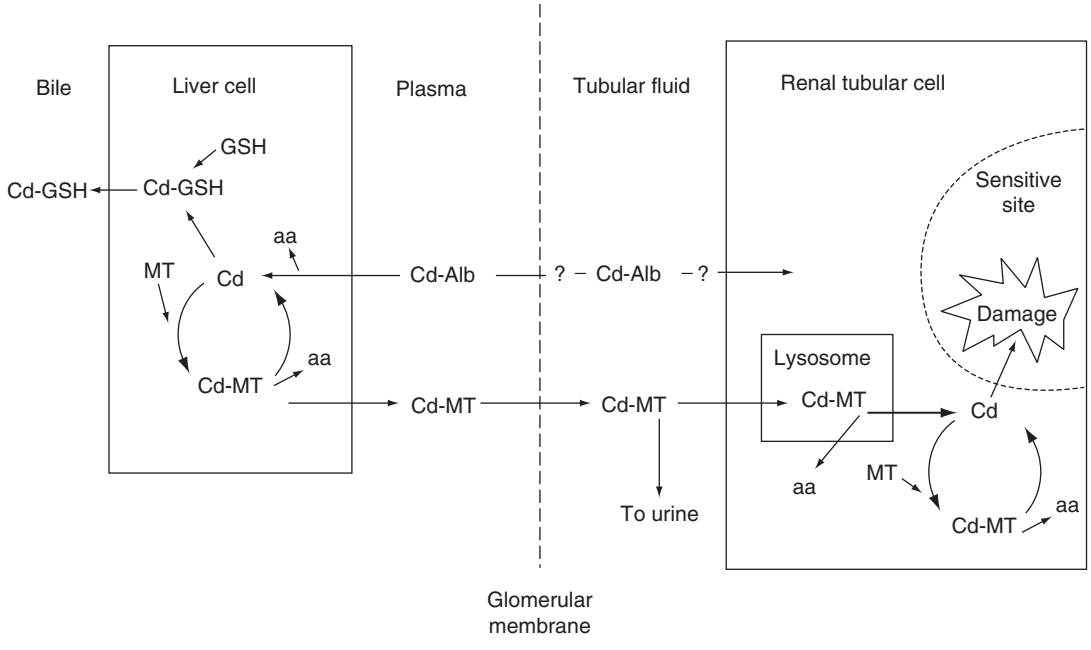

FIGURE 2 Basic flow scheme of cadmium in the body demonstrating the role of binding forms in blood and metallothionein synthesis and degradation. GSH: glutathione; MT: metallothionein; aa: amino acids; Alb:albumin (Modified from Nordberg, G., 1984).

synthesis of metallothionein in liver cells and gradually an increasing proportion of liver-Cd is bound to MT. In the early phase after a single administration of Cd (particularly if injected), plasma-Cd is mainly bound to albumin, and uptake of Cd by the kidneys is limited.

In studies by Nordberg *et al*. (1977), Cd was demonstrated to be excreted in bile mainly bound to glutathione (GSH). Biliary excretion of Cd was also studied (Sugawara *et al*., 1996) in mutant Eisai hyperbilirubinuric (EHB) rats with almost no biliary excretion of glutathione and in normal Sprague–Dawley (SD) rats. Biliary excretion of Cd in EHB rats was one-fortieth of that in SD rats. This finding supports previous conclusions that Cd in bile is bound to glutathione (cf. Nordberg, 1996b; Nordberg and Nordberg, 2000; Nordberg *et al*., 1985b).

A long time after a single exposure, or during long-term exposure, a considerable proportion of plasma-Cd is bound to MT. Because of its small molecular size, CdMT (Nordberg, M., 1978) is efficiently filtered through the glomerular membrane in the kidneys and taken up by renal tubular cells. Uptake of CdMT may be more efficient in cells preexposed to Cd relative to that of non-pre-Cd-exposed cells (Jin *et al*., 1986). In long-term exposure, there is a slow release of CdMT from the liver to the blood. This transport phenomenon has gained more support by studies where Cd-containing livers were transplanted to non-Cd-exposed animals, which demonstrated a gradual uptake of Cd in the kidney (Chan *et al*., 1993) and a lower Cd accumulation in the kidneys of MT-null mice (Liu *et al*., 1996). Uptake of CdMT into renal tubular cells occurs through adsorptive endocytosis at the same site where other low-molecular-weight proteins are reabsorbed (Bernard *et al*., 1987); in rats, it was demonstrated that CdMT and B2M could mutually inhibit tubular reabsorption. After entry into the tubular cells, MT is catabolized in lysosomes, releasing Cd ions (Fowler and Nordberg, 1978, Squibb and Fowler, 1984). Metallothionein binding in plasma and tissues is, therefore, of considerable importance for Cd distribution after uptake.

Similar mechanisms are likely to be the cause of the decreasing proportion of Cd body burden found in the kidneys when the daily exposure level increases (Nordberg, 1972; Nordberg, *et al*., 1985b).

5.2.2 Distribution

A number of animal studies that used autoradiography and analysis of individual organs have indicated that Cd is distributed to many of the organs in the body. In chronic exposure experiments, the greatest amounts of Cd were found in the liver and the kidneys (Nordberg, *et al*., 1985b; WHO/IPCS, 1992). In some experiments, approximately 75% of the body burden was found in these organs. The liver and the kidneys also have the highest concentrations of Cd. The distribution of Cd to the kidneys is of particular importance because the kidney is the critical organ after long-term exposure (see Section 7.2).

Single-exposure experiments (Gunn and Gould, 1957) have demonstrated that, initially, a very high proportion of the dose will be found in the liver and that, with time, a redistribution occurs from the liver to other tissues, particularly the kidneys. This phenomenon is probably due to an efficient MT synthesis in the liver; Cd bound to MT may subsequently be released into the plasma, filtered through the renal glomeruli, and reabsorbed in the tubuli as indicated in Figure 2. This process is of significance for humans in high-exposure situations. Even if the daily exposure ceases, the renal Cd concentration may be maintained for a long time, or it may even increase through transfer of Cd stored in the liver.

In humans subjected to normal low-level exposures, approximately 50% of the body burden at autopsy is found in the kidneys, approximately 15% in the liver, and approximately 20% in the muscles (Kjellstrom, 1979). The proportion in the kidney decreases as the liver Cd concentration increases (Nordberg, G. *et al*., 1985b) in accordance with the findings from animal studies. The Cd concentration in most tissues increases with age. This accumulation in the kidneys continues until the ages from 40–65 (Figure 3), probably depending on the biological half-life and the history of Cd exposure from the general environment (cf. Section 5.5). Accumulation in the muscles continues throughout the lifetime (Nordberg, G. *et al*., 1985b; WHO/IPCS, 1992). In the kidney, the highest concentration of Cd is found in the cortex (Livingston, 1972). The differences in the average renal-cortex Cd concentrations between populations in various countries (Figure 3) are associated with differences in their daily Cd intake from food (Section 4.1.1). In addition, smoking increases the Cd concentration regardless of the differences in food intake. Data in agreement with these observations have been reviewed by Jarup *et al*. (1998c). Recent data confirm these observations, but somewhat lower values for the Cd content in the kidneys of autopsy cases have been reported. In Canada, Benedetti *et al*. (1999) reported a mean concentration in the whole kidney of 29.5 mg/kg among persons 50–59 years of age. In Sweden, Friis *et al*. (1998) reported a geometric mean concentration in the kidney cortex lower that 14 mg/kg at ages of approximately 50 years. Nilsson *et al*. (2000) reported mean kidney cortex Cd levels of 9–18 mg/kg in nonsmoking Swedish farmers. In the UK, Lyon *et al*. (1999) reported a maximum mean kidney cortex

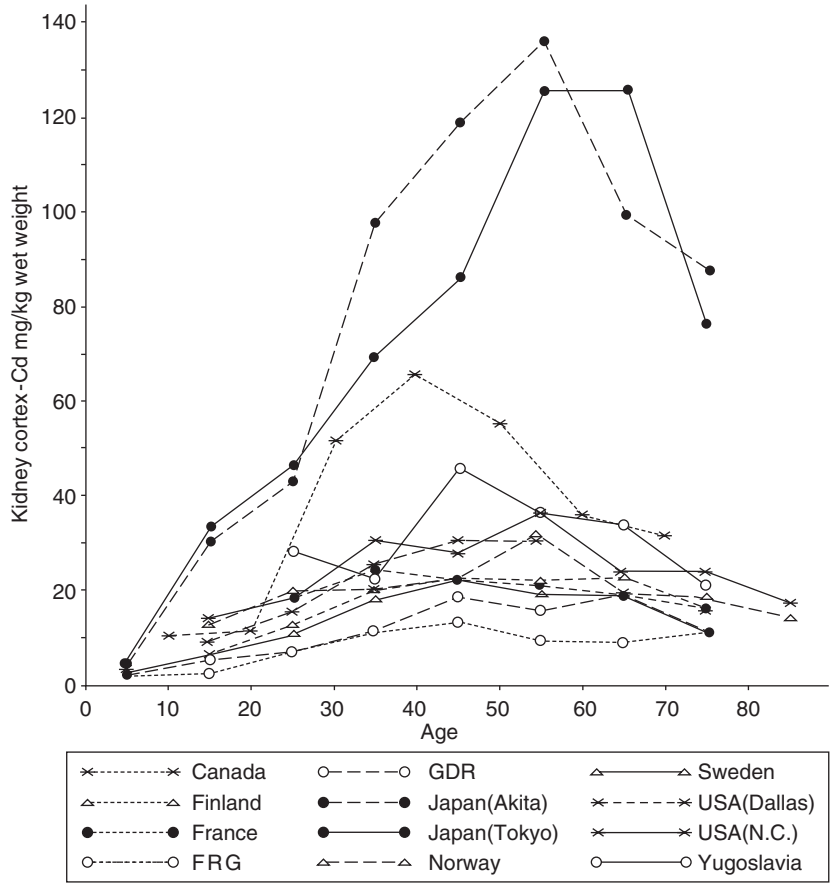

FIGURE 3 Average Cd concentrations in kidney cortex in relation to age. Results from 1970s and 1980s in ten countries are summarized from 12 different studies. The averages are based on smokers, non-smokers, males and females combined. For some studies kidney cortex concentrations in wet weight have been calculated from ash or dry weight or from whole kidney values (Elinder, 1985b).

concentration in middle-aged subjects of 23 mg/kg; 3.9% of the 2700 samples had values >50 mg/kg. In Poland, Hac *et al*. (1998) reported a maximum mean concentration in the kidney cortex of 47.9 mg/kg at ages of 41–50 years. In Australia, Satarug *et al*. (2002) reported a value of 15.5 mg/kg in the kidney cortex.

Although Cd can pass over to the embryo/fetus in animals early in gestation (Dencker, 1975), the transport of Cd across the fully developed placenta is normally low (Sonawane *et al*., 1975), and reported systemic effects of Cd on the embryo and fetus (see Section 7.2.8) may be associated with vascular changes in the placenta itself (Levin and Miller, 1981) and with changes in the fetal uptake of essential elements such as copper and zinc (Bakka *et al*., 1981). In humans, lower blood Cd levels (Lagerkvist *et al*., 1993) were found in umbilical cord blood than in the mother's blood.

When exposure is excessive, as in some industries and in several contaminated areas in Japan and China, liver concentrations of Cd may be up to 100 times greater than found normally. In many cases, kidney concentrations are also very high, but when kidney damage has occurred, Cd excretion increases considerably. This phenomenon explains the autopsy findings from the most severely poisoned workers and in *Itai-Itai* patients. In contrast to their liver levels, their kidney levels were low, often not higher than those in normal humans (Friberg *et al*., 1974; Nordberg, G., *et al*., 1985b; WHO/IPCS, 1992). *In vivo* NAA studies

(see Section 2) have confirmed the disproportionately low kidney levels in workers exhibiting severe renal dysfunction (Ellis *et al*., 1981; Roels *et al*., 1981).

5.3 Excretion

The considerable age-related accumulation of Cd in the body (Figure 3) indicates that only a small portion of the Cd absorbed from long-term low-level exposure will be excreted. The daily excretion that takes place through the feces and urine comprises only approximately 0.01–0.02% of the total body burden of Cd in human beings (Nordberg, G., *et al*., 1985b). The excretion through urine increases with age on a group basis and is proportional to the body burden of Cd (Figure 4); this Figure provides data from Sweden as an example. Reports from other countries (Kjellstrom, 1979; Nordberg, G., *et al*., 1985b; WHO/IPCS, 1992) support these findings. Individual variation is large. The dramatic increase in Cd excretion that occurs when renal damage appears was first demonstrated in rabbits by Friberg (1952); it has since been confirmed in several species of animals (Nordberg, G., *et al*., 1985b). As indicated in Figure 4, the total fecal output of Cd is not age-related in the same way as is that of Cd in urine. Because of the large proportion (90–95%) of ingested Cd being unabsorbed (Section 5.1.2), total fecal Cd is a close indicator of ingested Cd; its value is approximately 50 times greater than the daily urinary excretion (Fig. 4). Animal studies (Nordberg, G., *et al*., 1985b) have demonstrated that the true excretion of Cd in feces is dose-dependent; at low or moderate doses, it is about the same as the urinary excretion. True fecal excretion is partly proportional to the body burden, particularly at low doses. Fecal excretion arises mainly from the intestinal mucosa; only a smaller portion originates from the bile and pancreatic fluid (Nordberg, G., *et al*., 1985b).

5.4 Biological Half-Life

The low excretion rates of Cd lead to its very efficient retention in the body. The half-lives in mice and rats are approximately 200–700 days (Nordberg, G., *et al*., 1985b); in squirrel monkeys, it is longer than 2 years. The retention functions for Cd are multiphasic; the half-life of the slowest component is usually more than 20% of the life span of the animal (Nordberg, G., *et al*., 1985b). The different components are likely to reflect retention in different tissues. In humans, the longest half-lives have been reported for muscles (Kjellstrom, 1979), the kidney cortex, and the liver (Friberg *et al*., 1974); the half-lives in these tissues are 10–30 years, or 15–40% of the human life span (Nordberg, G., *et al*., 1985b). In blood the biological half-lives in humans are 100 days for the fast component and 7–16 years for the slow component (Jarup *et al*., 1983); see Figure 5.

Because of these long half-lives and the transfer of Cd by MT (Section 5.2.1) from other tissues to the kidney, after long-term low-level exposure (e.g., from natural levels of

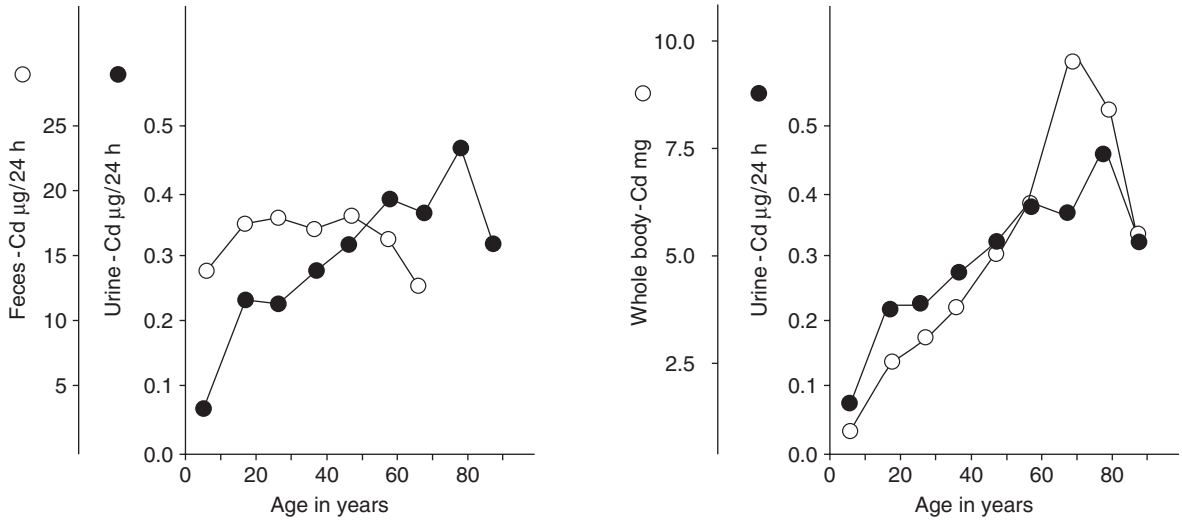

FIGURE 4 Estimated average daily cadmium excretion in urine for non-smoking Swedish men in different age groups (Elinder et al., 1978) compared with average daily amount of cadmium in feces, indicating daily intake (Kjellström et al., 1978), and estimated average body burden (Modified from Kjellström, 1977).

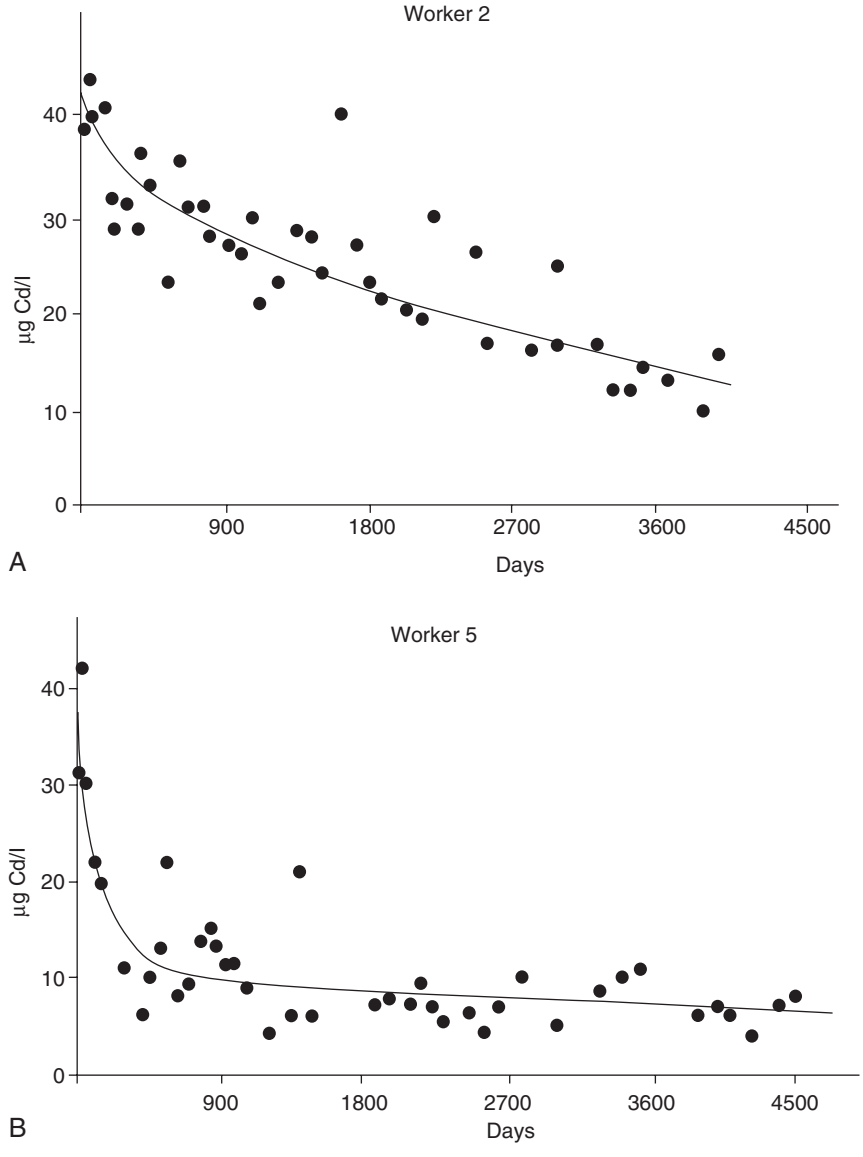

FIGURE 5 Decrease in blood cadmium in two workers after cessation of exposure (1 µg Cd/l=8.9 nmol Cd/l) (From Järup *et al.*, 1983).

Cd in the environment) accumulation in the kidney will occur during the major part of the human life span. This phenomenon is in accordance with observations of the Cd contents in human kidneys in Figure 3.

5.5 Mathematical Models for Cd Accumulation in Renal Cortex and Other Tissues

As mentioned previously, the kidney is the critical organ (see also Section 7). Of the total body burden in a normal human being, approximately one third to one half of the Cd accumulation will occur in the kidneys, with the concentration in the kidney cortex being 1.25 times that of the whole kidney (Nordberg, G., *et al.*, 1985b; Svartengren *et al.*, 1986). By use of equations describing the accumulation, the concentration in the kidney cortex after different types of exposure can be calculated.

Kidney weight and Cd intake vary with age. These features influence the shape of the accumulation curves when the kidney cortex concentrations in various age

groups are calculated (Friberg *et al*., 1974; Kjellstrom and Nordberg, 1978). The models for such changes were amended by Choudhury *et al*. (2001).

One-compartment models of Cd metabolism in humans have been used as a basis for such calculations (Kjellstrom, 1971; Tsuchiya and Sugita, 1971; WHO/IPCS 1992). By comparing calculated accumulation curves with empirical data, it was concluded from the one-compartment models that the whole-body (and renal cortex) half-life in humans was at least 20 years. A more elaborate eight-compartment physiologically based toxicokinetics (PBTK) model has been developed (Kjellstrom and Nordberg, 1978; Nordberg and Kjellstrom, 1979); it has also been used by Thun *et al*. (1991) for quantitative risk assessment.

The PBTK model takes into account the transfer between the muscles, liver, and kidneys; the best fit of the empirical data was achieved with shorter (8–14 years) half-lives for each compartment (Kjellstrom and Nordberg, 1978). The multicompartment PBTK model as amended by Choudhury *et al*. (2001), which is also described in Chapter 3, provides good agreement between the Cd levels in urine generated by the model and the urine Cd levels measured in a sample of the population in the United States.

For long-term low-level exposure, toxicity evaluations that use the multicompartment PBTK model do not differ substantially from evaluations performed using the one-compartment model. The latter was used in the risk assessment undertaken by the WHO/IPCS (1992). The value of the multicompartment model lies in the possibility of using it to calculate Cd concentrations in several tissues, including blood and urine, after both short-term and long-term exposure (cf. Chapter 3).

6 BIOLOGICAL MONITORING

6.1 Biomarkers of Exposure

Chapter 4 describes the current terminology. Because the biomarkers of exposure include biomarkers of the external and internal doses and the accumulation in critical organs, the utility of using the Cd content in blood and urine to indicate these three dose measurements are discussed sequentially under each heading, immediately after an account of references values for people in the general population who have not been subjected to excessive exposure.

6.1.1 Cd in Blood

Cadmium in blood occurs mainly in the blood cells. Although the binding of Cd in plasma has been studied in animals by the use of radioactive isotopes (cf. Section 5.2), the low levels occurring in humans have not made it possible to perform similar studies by use of chemical analyses. All studies referred to in the following text refer to levels of Cd in whole blood.

In blood, "normal" or reference levels in nonsmokers are below 1 µg/L in most countries, whereas considerably higher values, up to 7.6 µg/L, have been found in heavy smokers with the same intake from food (Friberg and Vahter, 1983; Stoeppler and Brandt, 1978; Ulander and Axelson, 1974). There are great discrepancies in the published data on "normal" or reference values of Cd in blood, but data with appropriate quality control from most countries agree with the levels presented previously (Elinder, 1985b; Friberg *et al*., 1974; Jarup *et al*., 1998c; WHO, 1979). Reference values for nonsmokers in the general population in Japan in the 1990s are higher (i.e., approximately 2 µg/L (Watanabe *et al*., 2000). Although, the relationship to age is less prominent for blood-Cd than for urine-Cd, generally somewhat higher levels of Cd in blood are found among older persons. In Sweden, nonsmoking men and women with a mean age of 87 years (Nordberg *et al*., 2000) had Cd concentrations in their blood of 3.9 nmol/L (0.43 µg/L). Previous smokers and current smokers had levels of 4.4 nmol/L (0.49 µg/L) and 7.5 nmol/L (0.83 µg/L), respectively. In a study (Akesson *et al*., 2005) of women aged 50–59 years in Sweden, nonsmokers had a mean blood-Cd level of 0.30 µg/L. Among 11-year-old children in Sweden (Lagerkvist and Lundstrom, 2004), lower values have been reported: a geometric mean of 0.8 nmol/L (0.09 µg/L). Somewhat higher levels have been reported in other countries. Children 7–8 years of age living near a nonferrous metal smelter in Poland had median levels of Cd in their blood of 0.5 µg/L, with a range of 0.3–0.8 µg/L, and pregnant women had a level of 0.7 µg/L, with a range of 0.4–1.3 µg/L (Osman *et al*., 1992). Among 8–9-year-old children living in an industrialized area of Poland, the median blood Cd level was 4 nmol Cd/L, with a range of 2–22 nmol/L (Osman *et al*., 1994).

Blood Cd can be used as an indicator of exposure levels, but the alteration after a sudden change of exposure is not as rapid as the alteration of fecal Cd after a change in daily ingestion. A study of blood Cd in newly used workers in a cadmium-battery factory (Kjellstrom and Nordberg, 1978) showed that during the first months of exposure (50 µg Cd/m^3 air), the blood Cd increased to levels many times higher than the initial level; similar findings were reported by Lauwerys *et al*. (1979): the half-life was approximately 2.5 months. The workers were studied during their first year of exposure; after the fourth month of exposure,

a very limited increase in blood Cd was observed (i.e., a steady state was reached). During the entire year of exposure, there was no apparent increase in the levels of urine Cd. Workers who were no longer exposed to Cd displayed a biphasic reduction in their levels of blood Cd (Figure 5). The half-life in the fast component was approximately 100 days on average; in the slow component it was 7–16 years. Most blood Cd is accumulated in the cells (see Section 5.2.1). The "fast" component may, therefore, merely reflect the turnover of cells in the blood, which is slow compared with the sudden changes in daily exposure that may occur.

The differences in average blood cadmium between smokers and nonsmokers (Jarup *et al*., 1998c, and data cited previously) may reflect differences in their daily Cd exposure, but, because of the effect of the slow component in blood, it may also be interpreted as reflecting differences in Cd body burden (see Section 5.2.2).

The Cd concentration in blood may be a useful indicator of the degree of exposure in recent months. After long-term Cd exposure, however, an increasing proportion of blood Cd will be related to the body burden, and blood Cd is a good indicator of internal dose and accumulation in the kidney in long-term orally exposed population groups (Nordberg *et al*., 2002). These considerations are in concordance with the general understanding of Cd kinetics and the toxicokinetic multicompartment model for Cd (see Section 5.5 and Chapter 3). Because of the "fast" compartment of blood Cd (Jarup *et al*., 1983; Figure 5), high blood Cd values may be found before critical levels are reached in the kidneys. The level of blood Cd is used for occupational biomonitoring (e.g., in Sweden, a level of 50 nmol/L [5.5 µg/L]) is used as an action level.

6.1.2 Cd in Urine

Urine levels of Cd often are adjusted for variations in volume that occur as a result of variable fluid intake. The most common adjustments are for creatinine or specific gravity, the latter usually to be preferred to Cd, as discussed in Chapter 4 and by Suwazono *et al*. (2005). The following discussion will nevertheless focus on creatinine-adjusted values, because such values are those available in published literature.

In urine, "normal" or "reference" levels will also vary with age, area, and smoking habits, but they are generally <1 µg/g CR (or 1 µg/L adjusted to specific gravity 1.024), although levels of 1–4 µg/g CR have been reported from some nonpolluted areas in Japan (Ezaki *et al*., 2003; Friberg *et al*., 1974; Suwazono *et al*., 2000; Watanabe *et al*., 2000). Figure 4 presents the variation with age in Sweden.

The Cd content in urine increases with the body burden of Cd and also after renal damage has occurred (see Section 5.3) and is usually not related to recent exposure (see Section 5.3).

After very high exposures, the Cd concentrations in urine may be high, even for short-term exposure (Piscator, 1974). Presumably, this phenomenon can be explained by considering "overloading" of the tubular reabsorption of MT (see Section 5.2.1). Such high exposures are very rare and are of only limited practical interest at present.

Among people who have not been subjected to occupational exposure (Elinder *et al*., 1978), there is no direct relationship between the average age-related changes in daily Cd intake (as indicated by fecal Cd elimination) and daily urinary excretion (Figure 4), but there is a continuous increase in urinary Cd with age. It was demonstrated a long time ago (Nordberg, G., 1972) from animal experiments that during the early phase of exposure, before renal tubular impairment has occurred, a correlation exists on a group basis between the body burden and the urinary concentration of Cd. Comparisons of group-average urinary Cd excretion in humans with group-average tissue amounts (Kjellstrom, 1977) indicated good agreement between the levels of urinary and kidney Cd, fair agreement between the levels of urinary and liver or whole-body Cd, and no agreement between the level of urinary Cd and the daily Cd intake (Figure 4). These observations have been confirmed in many more recent epidemiological studies; urinary Cd is presently widely accepted as an indicator of body burden and kidney accumulation of Cd (Jarup *et al*., 1998c; Jin *et al*., 2004; Kido *et al*., 2004; Nordberg *et al*., 2002).

Estimated relationships in the general population between the levels of urinary and kidney cortex Cd and the risk for developing tubular dysfunction are given in Table 5 and in Section 8.

6.1.3 Cadmium in Placenta

Cadmium accumulates in the human placenta, and placental samples can be used as an indicator of Cd exposure during pregnancy. Miller *et al*. (1988) reviewed the data available for the levels of Cd in human placenta. Mean levels in various countries ranged from 8–176 ng/g wet weight, with higher values observed in smokers than in nonsmokers. Lagerkvist *et al*. (1996) found levels of 2.6, 3.6, and 5.0 ng/g wet weight in Swedish nonsmokers, ex-smokers, and smokers, respectively. Placental levels were four times higher than those in the maternal blood. Moberg-Wing *et al*. (1992) reported values of 20 and 36 ng/g dry weight in nonsmokers and smokers, respectively. Ronco *et al*. (2005) reported levels of 20 and 60 ng/g dry weight in nonsmokers and smokers, respectively, in Chile.

TABLE 5 "Best Guess" of Prevalences of Tubular Effects in the General Population in Different Intervals of Cadmium in Kidney Cortex, Based on All Available Data Published Until Mid-1997 (From Jarup *et al.*, 1998c)

Cadmium in kidney cortex (mg/kg)	U-Cd (μg/g crea)	Percentage effect
<50	<2.5	0
51–60	2.75	1
61–70	3.25	2
71–80	3.75	3
81–90	4.25	4
91–100	4.75	5
101–110	5.25	6
111–120	5.75	8
121–130	6.25	10
131–140	6.75	12
141–150	7.25	14
151–160	7.75	17
161–170	8.25	20
171–180	8.75	23
181–190	9.25	26
191–200	9.75	30
>200	>10.25	>35

The levels just cited are all from nonoccupationally exposed populations. In a study of female smelter workers in the UK, Berlin *et al*. (1992) found a value of 21 ng/g wet weight in placental samples.

6.1.4 Cd in Hair, Feces, and Other Biological Materials

For people exposed to Cd almost exclusively from food, the average daily Cd content in feces is a good indicator of the daily intake, because the major part of the ingested Cd passes through the gastrointestinal tract unabsorbed and reaches the feces (Section 5.1.2); true fecal excretion is only a fraction of the daily intake (see Section 5.3).

The average daily fecal Cd (Figure 4) varies with age in a manner similar to the average daily energy intake (Kjellstrom *et al*., 1978). After a single oral exposure to Cd-polluted rice, almost 100% of the Cd was eliminated in the feces within 3 days (Kjellstrom *et al*., 1978). Fecal Cd has been used in several studies to measure the average daily intake from food in Cd-polluted areas (for a review, see: Elinder, 1985a) and in populations exposed to background levels of Cd (for a review, see Jarup *et al*., 1998c).

Cadmium in hair may be used as an indicator of exposure and of the internal dose in oral Cd-exposure (Nordberg and Nordberg, 1988). Because Cd levels in hair are fairly low, there is a risk of external contamination; thus, Cd in hair has not been used significantly for biological monitoring.

6.1.5 Cd in Kidney and Liver, Measured In Vivo, *Body Burden*

Newborn babies are almost free from Cd; the total body burden is only approximately 1 μg (Henke *et al*., 1970). A man who is 50 years of age in the United States, Sweden, or Germany will have a total body burden of Cd between 10 and 30 mg. These values correspond to concentrations in the liver of approximately 1–3 mg/kg weight and in the kidney cortex of 15–50 mg/kg (corresponding to approximately 10–30 mg/kg calculated for a whole kidney). Higher values are found among smokers than among nonsmokers.

"Normal" or "reference" values in Japan are usually higher than in other countries (Figure 3). Present-day values are somewhat lower than those displayed in Figure 3, because daily intake of Cd has decreased in Japan during the past two decades as a result of decreased rice consumption.

In vivo NAA or *in vivo* X-ray fluorescence measurements of liver and kidney Cd has made it possible to measure the correlation between the levels of Cd in the indicator media and in the critical organ, the kidney cortex. Roels *et al*. (1981) found no increase in the blood Cd with increased body burden. The generally high blood levels of Cd (5–30 μg/L) among the workers studied reflected their high daily exposures. The levels of urinary Cd, on the other hand, correlated with the body burden in workers not exhibiting renal damage (Figure 6). Ellis *et al*. (1984) calculated the relationship between the Cd levels in the kidney or liver measured with *in vivo* NAA and the prevalence of renal tubular dysfunction (see also Section 8). Other studies have not succeeded in establishing these relationships because of the lower levels measured and the related larger proportion of values falling below the detection limit (for a review, see Jarup *et al*., 1998c).

6.2 Biomarkers of Effects

Biomarkers of kidney effects of Cd are well established. For glomerular kidney damage, useful biomarkers are those of the glomerular filtration rate (GFR) and of albumin in urine (UAlb). For the tubular kidney effects, crude indicators are glucosuria and aminoaciduria. More sensitive indicators are the urinary excretion of RBP, B2M, ProtHC (a_1-microglobulin), NAG and its isoenzymes A and B, as well as CC16. Each protein indicates quite specifically where in the tubule the effect occurs. Metallothionein can also serve as a biomarker of the effect; Table 6 lists the concentration values of MT. Section 8.2 of this chapter describes the dose-response relationships for these effect biomarkers.

As a biomarker of bone effects (i.e., in terms of osteomalacia and osteoporosis) bone mineral density

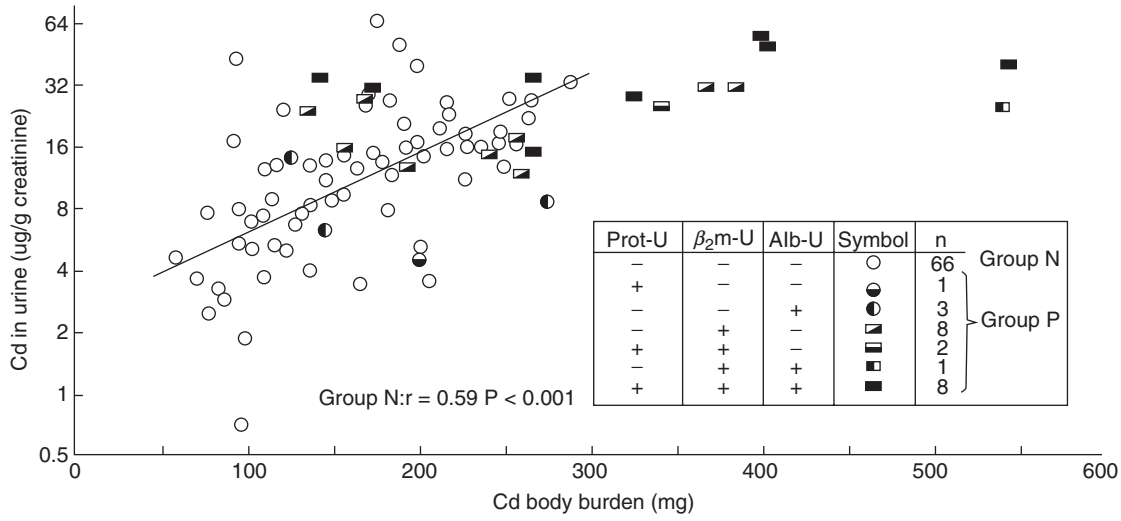

FIGURE 6 Urinary cadium level as a function of estimated cadmium body-burden for cadmium workers (active, retired, removed from cadmium exposure) with liver and renal-cortical cadmium above the detection limits (CdL ≥ 10 p.p.m., CdKc ≥ 50 p.p.m.). Regression line for subjects without renal dysfunction: o—o, group N. The individual values of the subjects with renal dysfunction(s) (Group P, n = 23) are plotted with different symbols to distinguish between those with increased proteinuria (Prot-U) β_2-microglobulinuria (β_2 m-U) or albuminuria (Alb-U), or a combination of these (From Roels *et al*., 1982).

TABLE 6 Tissue Levels of Metallothionein in Humans and Rats

Method	Media	Concentration ng/mL	Status	Reference
RIA	Sera (human)	0.01–1	Normal*	Nordberg *et al*. (1982)
RIA	Sera (human)	>2	Abnormal*	Nordberg *et al*. (1982)
RIA	Urine (human)	1–10	Normal*	Nordberg *et al*. (1982)
RIA	Urine (human)	>10	Abnormal*	Nordberg *et al*. (1982)
RIA	Urine (human)	1880 µg/g CR	Itai-Itai patients	Tohyama *et al*. (1981)
		880 µg/g CR	Cd-polluted area	
		394 µg/g CR	Nonpolluted areas	
ELISA	Liver (rat)	18 µg/g		Chan *et al*. (1993)
ELISA	Kidney (rat)	30 µg/g		Chan *et al*. (1993)
ELISA	Kidney (rat)	35 µg/g		Liu *et al*. (1994)
	Urine (human)	120 and 210 µg/g CR	Normal* Abnormal*	Shaikh *et al*. (1987)
		320 and 1050 CR	Abnormal*	

*Occupational exposure.
Abnormal indicates presence of low mol weight proteinuria.
Source: modified from Nordberg and Nordberg (2002).

(BMD) is usually measured; serum calcium and serum alkaline phosphatase measurements are also classical indicators of osteomalacia (cf. Section 7.2.7).

7 EFFECTS AND DOSE-RESPONSE RELATIONSHIP

Cadmium may exert both acute and chronic influences on human health. In recent years, the former has become rare, whereas the latter has attracted increasing attention. In this section, we focus mainly on the chronic influences of Cd. To help explain the mechanisms underlying the effects of Cd on human health, we refer to the results of animal experiments and *in vitro* studies, when indicated.

7.1 Acute Poisoning

7.1.1 Inhalation

Acute poisoning has occasionally occurred in the past mainly because of inhalation of fumes engendered at the time of dissolving or breakage of substances containing Cd or when soldering with silver-cadmium solder. The first case was reported by Sovet (1858). The initial symptoms that develop 24 hours after exposure include irritation, dryness of the nose and throat, coughing, headaches, chills, fevers, and chest pains. These symptoms strongly resemble those of metal fume fever. When Cd causes these symptoms, serious adverse effects may develop; in contrast, acute symptoms without serious adverse effects are usually caused on exposure to Zn. In severe cases, there is a progression to pulmonary edema and chemical pneumonitis, sometimes culminating in death. The prognosis is usually favorable in cases of recovery, although lethal cases resulting from pulmonary edema and acute tubular necrosis have occurred (Beton *et al.*, 1966). Cases progressing to pulmonary fibrosis after 17 years have also been described (Townshend, 1982). Hitherto, more than 100 cases of acute poisoning, including at least 17 fatal cases, have been reported. The dose-response relationship of the occupational environment where acute poisoning has occurred has yet to be clarified. In estimates from such cases, the lethal dose of cadmium oxide during an 8-hour exposure was surmised to be 5 mg/m^3, and the lethal dose has been estimated in general terms to be 2500 minutes × mg/m^3. Approximately 1 mg/m^3 inhaled over the same period will lead to clinically evident symptoms in sensitive subjects (Elinder, 1986).

7.1.2 Ingestion

Sovet (1858) described the acute gastrointestinal symptoms of Cd after ingestion of cadmium carbonate dust. Acute symptoms may also occur if Cd-containing cooking utensils are used. This situation is facilitated by the dissolution of Cd by acidic juices or foodstuffs or when Cd leaches into water from sources such as solders in water pipes, refrigerators, and pottery. Symptoms result when the Cd concentration in drinks exceeds 15 mg/L (Nordberg *et al.*, 1973). Cadmium ions in gastric juice strongly stimulate the gastrointestinal mucosa, provoking nausea, vomiting, abdominal pain, and diarrhea within a few minutes of ingestion, followed by the development of shock in some severe cases. Thanks to the vomiting of the gastric contents and the low absorption rate of Cd, recovery is, however, rapid and fatalities usually do not occur. Cadmium concentrations in water of approximately 15 mg/L induce vomiting. In the case of protein-containing foods, higher concentrations seem to be necessary to induce vomiting.

7.2 Chronic Poisoning

7.2.1 General Aspects

Although acute Cd poisoning is rare at present, chronic poisoning occurs frequently under conditions when there is inadequate protection in industries handling Cd-containing material and in situations where environmental contamination causes elevated levels of Cd in food. The general clinical picture of chronic Cd poisoning was first investigated by Friberg (1950) in a group of 58 Swedish Cd workers. Shortness of breath, pulmonary dysfunction associated with an increase in residual volume, and renal tubular dysfunction associated with low-molecular-weight proteinuria were described. With airborne exposure, pulmonary emphysema and renal dysfunction, as well as rare bone injury, were noted; with oral exposure, renal dysfunction and in some cases bone injury were observed. The renal injury is characterized initially by proximal tubular dysfunction, and then complicated by glomerular dysfunction with increasingly severe disease. The bone injury is characterized by osteomalacia and osteoporosis, which in its well-recognized severest form has been manifested in Japan as *Itai-Itai* disease. *Itai-Itai* disease patients also present severe anemia. Pulmonary and renal injury induced by Cd exposure adversely affects life prognosis (see Section 9).

7.2.2 Pulmonary Disorders

In the upper airway, chronic inflammation of the nose, pharynx, and larynx, as well as olfactory disturbances, are observed. In the lower airway, chronic obstructive lung disease of varying severity

is found. In reports subsequent to that of Friberg (1950), impaired pulmonary function was also noted in Cd workers (Lauwerys *et al*., 1974; Sakurai *et al*., 1982; Smith *et al*., 1976). In a survey of 16,024 subjects selected from the general American population, a negative correlation was identified between pulmonary function and urinary Cd levels, and Cd exposure was implicated in the exacerbation of pulmonary disorders associated with cigarette smoking (Mannino *et al*., 2004).

7.2.3 Kidney Damage

The critical organ in Cd poisoning is the kidney. After Friberg's report (1950) describing how the proteinuria was found in Cd workers comprised mainly of low-molecular-weight proteins, numerous studies have demonstrated that Cd-induced renal damage is characterized by proximal tubular reabsorptive dysfunction. The earliest manifestations of Cd-induced renal damage are increased urinary excretion of low-molecular-weight proteins, in particular β_2-microglobulin (β_2-mg) and α_1-microglobulin (a_1-mg), also called protein HC. The amount of β_2-mg excreted into the urine in subjects with Cd-induced renal damage is proportional to the severity of damage. In severe tubular damage occurring, for example, in cases of *Itai-Itai* disease levels of 100,000 μg/g creatinine may be found. The urinary excretion of the enzymes *N*-acetyl-β-D-glucosamidase (NAG) and lysozyme also increases. Severe Cd-induced renal damage results also in depressed glomerular function, with rises in the levels of serum creatinine and serum β_2-mg, and culminates in uremia in some cases. The amount of β_2-mg excreted into the urine has been found to correlate closely to the creatinine clearance, percent TRP, and the arterial blood bicarbonate; it is recognized as an excellent marker of Cd-induced renal damage (Aoshima *et al*., 1988a). Glomerular dysfunction has also been described as an early influence because of the finding of increased albuminuria after mild Cd exposure (Bernard *et al*., 1976; 1979; Lauwerys *et al*., 1974). In Cd workers, Cd-induced renal injury is irreversible, with progressive deterioration occurring even after cessation of Cd exposure (Elinder *et al*., 1985a; Roels *at al*., 1982; 1989; 1997). In investigations of inhabitants of Cd-polluted areas in Japan, irreversible injury was noted to occur when β_2-mg urinary excretion exceeded 1000 μg/g CR (Cai *et al*., 2001; Iwata *et al*., 1993; Kido *et al*., 1988). Decreased arterial blood pH and elevated serum creatinine have been reported to persist even after cessation of Cd exposure (Kido *et al*., 1990a). Metallothionein, which transports Cd in the blood, has been implicated in the mechanism by which Cd induces tubular damage. When Cd accumulation in the kidney is excessive (Nordberg *et al*., 1975), the level of Cd not bound to MT increases in the renal cells and injures the renal tubules because of its marked toxicity (Figure 2; Nordberg, 1984). Accordingly, the equilibrium between MT-bound and MT-unbound Cd in the kidney is believed to be a determinant of the development of renal damage. When renal damage is manifest, MT excretion in the urine is increased (Nordberg M., 1998, Nordberg and Nordberg, 2000). Approximately 30–40% of the total Cd, as well as total Cu, was found to be bound to MT in the urine of *Itai-Itai* disease patients. It is very common that the levels of urinary MT and Cu (as well as Cd, but not Zn) exhibit a significantly statistical relationship; the same is true for B2-MG (Mitane *et al*., 1986).

That renal injury induced by Cd exposure is an extremely distinctive disorder was proven from a survey of 12,559 inhabitants of a Cd-polluted region and 6435 inhabitants of a non-Cd-polluted region in Japan. In that investigation, suspected proximal tubular dysfunction (based on the finding of one or more of the following items—urinary β_2-mg ≥ 10 mg/L; RBP ≥ 4 mg/L; lysozyme ≥ 2 mg/L—as well as glucose ≥ 100 mg/L and generalized aminoaciduria ≥ 20 mmol/L) was detected in 333 of the inhabitants of the Cd-polluted region, in contrast to only a single inhabitant of the Cd-unpolluted region; in addition, definite proximal tubular dysfunction (%TRP < 80% and arterial blood bicarbonate < 23 mEq/L in the subjects with suspected proximal tubular dysfunction) was detected in 202 of the inhabitants of the Cd-polluted region and in none of the inhabitants of the non-Cd-polluted region (Shigematsu, 1989). The appearance of kidney stones occurs more frequently in Cd workers; this situation has been attributed to the increased urinary calcium excretion associated with tubular dysfunction (Elinder *et al*., 1985b). It has also been suggested that exposure to occupational and relatively low environmental levels of Cd is a determinant for the development of end-stage renal disease (Hellstrom *et al*., 2001).

Epidemiological evidence is available on an increased susceptibility of diabetics to the development of Cd-induced renal dysfunction on Cd exposure (Akesson *et al*., 2005; Buchet *et al*., 1990). Recent epidemiological studies have demonstrated an increased prevalence of tubular dysfunction related to Cd levels in the urine of type II diabetics. Persons exhibiting increased levels of serum antibodies against MT had increased risks of tubular proteinuria at similar levels of Cd in their urine (Chen *et al*., 2006c). Data from animal experiments support the possibility of an increased susceptibility for Cd nephropathy in diabetics. Streptozotocin (STZ)-induced diabetes in animals is similar to insulin-dependent diabetes or type I diabetes in humans. An increased resistance to CdMT nephrotoxicity was

demonstrated in experiments performed on STZ diabetic rats during short time intervals (Jin *et al*., 1996). Increased binding of Cd to MT occurred in the liver of the STZ-injected animals 24 hours after the injection of CdMT and parallel to an increased resistance to nephrotoxicity. Induction of MT by STZ thus protects against nephrotoxicity. In long-term experiments of the effects of Cd in drinking water (Bernard *et al*., 1991; Jin *et al*., 1999), the response of rats having STZ-induced diabetes was compared with that of similar animals without diabetes. Compared with nondiabetic animals, animals with diabetes developed more prominent nephropathy. Another diabetes model is the use of Umeå obob obese mice, which are similar metabolically to type II diabetic humans. It was demonstrated (Jin *et al*., 1994) that there is an increased susceptibility to the development of Cd-MT-induced proteinuria and calciuria in the Umeå obob mice relative to that of normal mice (Jin *et al*., 1994).

Cadmium workers exhibiting increased plasma levels of antibodies against MT developed renal tubular dysfunction at lower levels of urinary Cd than did workers without such increased plasma levels (cf. Section 7.5.1. and Chen *et al*., 2006b). Cadmium exposure is of concern for subjects with thalassemia, because it cannot be ruled out that such persons may be particularly sensitive to the adverse effects of Cd exposure (cf. Section 7.2.4).

7.2.4 Anemia

Slightly decreased hemoglobin concentrations were observed in Cd workers but were found to be reversible changes unrelated to renal injury (Friberg, 1950; Toda *et al*., 1984). However, in *Itai-Itai* disease patients who have the most severe form of Cd poisoning, extremely severe anemia with red blood cell counts of 1,155,000/mm^3 and hemoglobin of 4.2 g/dL (42 g/L)—is found. In a group of 10 *Itai-Itai* disease patients in whom serum iron, ferritin, and erythropoietin levels and renal function were determined, as well as bone marrow aspiration performed, the anemia was noted to be normochromic, with no decreases in the iron or serum ferritin levels and no particular abnormalities noted from smears of bone marrow aspirates. On the other hand, serum erythropoietin concentrations were markedly decreased, the cause of which was ascribed to renal anemia (Horiguchi *et al*., 1994). In patients with *Itai-Itai* disease, normal liver Fe concentrations are found (Nogawa *et al*., 1984). In animal experiments, the administration of Cd has been reported to result in the development of iron-deficiency anemia and renal anemia (Hiratsuka *et al*., 1996).

Subjects with thalassemia and hematochromatosis exhibit disturbed patterns of their hemoglobin. Carriers of the thalassemia gene are found around the Mediterranean (i.e., in populations originating from Cyprus, Egypt, Greece, Italy, Lebanon, Malta, Turkey, and the Middle East) as well as in India and some parts of South East Asia, including Thailand, China, and the Philippines. In areas with intake of foodstuffs that contain Cd, these populations will probably be at increased risk of toxicity from Cd because of the increased risk of hemolysis. No studies, however, have examined the potentially increased Cd susceptibility of persons with these genetically inherited disorders.

7.2.5 Blood Pressure

Since the first report describing that the administration of Cd elevated the blood pressure of rats (Schroeder and Vinton, 1962), numerous animal experiments have been conducted that variously support or dispute this observation. Although mechanisms devised to explain the elevation of blood pressure suggest the involvement of the renin-angiotensin system (Perry and Erlanger, 1973), monoamine oxidase (Revis, 1978), catechol-O-methyltransferase (Revis, 1978), endothelin (Ozdem and Ogutman, 1997; Wada *et al*., 1991), and nitric oxide (Demontis *et al*., 1998), no consensus has yet been reached.

With respect to the relationship between Cd and blood pressure in humans, whereas some reports have described that renal Cd concentrations and Cd/Zn ratios were elevated in hypertensive autopsied cases (Schroeder, 1965) and that blood Cd concentrations of hypertensive subjects exceeded those of normotensive subjects (Glauser *et al*., 1976; Luoma *et al*., 1995), others have reported no differences between the two groups (Beevers *et al*., 1976; Fontana and Boulos, 1986; Staessen *et al*., 1991). No reports have noted the development of hypertension in Cd workers. In a survey of inhabitants of a Cd-polluted region in Japan, a tendency for low blood pressure was found in 471 women with renal injury compared with 2308 women in a control area (Nogawa and Kawano, 1969). Furthermore, hypertension has not been observed in *Itai-Itai* disease patients or in persons requiring observation for such disease (Kagamimori *et al*., 1986; Shinoda *et al*., 1977). These authors noted low systolic and diastolic pressures among *Itai-Itai* disease patients. The Cd concentrations in the blood (Basun *et al*., 1994) of an aged population was found to be related to the diastolic blood pressure in nonsmoking and nondemented individuals. The observed differences in the Cd concentrations in blood were related to smoking habits (Basun *et al*., 1994); thus, the relationship to blood pressure may be explained by considering the effects of smoking. In a follow-up study (Nordberg *et al*., 2000) of 804 subjects, both men and women, aged 77 years and older, with a mean age of 87 years, blood-Cd was analyzed among

763 subjects and its relationships to age, blood pressure, BMI, cognitive function, gender, and smoking were examined. No statistically significant relationships were found between the levels of Cd in the blood and the mentioned recordings or measurements.

In inhabitants of a mildly Cd-polluted district in Belgium, the amount of 24-hour urinary Cd excreted was found to correlate with low blood pressure (Staessen *et al*., 1984). In a large-scale epidemiological survey conducted in the United States, no association was noted between urinary Cd and blood pressure (Whittemore *et al*., 1991). With regard to cardiovascular manifestations, in a group of 52 *Itai-Itai* disease patients and persons requiring observation compared with an age-matched control group, the ECG findings were characterized by a scarcity of ischemic changes and an increasing of the PR/RR ratio (Kagamimori *et al*., 1986). Peripheral arterial disease was associated (not statistically significant) with blood Cd in a cross-sectional study in the United States (Navas-Acien *et al*., 2005).

In summary, human studies consistently indicate decreased blood pressure in persons with severe renal injury, but the effect of mild Cd exposure on blood pressure has yet to be resolved.

7.2.6 Liver Disturbances

Although morphological changes in the liver and fluctuations of enzyme activity have been demonstrated in animal experiments, particularly after high-level, short-term exposure, no reports have clearly described the development of hepatic injury in either Cd workers or inhabitants of Cd-polluted regions.

7.2.7 Effects on Bone

The frontier interest in health effects caused by Cd relates to bone effects. An increasing incidence of osteoporosis is occurring in industrialized countries worldwide. A high number of such cases are smokers, suggesting a link between Cd in tobacco and osteoporosis. Studies in humans who had been exposed occupationally to Cd for almost 20 years have suggested relationships not only between Cd dose and decreased bone mineral density but also between Cd dose and osteoporosis (Jarup *et al*., 1998a). Nordberg *et al*. (2002) and Wang *et al*. (2003) found decreased bone mineral density in Chinese farmers exposed for more than 30 years to Cd from rice.

The influence of Cd on bone matter can be divided into osteomalacia and osteoporosis. The former has been observed in Cd workers and inhabitants of Cd-polluted regions in Japan. Since 1942, a number of cases of bone effects, primarily osteomalacia, have been reported for Cd workers (Table 7): 5 cases in France (Nicaud *et al*., 1942), 10 cases in Poland, 1 case of osteomalacia, and 4 cases of osteoporosis in England, all with proteinuria and calciuria in workers exposed to CdS (Kazantzis, 1979; 2004), and 10 cases in Russia (Kjellstrom, 1986b). In Sweden, kidney stones, calciuria, and proteinuria

TABLE 7 Total Reported Number of Itai-Itai Patients and Cases of Suspected Cadmium Poisoning with Bone Effects

Area	Jinzu River basin	Ichi River basin	Kakehashi River basin	Tsusima Island
Year	Toyama Prefecture	Hyougo Prefecture	Ishikawa Prefecture	Nagasaki Prefecture
1967–1970	124(3)			
1971–1980	7	5	1	1
1981–1990	23		1	8(1)
1991–2000	29			
2001–2004	5			
Total	188(3)	5	2	9(1)
	( ) is a male case			
	Itai-Itai disease is officially recognized only in the Jinzu River basin			
Work environment				
Suspected cadmium poisoning				
1942	6	France		
1955	1	England		
1963	8	France		
1966	26	Poland		
1969–1980	3	England		
1973–1975	10	Soviet Union		
1980	1	France		

have been reported in Cd/Ni battery workers (Friberg, 1950). An increased occurrence of osteoporosis in these workers has been reported by Alfven *et al*. (2000).

In Japan, the highest prevalence of osteomalacia is found in the Cd-polluted Jintsu River basin in Toyama Prefecture, with hitherto 188 cases confirmed and recognized as *Itai-Itai* disease (see Section 7.2.9). Characteristics of the *Itai-Itai* disease are osteomalacia, osteoporosis, renal tubular dysfunction, malabsorption, and anemia (WHO/IPCS, 1992). In addition, two cases of Cd-induced osteomalacia have been reported in the Kakehashi River basin in Ishikawa Prefecture, five in the Ichi River basin in Hyogo Prefecture (Nogawa *et al*., 1975) and nine in Tsushima in the Nagasaki Prefecture (Takebayashi *et al*., 2000). All of these cases manifested tubular dysfunction, with most also exhibiting glomerular dysfunction. In the Cd-polluted Jinzu River basin in the Toyama Prefecture and the Kakehashi River basin in the Ishikawa Prefecture, renal dysfunction and decreased bone density were found to be closely related (Aoshima *et al*., 1988b; Kido *et al*., 1990b), clarifying that, in Japanese Cd-polluted areas, numerous residents sustained the bone effects of Cd.

In a group of 508 inhabitants of mildly to moderately Cd-polluted districts in Belgium, the relationships between forearm bone density, bone fracture incidence, and 24-hour urinary Cd excretion were investigated. In postmenopausal women, proximal and distal bone densities decreased by nearly 0.01 g/cm^2 with a doubling of the 24-hour urinary Cd excretion at baseline (median, 6.6 years earlier). A doubling of the Cd excretion at baseline was associated with a 73% increase in the risk of fractures in women (Staessen *et al*., 1999).

In a survey of 43 Swedish Cd workers, the mineral densities in the bones of the forearm, lumbar spine, and hip, even when adjusted for confounding factors, correlated inversely with the levels of blood Cd and urinary Cd (Jarup *et al*., 1998a). In a survey of 520 men and 544 women exposed to Cd, a dose-response relationship was found between urinary Cd and decreased bone mineral density; it was further clarified that Cd exposure increased the incidence of bone fracture (Alfvén *et al*., 2000; 2004). The mean urinary Cd concentration in this population at the time bone density was determined was 0.74 µg/g CR.

Results from China (Nordberg *et al*., 2002; Wang *et al*., 2003) have indicated bone effects—measured as decreased mineral density in the bone of the forearm and increased prevalence of fractures—at cumulative doses somewhat lower than those giving rise to the classical *Itai-Itai* cases in Japan among residents in a Cd-polluted region. In a survey of 908 women in a non-Cd-polluted region of Japan, calcaneal bone mass displayed a significant negative correlation with the urinary Cd concentration, even after adjusting for factors such as age, body weight, and menstrual status (Honda *et al*., 2003). Although a recent survey of 1380 women in a mildly Cd-polluted region of Japan found no association between bone density and urinary and blood Cd concentrations (Horiguchi *et al*., 2005), numerous other studies have demonstrated that mild Cd exposure is associated with decreased bone density.

The following mechanisms have been proposed for the influence of Cd on bone matter: (1) impairment of vitamin D activity in the kidney, (2) the action of Cd within the digestive tract interfering with the absorption of calcium, and (3) the direct action of Cd on the bone with derangement of collagen metabolism. Figure 7 depicts the different possible points of action of Cd on calcium and vitamin D metabolism; Kjellstrom (1992) has reviewed the animal and *in vitro* evidence concerning these possible points of action.

The 1α25(OH)$_2$D levels measured in five *Itai-Itai* disease patients and 36 inhabitants of a Cd-polluted area manifesting renal injury were significantly lower than those of a group of 17 inhabitants of a non-Cd-polluted area. Moreover, the level of 1α,25(OH)$_2$D correlated negatively with the levels of serum β_2-mg and PTH and positively with the %TRP (Nogawa *et al*., 1987). These associations exhibited a gender difference; it was pronounced in women, but not evident in men (Tsuritani *et al*., 1992). The levels of 1α,25(OH)$_2$ D measured in 21 men and 13 women residing in the Cd-polluted Jintsu River basin of the Toyama Prefecture correlated significantly with creatinine clearance (for both sexes), the fractional excretion of β_2 microglobulin (for women), the serum creatinine (for men), and the %TRP (for men), with the level of 1α,25(OH)$_2$D reported to decrease in parallel with decreasing renal function (Aoshima *et al*., 1993). Although no comparison was performed with a control group, it was emphasized, however, that the magnitude of this decrease remained within the normal range. Chalkley *et al*. (1998) measured the levels of vitamin D metabolites in workers exposed to lead and Cd (blood-Cd: 6-145 nmol/L; urine-Cd: 3-161 nmol/L). Plasma 24R,25(OH)2D levels were depressed below the normal range when the levels of blood and urinary Cd increased. High Cd levels were associated with decreased levels of 1α,25(OH)$_2$D when lead concentrations were also elevated. Accordingly, there is evidence supporting a Cd-related decrease in 1α,25(OH)$_2$D in humans when there is combined exposure to lead and when there is pronounced Cd-induced renal injury. The significance of these findings for the development of bone injury has not, however, been clarified sufficiently. As for the second and third pathways, no evidence is available to support their existence in man. Most animal studies have indicated that Cd may exert

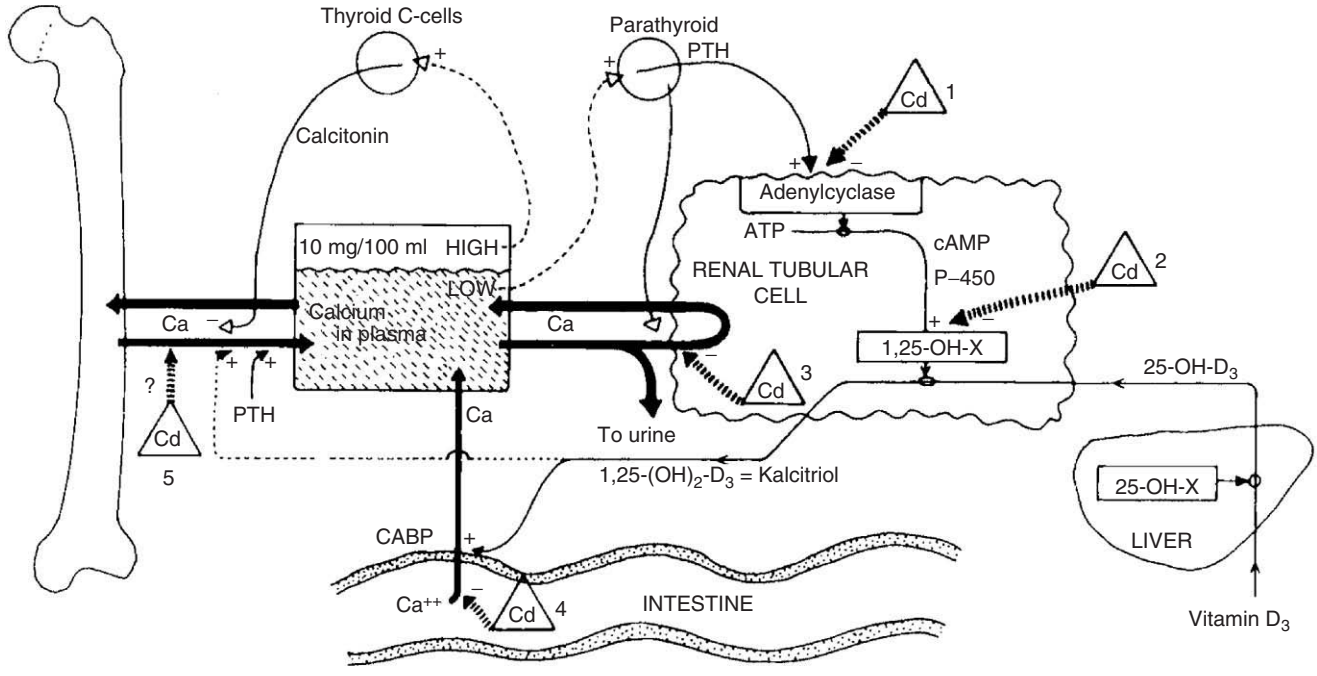

FIGURE 7 Schematic drawing of Vitamin D metabolism and how it may be affected by Cadmium. (1) Cadmium decreases PTH stimulation of adenylcyclase. (2) Cadmium inhibits hydroxylation of 25-OH-D_3. (3) Cadmium increases urinary calcium excretion. (4) Cadmium decreases gastrointestinal calcium absorption. (5) Cadmium affects bone mineralization and bone collagen directly. CABP = calcium binding protein.

a direct effect on bone mineralization, possibly related to calcium deficiency. There are also indications that Cd affects the enzyme lysyloxidase and, indirectly, bone collagen metabolism (Iguchi and Sano, 1982).

In animal experiments, no reports have described the development of severe osteomalacia similar to that found in subjects with *Itai-Itai* disease. When Cd was administered to vitamin D–deficient rats (Nogawa and Kobayashi, 1980) or to ovariectomized rats, increased osteoid formation was detected (Umemura, 2000). There is also a report of extreme demineralization, similar to that seen in *Itai-Itai* disease, evoked by gestation or lactation when a calcium-poor diet containing 5 mg/kg (ppm) or 25 mg/kg (ppm) Cd was administered to mice (Wang *et al*., 1994).

In ovariectomized animals, Bhattacharyya *et al*. (1992) demonstrated increased bone loss in Cd-exposed animals compared with that of non-Cd-exposed ones. This effect occurred before any renal damage was induced through Cd exposure.

7.2.8 Itai-Itai Disease

The most severe form of chronic Cd poisoning caused by prolonged oral Cd ingestion is *Itai-Itai* disease. The clinical picture of *Itai-Itai* disease shows renal injury manifested by tubular and glomerular dysfunction and bone injury consisting of a combination of osteomalacia and osteoporosis. Serum and hematological findings include elevated levels of serum alkaline phosphatase, decreased levels of serum Ca and inorganic P, and moderate-to-severe anemia; urinary findings characteristically include increased excretion of proteins—especially low-molecular-weight proteins (by several thousandfold of normal)—and of glucose and (general) amino acids (Shinoda *et al*., 1977). In patients with *Itai-Itai* disease, kidney histopathological examination reveals no abnormalities in the glomeruli, but atrophy and dilatation of the tubules, as well as marked degeneration of the epithelium; these features are considered to be characteristic effects of Cd poisoning.

The cause has been ascribed to the inflow of effluent from a zinc mine located in the upper reaches of the Jinzu River in the Toyama Prefecture, with the Cd contained therein contaminating the surrounding rice paddies and fields; the inhabitants were then exposed to Cd through their ingestion of rice and other produce grown there. Up to 2004, the number of recognized patients totalled 188 (3 men and 185 women; Table 7), with 256

subjects (46 men and 210 women) requiring observation. Renal tubular injury is highly prevalent among the inhabitants of the Cd-polluted Jinzu River basin, with urinary β_2-mg concentrations in the most heavily Cd-polluted district exceeding 4 mg/L for 71–74% of those aged in their 50s and 91–100% of those aged 70 years or above (Kobayashi, 1981). The rate of prevalence of *Itai-Itai* disease in the Jinzu River basin is high, but it is low in surrounding regions, with a clear regional accumulation found (Figure 8). In addition, rice-Cd concentrations correlate highly with the rates of prevalence of renal injury and *Itai-Itai* disease (Ogawa *et al*., 2004). In Cd-polluted regions other than the Jinzu River basin, at least 16 cases resembling *Itai-Itai* disease have been reported, with tubular injury prevalent in the inhabitants of each of these Cd-polluted regions as well (Table 7). One of the criteria for diagnosing *Itai-Itai* disease is that the subject is an inhabitant of a Cd-polluted region, but it does not mean that subjects with the clinical signs of *Itai-Itai* disease cannot be found elsewhere.

7.2.9 Cadmium and the Central and Peripheral Nervous System

There are only limited data from animals and humans (Friberg *et al*., 1986; Jarup *et al*., 1998c; WHO/IPCS, 1992) suggesting that exposure to Cd can give rise to adverse effects on the central nervous system (CNS). Oral exposure of pregnant or lactating rats to low levels of Cd caused alterations in the brain serotonin levels of their offspring (Andersson *et al*., 1997; Oskarsson *et al*., 2004; see also Section 5.2).

A direct toxic effect of Cd on primitive nervous tissue seems possible at early stages of gestation, before the blood–brain barrier is established (Dencker, 1975). At later stages of pregnancy and in adult animals, the blood–brain barrier offers considerable protection of the CNS from the effects of Cd, as demonstrated in animals (Sonawane *et al*., 1975). One explanation why there is limited transfer of Cd across the blood–brain barrier might involve its binding to MT and larger proteins in blood plasma (see Section 7.9). The reported neurotoxic effects of Cd during development are, therefore, likely to be secondary to the interference of Cd toward Zn metabolism or other factors of importance for the development of the CNS (e.g., hormones) and not to a direct effect of Cd on brain cells. Metallothionein-3 (MT-3) a form of MT found in the brain is rich in zinc and the interference of Cd in the placenta with the uptake of Zn in MT-3, MT-1, or MT-2 in fetal brain tissue seems to be a possible mechanism for induction of brain damage by

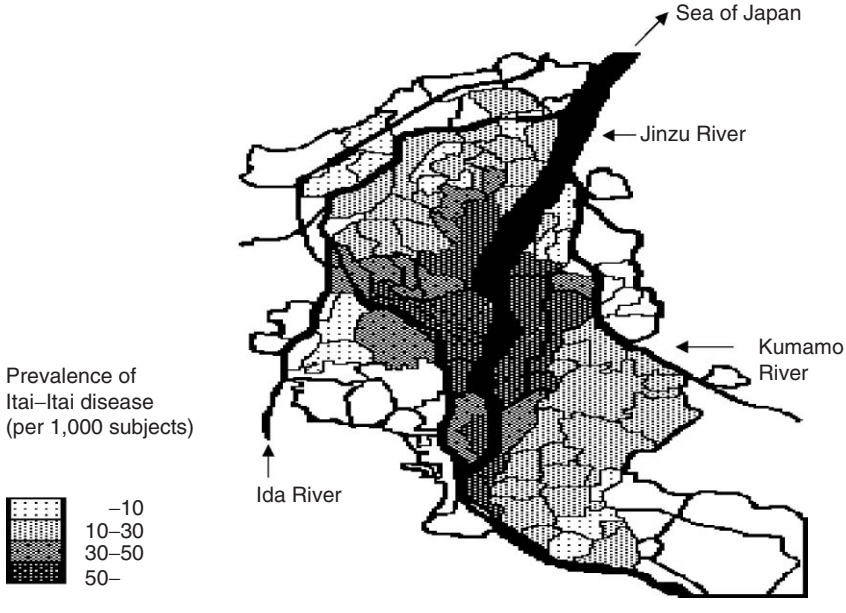

FIGURE 8 Geographical distribution of Itai-Itai disease in the Jinzu River basin (Nogawa, K., unpublished).

substances, like Cd, that do not pass the blood–brain barrier. Active axonal transport of airborne Cd through the nose and the olfactory bulb (Tjalve *et al*., 1996) may also be of importance. Here we only mention briefly the available evidence for zinc and MT in the brain. MT-3, first identified as a growth inhibitory factor (GIF), is present in the brain; its involvement in neurotoxicity and the development of a number of neurodegenerative disorders (e.g., Alzheimer's disease) has been suspected (Uchida *et al*., 1993). MT-3 is not inducible by CD, but both MT-1 and MT-2 are induced by Cd, Zn, and other metal ions, and all three forms are expressed in brain tissue (Aschner *et al*., 1997; Jin *et al*., 1998). MT-3 is expressed mainly in large Zn-ergic neurons sequestering Zn in synaptic vesicles, whereas MT-1 is predominant in regions rich in glia (Masters *et al*., 1994). All three forms of MT exist in the olfactory bulb (Choudhuri *et al*., 1993), where they might play a protective role, because the olfactory bulb provides a direct route of entry of metals into the CNS. In transgenic mice that cannot synthesize MT-3 (MT-$3^{-/-}$) or that overexpress human MT-3 (hMT-3; Erickson *et al*., 1997), it was found that MT-3 might participate in the use of zinc as a neuromodulator (Master *et al*., 1994) even though zinc and cadmium toxicity were unchanged in MT-$3^{-/-}$ or hMT-3 mice (Erickson *et al*., 1997). No differences in the Cd levels in blood between Alzheimer's disease sufferers and nondemented persons were found in a Swedish study (Basun *et al*., 1994), and no relation to cognitive function was observed in a follow-up study (Nordberg *et al*., 2000).

Until recently, there has been very limited evidence for the effects of Cd on the CNS in humans. In a study by de Burbure *et al*. (2006), the effects of low levels of Cd and other metals on the dopaminergic biomarkers—serum prolactin and urinary homovanillic acid—were reported in children residing in three European countries. Statistically significant effects were observed at blood Cd levels lower than 0.5 μg/L. It should be noted that other toxic metals also occurred in the exposed areas in all three countries and that levels of metal exposure had decreased considerably during the past 10 years. Thus, it cannot be excluded that the observed effects might have been caused by earlier high exposure. Further studies will be necessary to confirm these findings in children. The effects on the central and peripheral nervous system in Cd workers have been reported on the basis of a study using a questionnaire (Viaene *et al*., 2000).

7.2.10 Reproductive and Developmental Effects

In animal experiments focusing on the influence of Cd on the male reproductive system, Parizek and Zahor (1956) reported that a single injection of cadmium chloride induced testicular necrosis; subsequent reports confirmed that administration of large amounts of Cd through different routes also resulted in similar damage. Long-term or short-term administration of Cd results in changes in the levels of male sex hormones and related changes in accessory reproductive organs in animals (Gunnarsson *et al*., 2004; Nordberg, 1975). Zeng *et al*. (2004) detected changes in the levels of male reproductive hormones in a Cd-exposed population (cf. Section 7.3). With regard to male infertility, studies have been conducted on Cd workers (Gennart *et al*., 1992) and also with respect to the Cd concentrations in semen in the general population (Keck *et al*., 1995) and in male reproductive organs (Oldereid *et al*., 1993). None of these studies was supportive, however, of the contention that Cd is a cause of infertility.

In female rats, hemorrhagic changes in the ovaries, changes in ovarian hormonal production, endothelial changes in uterine blood vessels, and placental necrosis have all been induced after injection of Cd (Copius Peerebom-Stegeman *et al*., 1987; Parizek, 1964; Piasek and Laskey, 1994; Parizek *et al*., 1968). Exposure to Cd in female animals can increase the weight of the uterus and cause proliferation of the mammary gland. It was suggested that these changes might be related to the finding that Cd can bind to estrogen receptors in breast cancer cells (Johnson *et al*., 2003). When Cd is injected during gestation, teratogenic effects are observed in rats, mice, and hamsters (Chernoff, 1973; Ferm and Carpenter, 1968; Ishizu *et al*., 1973). Furthermore, when Cd is administered to pregnant or lactating rats, brain developmental injury and changes in brain enzymes are found in rat pups (Gupta *et al*., 1995; Oskarsson *et al*., 2004; Pelletier and Santinder, 1991); an influence on the immune response in the offspring has also been reported (Soukupova *et al*., 1991).

No relationship has been observed between the level of placental Cd and the birth weight of humans (Berlin *et al*., 1992; Loiacono *et al*., 1992). On the other hand, associations have been described between increased Cd concentrations in the hair of newborns and decreased birth weight (Frery *et al*., 1993) and between increased Cd concentrations in the maternal blood and cord blood and decreased infant birth weight (Salpietro *et al*., 2002). In Japan, a study focusing on the relationship between the Cd concentrations in breast milk and urine of 57 mothers and the infant growth and gestational age at birth found that maternal exposure to Cd led to an increase in early delivery, thereby leading to lower birth weight (Nishijo *et al*., 2002). Nishijo *et al*. (2004) observed an inverse relationship between the level of maternal blood Cd and infant height.

The Cd levels in the mothers studied by Nishijo were higher than those in smokers. Although it is well known that Cd levels in placenta are higher in

smokers than in nonsmokers (cf. Section 6.1.3), Cd is not likely to be the main cause of the decreased birth weight observed among babies born to smoking mothers.

7.3 Carcinogenic Effects

After Cd administration to rats through injection, inhalation, or oral routes, tumor formation has been detected in the testes, lungs, prostate, hematopoietic system, and at sites of subcutaneous and intramuscular injections (Heath *et al*., 1962; Poirier *et al*., 1983; Takenaka *et al*., 1983; Waalkes, 2000; Waalkes *et al*., 1988; 1992ab; 1997; 1999). Prostate carcinogenesis depends on an intact testicular production of testosterone. Cadmium-induced changes in the expression of MT, p53, and protooncogenes such as *c-jun* may be important for the development of prostatic and testicular tumors in rats (Xu *et al*., 1999, Zhou *et al*., 1999). A dose-related increase in lung cancer was reported in rats exposed to $CdCl_2$ through inhalation (Takenaka *et al*., 1983). Human cancer-related mortality was not found to be excessive in surveys conducted into the causes of death in inhabitants of any of the Cd-polluted regions in Japan (Arisawa *et al*., 2001; Nishijo *et al*., 1995); similar findings were obtained with regard to the inhabitants of a Cd-polluted region in England (Inskip *et al*., 1982). Because of the limited number of persons studied (e.g., Arisawa *et al*. studied 275 persons), however, conclusions regarding the absence of cancer cannot be drawn.

In humans, cohort studies have been undertaken regarding the development of lung and prostate cancers in Cd workers in England, Sweden, and the United States. In serial studies on English nickel-cadmium battery workers, Cd exposure was found not to be significantly associated with lung or prostate cancers (Sorahan, 1987; Sorahan and Esmen, 2004; Sorahan and Waterhouse, 1983); in the study by Sorahan and Esmen (2004), the mortality from cancers of the pharynx was, however, significantly higher than that in the general population of England and Wales. In a study of Swedish battery workers that also considered smoking status (Jarup *et al*., 1998b), the incidence of lung cancer was significantly increased in male workers (SMR 176; 95% confidence interval [CI], 101–287). In a study of copper-cadmium alloy workers in England conducted between 1946 and 1992, the SMR for chronic nonmalignant disease, other than lung cancer, of the respiratory system was increased significantly, with a dose-response relationship found with respect to the amount of cumulative exposure to Cd, although no significant increase in SMR was found for lung cancer (Sorahan *et al*., 1995). In a series of surveys focusing on Cd workers in English factories, no clear association was noted between Cd exposure and the development of lung or prostate cancers (Ades and Kazantzis, 1988; Armstrong and Kazantzis, 1983, 1985; Kazantzis *et al*., 1988; 1992).

In investigations of an American Cd recovery plant, Thun *et al*. (1985) and Stayner *et al*. (1992) reported that lung cancer SMRs were significantly increased, with a dose-response relationship between the exposure to Cd and the development of lung cancer. On the other hand, Lamm *et al*. (1992) and Sorahan and Lancashire (1997), who surveyed the same population, found that the development of lung cancer in this plant was more closely associated with exposure to arsenic and smoking than with exposure to Cd. Verougstraete *et al*. (2003) pointed out that data for occupational exposure obtained before 2003 indicated a lower relative risk in groups exposed to Cd in the absence of arsenic and nickel than in the presence of these agents; nevertheless, these authors considered these data indicative of a role for Cd as a human carcinogen. All these data on humans concern occupational exposure; until recently, there were no data from environmental Cd exposure with individual-related exposure measurements. Nawrot *et al*. (2006) found an association between an increased risk of lung cancer and exposure to environmental Cd in a prospective population-based cohort study in Belgium. These authors included information on urinary Cd excretion in each person, as well as data on several potential confounding factors, such as smoking and concomitant arsenic exposure, in their analysis. As noted by Nordberg (2006) in a commentary, arsenic exposure is of particular interest as a potential confounding factor because oral exposure to As from drinking water has been demonstrated to increase the incidence of lung cancer (Chan and Ahsan, 2004). The adjustments for arsenic made by Nawrot *et al*. (2006) need further comment. The investigators did not determine urinary As for all participants, but they did calculate probable values using two different models. In those subjects who were exposed environmentally, urinary Cd remained a significant predictor for lung cancer with only one of the models. Although the studies by Nawrot *et al*. (2006) give support to Cd as an environmental carcinogen, further studies will be necessary to confirm these findings.

Early observations of an increased occurrence of prostate cancer in Cd workers (Kipling and Waterhouse, 1967) were not confirmed in some later studies (Kazantzis *et al*., 1988); more recent epidemiological studies have reported variable results (Rooney *et al*., 1993; West *et al*., 1991). Cadmium has been demonstrated to induce malignant transformation of human prostate epithelial cells *in vitro* (Achanzar *et al*., 2001). Zeng *et al*. (2004a) performed studies in a Cd-polluted area in China and found a statistically sig-

nificant relationship between high testosterone levels and urinary Cd, but not to blood Cd. There were also indications of a possible increase in prostatic lesions and a statistically significant relationship between urinary Cd and presence of increased levels of prostate specific antigen (Zeng *et al.,* 2004b).

The IARC (1993) concluded that there was sufficient evidence to classify Cd as a human carcinogen; thus, it belongs to group 1 of the IARC classification system. The classification was based on the animal data and epidemiology cited previously (Stayner *et al*., 1992; Thun *et al*., 1985), demonstrating that the presence of Cd can lead to lung cancer in industrial workers. The US Department of Health and Human Services (DHHS) has determined that Cd and Cd compounds may reasonably be anticipated to be carcinogens. This conclusion is based on the increased prevalence of lung cancer in humans caused by the inhalation of Cd. The evidence available at the time of the IARC evaluation was compiled and discussed by Nordberg *et al*. (1992); the difficulties in reaching clear conclusions were pointed out. Although, there is sufficient evidence relating the inhalation of Cd to lung cancer in animals, human evidence is less clear. It may, however, still be prudent to consider Cd as a human carcinogen.

7.4 Genetic Effects

No Cd-induced mutagenicity was evident from routine mutagenicity trials using *Salmonella typhimurium* and *E. coli*. In recent years, numerous studies have focused on Cd-induced DNA damage using cultured cells. To summarize the results of these studies, Cd was found to induce chromosome anomalies (Han *et al*., 1992; Ochi and Ohsawa, 1985), to breaks strands in DNA (Hartwig, 1994; Lloyd *et al*., 1998), and to mutate suppressor oncogenes (Abshire *et al*., 1996; Meplan *et al*., 1999), as well as to hinder the repair of gene damage (Mandel and Ryser, 1984; Takahashi *et al*., 1988). From investigations of humans, some studies have detected no chromosome anomalies in *Itai-Itai* disease patients or in Cd workers (Bui *et al*., 1975; Nogawa *et al*., 1986), whereas others have described significant increases in such anomalies (Deknudt and Leonard, 1975).

7.5 Interaction Between Cadmium and Other Metals

7.5.1 Cadmium-Zinc-Metallothionein, Iron, and Calcium

Cadmium–zinc interactions are believed to be of special importance in Cd toxicity (early data reviewed by Task Group on Metal Interactions, 1978). For example, Cd-induced acute testicular effects can be prevented by the administration of Zn (Parizek, 1957). Lethal toxicity can be prevented by pretreatment with Cd or Zn (Leber and Miya, 1976; Nordberg, 1971). Metallothionein is deeply implicated in the development of Cd toxicity and in intracellular protection against Cd. By previously administering a small amount of Cd or Zn, thereby inducing the *in vivo* synthesis of MT, acute Cd toxicity is mitigated as a result of the exchange of Cd for Zn in the synthesized zinc–MT complex. Recently, research has been undertaken using MT-transgenic and MT-null mice. In mice excessively expressing MT, sensitivity to acute lethal toxicity and hepatic toxicity was markedly lower than that in wild-type mice (Liu *et al*., 1995). In MT-null mice, the renal Cd concentration at the time of appearance of renal injury was approximately one eighth of that of wild-type mice (Liu *et al*., 1998). In addition, in MT-null mice, the long-term subcutaneous administration of Cd was reported to be associated with enhanced susceptibility to bone injury (Habeebu *et al*., 2000), hematotoxic effects (Liu *et al*., 1999), and immunotoxic effects (Liu *et al*., 1999), relative to those of wild-type mice.

Metallothionein-gene expression was studied in humans exposed to Cd in either the work environment or the general environment. The aim was to validate the possibility of using MT-gene expression in peripheral blood lymphocytes (PBLs) as a biomarker of Cd exposure and related susceptibility to renal dysfunction; MT mRNA levels were measured using RT-PCR in PBLs among subjects exposed either occupationally or environmentally to Cd (Ganguly *et al*., 1996; Lu *et al*., 2001; 2004; 2005). A dose-effect relationship between the internal dose of Cd and the MT mRNA level confirmed the validity of MT expression in PBLs as a biomarker of Cd exposure.. It was demonstrated both in Cd workers and in persons environmentally exposed to Cd that a negative correlation existed between the levels of urinary *N*-acetyl-β-D-glucosaminidase (UNAG), a renal effect indicator, and that of *in vitro*–induced MT mRNA in subjects exhibiting a high UCd level (>10 μg/g CR).

The lower proportion of individuals expected to have exceeded their individual critical concentration in the renal cortex at UCd levels <10 μg/g CR explains why no observed statistically significant correlation exists between the levels of *in vitro*–induced MT mRNA and UNAG in the groups exhibiting 2–10 μg/g CR UCd. UNAG is considered to be a sensitive renal effect indicator of Cd exposure. The reverse relationship between the levels of *in vitro*–induced MT-mRNA in PBLs and UNAG indicates that MT gene expression in PBLs can be used as a biomarker inversely related to the susceptibility to renal toxicity of Cd. It has been suggested that MT gene expression in PBLs should be applied

to the practice of risk assessment of Cd exposure (Lu *et al*., 2001; 2004; 2005).

In a recent study of occupationally Cd-exposed persons in China (UCd 2.5–4.1 μg/g CR as 25–75 percentiles) and a control group (UCd 1.0–1.8 μg/g CR as 25–75 percentiles), Chen *et al*. (2006b) found that persons with elevated plasma levels of autoantibodies against MT (MTAb) more readily developed Cd-induced renal tubular dysfunction. They found an odds ratio of 4.2 (CI, 1.2–14) for tubular dysfunction between persons with elevated levels of MTAb relative to those with low levels of MTAb. The authors suggested that plasma MTAb may be used as a biomarker of susceptibility to renal dysfunction in occupational Cd exposures.

In normal human beings, the increase in Cd in the renal cortex with age is accompanied by an equimolar increase in Zn. This phenomenon is believed to be due to MT stored in the kidneys containing equimolar amounts of the two metals.

In humans, iron deficiency has been shown to increase Cd absorption (Flanagan *et al*., 1978; see Section 5.1.2). Akesson *et al*. (2002) found that blood and urine values in pregnant Swedish women were higher among those with iron deficiency. Jin *et al*. (2002) found higher blood and urine levels in women than in men in a highly polluted area in China. A few epidemiological studies in Japan have been unable to find this effect (Horiguchi *et al*., 2004a; Tsukahara *et al*., 2003), and further well-designed epidemiological studies are needed.

It is well known from animal experiments (cf. review by Nordberg *et al*., 1985) that the uptake of Cd from a calcium-deficient diet is higher than that from a normal diet. A possible contributing role of low dietary calcium levels for the development of *Itai-Itai* disease has been suggested. The relatively low calcium levels in Japanese diets, as well as low dietary intake of vitamin D compared with western European diets, are discussed in Chapter 7.

7.5.2 Interaction Between Arsenic and Cadmium

In animal studies, more pronounced renal toxicity was observed after exposure to inorganic As and Cd combined, relative to those detected after exposure to either of the agents alone. Studies in China examined whether a similar situation occurs in humans. A study of the general population in Guizhou Province, China, included 122 subjects in an arsenic- and cadmium-polluted area and 123 in a nonpolluted area. Levels of urinary B2M, ALB, and NAG in the polluted area were significantly higher than those in the nonpolluted area ($P < 0.01$) and that the increased levels of the biomarkers of tubular damage occurred at lower urinary Cd levels (Hong *et al*., 2004) than in previous studies of Cd-exposed areas (Jin *et al*., 2004).

Urine samples of population groups (619 persons in total) living in two metal-contaminated areas in China—one mainly contaminated with Cd (in Zhejiang Province) and the other mainly contaminated with As (in Guizhou Province)—and two control areas without excessive metal exposure were analyzed for their levels of B2MG, NAG, RBP, ALB, and Cd and As. Higher levels of these markers after combined exposure to As and Cd showed an enhanced effect on the kidney compared with Cd exposure alone, thus demonstrating an interaction effect between As and Cd. A gender difference was indicated by a statistically significant relationship between the levels of As and B2MG in the urine from women but not from men (Nordberg *et al*., 2005). Further information concerning these findings is given in Chapter 7.

Thus, simultaneous exposure to both Cd and As has a multiplicative effect on the renal tubular toxicity in general population groups, as demonstrated from a population in China (Hong *et al*., 2004; Nordberg *et al*., 2005).

8 DOSE-RESPONSE RELATIONSHIPS

Dose-response relationships and tolerable values for oral and respiratory Cd exposure have been determined on the basis of a metabolic model, as well as on directly observed data. The critical organ of Cd exposure is the kidneys, particularly the kidney cortex, with the critical Cd concentration in the kidney cortex being important. The critical concentration is individual and for the purpose of dose-response analysis the distribution of the critical concentrations needs to be known. The concept of population critical concentration (PCC) is, therefore, used (see Chapter 14). Estimates of the distribution of the critical concentration in the population, as well as the distribution of toxicokinetic parameters, makes it possible to calculate the dose-response relationship (Diamond, *et al.,* 2003; Nordberg and Strangert, 1985). Such calculations were of particular importance in the past, when epidemiological data on dose-response relationships were scarce or nonexistent, but they are also of importance at present for a more complete understanding of such relationships.

8.1 Critical Concentration in the Kidney and Toxicokinetic Model

In animals, although differences are observed in the renal Cd concentration eliciting renal injury depending on factors such as the type of animal studied, the amount of Cd ingested or injected, and the period of

administration, renal injury generally develops in half of the Cd-exposed animals when values exceed 100–150 mg/kg wet weight (review by Kjellstrom, 1986a; Stowe *et al*., 1972; Tohyama *et al*., 1981; 1987). On the basis of a comprehensive evaluation of Cd concentrations obtained through biopsy or autopsy from persons exposed occupationally to Cd, the presence or absence of proteinuria, and renal pathological findings, as well as data derived from animal experiments, Friberg *et al*. (1974) concluded that, for humans, the critical Cd concentration in persons highly susceptible to renal injury is approximately 200 mg/kg. From measurements of renal Cd concentrations in Cd workers, on the basis of the *in vivo* NAA method (Ellis *et al*., 1984; Roels *et al*., 1981), the mean PCC (PCC-50) in the renal cortex was found to be approximately 250–300 mg/kg, with the level at which 10% of workers exceeded their critical concentrations (PCC-10) estimated to be approximately 170–200 mg/kg (Kjellström, 1986a). In the IPCS document, the critical Cd concentration in the renal cortex for inducing renal tubular dysfunction in 10% of Cd-exposed populations was estimated to be 200 mg/kg wet weight (WHO/IPCS, 1992). In calculations from a metabolic/toxicokinetic model based on data from human and animal studies (Kjellström and Nordberg, 1978; 1985) and the statistical distribution of renal cortex Cd concentrations, a 10% response rate (prevalence of effects above background) was estimated to occur at a daily intake of approximately 200 µg from food after 45 years of exposure; it was surmised that, in Cd workers, a 10% response rate would be found after 10 years' exposure at a Cd concentration of approximately 50 µg/m^3 (Kjellström and Nordberg, 1985). Thun *et al*. (1991) reviewed studies up to 1991 using the metabolic model and critical organ concept for calculating the risk of renal damage after occupational exposure to Cd. They found that cumulative exposure, corresponding to 45 years of exposure, to between 5 and 10 µg of Cd in industrial air would be expected to lead to a distinct increase in tubular dysfunction in a small proportion of exposed workers. In view of the recent estimates of lower levels of Cd as the PCC-10 in the general population (cf. Section 8.2), estimates of response rates based on calculations using a metabolic/toxicokinetic model will be correspondingly lower compared with the calculations presented previously, which were based on the higher values for PCC-10 cited in the foregoing text. At present, however, there are no published articles presenting such values for occupational exposure. The estimated values for PCC-10 cited previously for a male working population may still be valid, but in view of the fact that occupational exposure frequently lasts for more than 10 years and that nowadays an increasing proportion of workers are women, some of child-bearing age, lower permissible occupational exposure limits than 50 µg/m^3 should be recommended; indeed, they have been recommended in several countries (see Section 4.2).

For environmental exposure, Diamond *et al*. (2003) estimated a lower confidence limit for PCC-10 (in their terminology, "RC10L") of 84 µg/g kidney cortex. Using the toxicokinetic model described by Choudhury *et al*. (2001)—cf. Section 5.5 in this chapter and Chapter 3 in this Handbook—these authors estimated that such a kidney cortex level would be attained at a constant chronic intake of 1 µg/kg/day in females and 2.2 µg/kg/day in males. The RC10L was 1.6-3.0 times higher than the 95th percentile of renal cortical levels in the US population (50 µg/g in females; 27 µg/g in males), and the authors stated that, for most of the US population, diet-derived risks are likely to be negligible in the absence of exposure from other sources.

8.2 Direct Observations of Dose-Response and Risk Characterization

A number of studies of workers who have experienced prolonged Cd exposure have been published (Kjellstrom *et al*., 1977a; reviews by Thun *et al*. [1991] and Jarup *et al*., [1998c]). The conclusions from the evaluations and calculations by Thun *et al*. (1991) are mentioned in Section 8.1. The further studies reviewed by Jarup *et al*. (1998c) pointed out the greater sensitivity to development of renal effects among older workers.

Numerous reports from Japan have focused on the dose-response relationships of the inhabitants of Cd-polluted areas. In Japan, because 50–70% of the amount of Cd ingested orally derives from rice (Tsuchiya and Iwao, 1978), the Cd concentration in rice and the lifetime Cd intake are used as indices of Cd exposure. These dose-response relationships have been most thoroughly investigated for the inhabitants of the Jinzu River basin, Toyama Prefecture, where *Itai-Itai* disease occurred, and for those in the Cd-polluted Kakehashi River basin of the Ishikawa Prefecture. In the former area, significant associations have been identified between the urinary finding positive rates (protein, glucose) in 13,183 persons in 1967–1968 and the Cd concentrations in 2446 rice samples investigated from 1971–1976 (Figure 9) and between the lifetime Cd intake (calculated from Cd concentrations in the rice samples) and the period of residence (Chiyoda *et al*., 2003; Osawa *et al*., 2001;Watanabe *et al*., 2002; 2004). Permissible values determined by the authors from regression lines of dose-response relationships using proteinuria plus glucosuria as an index were estimated to be 0.11 mg/kg (ppm) for the rice Cd concentration

and 1.5–1.6 g for the lifetime Cd intake. In the studies performed in the Kakehashi River basin, similar dose-response relationships were identified between each of the urinary finding positive rates (β_2 -mg, MT, protein, glucose) and the mean Cd concentration in rice in each hamlet, with permissible values determined from the regression lines of dose-response relationships (using β_2-mg as an index) of <0.05 μg/g (ppm) for rice Cd concentrations and 1.7–2.1 g for the lifetime Cd intake (Hochi *et al*., 1995; Kido and Nogawa, 1993; Nakashima *et al*., 1997; Nogawa *et al*., 1989). In 1997–1998, the mean Cd concentration in 37,250 rice samples obtained from the general environment in Japan was 0.06 ppm (mg/kg), with values exceeding 0.2 ppm (mg/kg) noted in 3.2% of the samples.

Urinary Cd is recognized as a useful index of the internal dose of Cd (Borjeson *et al*., 1997; Kido *et al*., 1992, 2004; Nordberg and Nordberg, 1988; Shimbo *et al*., 2000; see also Sections 5.3 and 6.1.2). Investigations using urinary Cd concentrations as an index of the internal Cd dose have been undertaken in various countries. In the Belgian Cadmibel study, 24-hour urine samples from 1699 residents of areas polluted with Cd to various degrees were investigated, with significant associations detected between the amounts of urinary Cd excreted and the levels of retinol-binding protein, NAG, β_2-mg, amino acids, and calcium; urinary finding abnormality rates of 10% were found for each of these parameters at urinary Cd excretion rates of 2–4 μg/24 hour (Buchet *et al*., 1990). In a follow-up study by Hotz *et al*. (1999), the urinary levels of Cd were lower, and some subjects who previously had displayed biomarkers of renal dysfunction presented normal values, indicating that slight deviations in these renal biomarkers might be reversible. In Belgium, statistically significant effects on the urinary excretion of Clara cell protein 16 (CC16) in women were reported at very low urinary Cd levels by Bernard *et al*. (1994). In the Swedish OSCAR study, which focused on 1021 persons, who were subjected to low-level occupational or environmental Cd exposure, the a_1-microglobulin concentrations were significantly associated with the urinary Cd concentrations, with tubular proteinuria found in 10% of subjects exhibiting a urinary Cd concentration of 1.0 μg/g CR (Alfvén *et al*., 2000; Jarup and Alfvén, 2004; Jarup *et al*., 2000). Furthermore, in a study on 72 subjects living in the vicinity of a Cd factory, increased NAG excretion was reported at urinary Cd concentrations of 0.5 μg/g CR (Jarup *et al*., 1995). It should be noted that the exposure to Cd among those living in this area was higher in the past and that the reported dose-response relationship might be a result of remaining renal dysfunction caused by such higher past levels of exposure. Estimates of past exposure levels in this area were presented by Alfvén (2002). De Burbure *et al*. (2006) reported the increased excretion of NAG in children at UCd levels of 0.6–1.3 μg/g CR, but those children lived in areas contaminated by

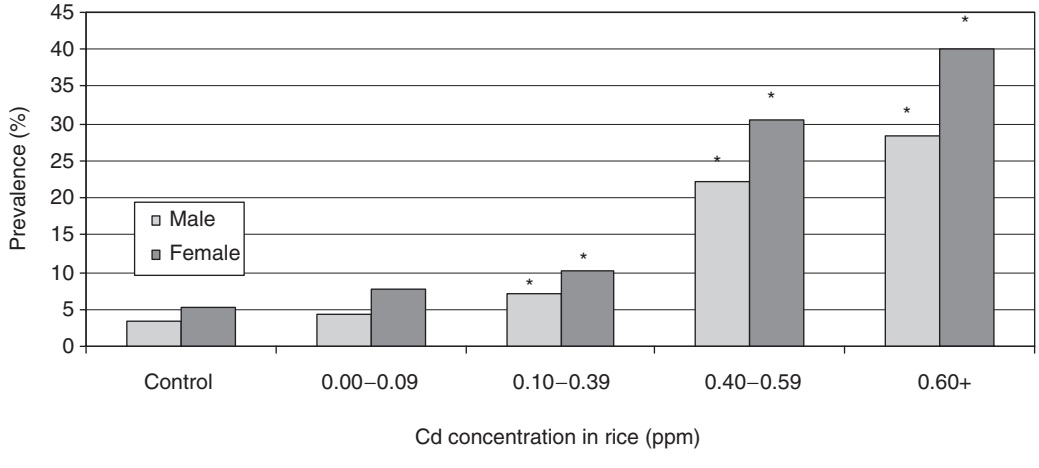

FIGURE 9 Prevalence of proteinuria with glucosuria according to sex and Cd concentration in rice (people living in the same hamlet over 30 years and aged over 50 years). *Significant difference compared with the prevalence of control group (P<0.05) (Osawa *et al*., 2001).

several toxic metals (i.e., combined exposure to Cd, Hg, and Pb), and their exposure levels had decreased constantly during the past 10 years. In a Swedish study of an area (Skane) where exposure most probably had not decreased, Olsson *et al*. (2002) reported a statistically significantly increased age-adjusted urinary beta$_2$-microglobulin clearance in relation to urinary Cd at UCd levels below 1 µg/g CR. There were no statistically significant increases in other indicators of renal tubular dysfunction, such as protein HC (α1-microglobulin), after adjustment for age. In another recent study, Akesson *et al*. (2005) reported statistically significant increases in the urinary levels of NAG and protein HC, as well as decreases in the creatinine clearance (from 75 to approximately 70 mL/min) and glomerular filtration rate (from 100 to approximately 95 mL/min) among women in Skane, Sweden, exhibiting urinary Cd of 0.8 µg/g CR. These changes were statistically significant when compared with women having on average 0.48 µg/g CR. Interactions with diabetes were indicated.

In Japan, a study of 3178 inhabitants of the Cd-polluted Kakehashi River basin indicated an association between urinary Cd concentrations and urinary β_2-mg concentrations. "Permissible values" were reported to be 1.6–3.0 µg/g CR for men and 2.3–4.6 µg/g CR for women (Hayano *et al*., 1996). Because the mean urinary Cd concentrations of adults living in the general environment in Japan are in the range 1–4 µg/g CR, there is concern about the possibility of adverse renal effects of Cd exposure, even in so-called non-Cd-polluted regions of Japan.

Jarup *et al*. (1998c) reviewed the available studies up to 1997 on dose-response relationships between internal doses of Cd, accumulation in renal cortex, and renal tubular dysfunction (Table 5; Section 6.1.2). These authors emphasized that the lowest level of Cd in the kidneys at which renal effects can be detected in a small percentage of the general population is approximately 50 mg/kg, whereas the value of PCC-10 would be approximately 125 mg/kg (6.25 µg/g CR in urine). A long-term daily oral intake of 70 µg of Cd (in persons of 70-kg body weight) was suggested to give rise to a renal dysfunction rate of 7% in a human population, with higher percentages in particularly sensitive groups. Furthermore, the authors asserted that to prevent the development of renal tubular dysfunction, Cd concentrations in the renal cortex and urine must be maintained at levels <50 mg/kg and 2.5 µg/g CR, respectively (Table 5), and they also recommended that the PTWI be lowered.

In investigations on residents in areas of Japan not considered polluted by Cd, researchers from Chiba University, Japan, have demonstrated the existence of dose-response relationships between urinary Cd concentrations and the urinary excretion of low-molecular-weight proteinuria or NAG (Oo *et al*., 2000; Suwazono *et al*., 2000; Yamanaka *et al*., 1998); a group of scientists from the Kyoto Industrial Health Association and Jichi Medical University have disputed these findings (Ezaki *et al*., 2003; Horiguchi *et al*., 2004b; Ikeda *et al*., 2000), although Ikeda *et al*. (2000) considered their evidence concerning a Cd-induced effect on renal dysfunction to be of "borderline significance" (i.e., not a negative finding). In addition, Ezaki *et al*. (2003) concluded that there was "no clear-cut evidence" for Cd-induced renal dysfunction in their study of more than 10,000 women in the Japanese general population, but they found a statistically significant ($P < 0.01$) higher level of α_1-microglobulin and β_2-microglobulin in groups of women having the same mean age and high (3.4 µg/g CR) geometric mean UCd compared with that of those having a low (i.e., below detection limit) level of UCd. The reason why Ezaki *et al*. (2003) concluded that the evidence was not clear-cut was the absence of statistically significant increases in the number of women with β_2-microglobulin levels in urine as high as 1000 µg/g CR in the "high"-UCd group. In the study by Horiguchi *et al*. (2004b), the geometric mean UCd value (µg/g CR) was 4.08 in the group exhibiting the highest values. This result would correspond to an expected increase in prevalence of renal dysfunction of 3–4% above the background prevalence (according to the estimates by Jarup *et al*. [1998c]), and it is not surprising that the authors were unable to detect such a small expected increase, when they had no subgroup with UCd levels <2.6 µg/g CR (corresponding to an expected prevalence of approximately 1% according to Jarup *et al*. [1998c]). This study, therefore, is inconclusive in relation to the assessment of risk at the present PTWI level. Uno *et al*. (2005), from studies of 828 subjects living in areas in Japan without any known Cd pollution, calculated the values of LBMD-10 for increased urinary excretion of NAG and B2M of 0.6–1.2 µg/g CR in men and 1.2–3.6 µg/g CR in women. Because they did not have a control group, it is not completely clear how they were able to account for such confounding factors as age and smoking; confirmatory studies are required.

In summarizing the studies of renal tubular dysfunction in Japanese areas not considered to be Cd contaminated, it can be said that the risk of dysfunction giving rise to >1000 µg/g CR of β_2-microglobulin is low, but that there is evidence of a dose-response relationship between urinary Cd levels and less pronounced low-molecular-weight proteinuria. The evidence is not contradictory to the estimates presented by Jarup *et al*. (1998c).

The "permissible values" for rice Cd concentrations, lifetime Cd intake, and urinary Cd concentrations (cited in the foregoing text) are all low and do not differ appreciably from the degree of Cd exposure in the general environment. This situation is supported by the reported benchmark doses and lower confidence limits of the benchmark dose (LBMD) of Cd in urine giving rise to tubular dysfunction in Chinese population groups exposed to Cd (Jin *et al*., 2004). In that study, the authors reported LBMD values (corresponding to the lower confidence limit of PCC-5) for UCd to be 3–4 μg/g CR when using bioindicators of renal dysfunction, such as NAG, NAG-B, β_2-microglobulin, and RBP. The background levels of urinary Cd in the control group were relatively high, and only 64 of the 790 subjects in this study had UCd levels < 1 μg/g CR; these features may have influenced the calculated LBMD values upwards (cf. the discussion in Chapter 14). Chen *et al*. (2006a) studied an occupationally Cd-exposed group in China and found that the LBMD for metallothioneinuria was 3.1 μg/g CR and for urinary NAG 2.7 μg/g CR. In a study by Uno *et al*. (2005), even lower benchmark doses were reported in Japanese population groups.

The Joint FAO/WHO Expert Committee on Food Additives (WHO/FAO, 2003; 2005) has set the provisional tolerable weekly intake (PTWI) of Cd at 7 μg/kg body weight. This value corresponds to 1 μg/kg body weight for each day of the week (i.e., 70μg/day for a person of 70-kg body weight).

The PTWI is set to allow a certain variation of intake during a week, provided that the weekly intake is not exceeded. Because the critical effect of long-term oral intake is renal dysfunction, short-term exceedances can, in principle, be accepted. The extent to which excursions above the PTWI can be allowed during various time intervals was discussed by Nordberg (1999). Oral intake of Cd in a high single dose from food or drink gives rise to gastrointestinal symptoms (see Section 7.1). A single oral dose of 4–14 μg/kg body weight or a dose of 3 μg/kg body weight during periods of months was considered tolerable by adult persons without the appearance of gastrointestinal symptoms when PTWI is temporarily exceeded. To avoid the risk of renal dysfunction, there should be a period of compensatory intakes lower than the PTWI. For children and pregnant or lactating women, daily intakes should not exceed 1 μg/kg body weight in view of potential adverse effects in the offspring, as indicated in animal experiments (see Section 7.2.8).

Our risk characterization of Cd exposure in the general population, on the basis of presently available data, uses increased urinary excretion of β_2-microglobulin as the criterion for renal tubular dysfunction. This indicator has been used widely in epidemiological studies (referred to in the foregoing text), and it is known to be of importance for health (cf. Section 9). Our quantitative evaluation is similar to the one presented by Jarup *et al*. (1998c), but we specify the dose-response relationship (Table 7) as β_2-microglobulinuria above the normal range (i.e., above approximately 300 μg/g CR), whereas Jarup *et al*. (1998c) used B2M and other indicators, such as NAG, as the basis for their assessment of "tubular effects."

In adult nonsmokers having a body weight of 70 kg, an average daily intake of 30 μg Cd over a lifetime (implying that only a small proportion of persons in a population will exceed 50 μg/g in the kidney cortex) will increase the degree of renal dysfunction by approximately 1% (β_2-microglobulinuria). In high-risk groups, such as females with depleted iron stores, diabetics, or subjects with increased levels of MT antibodies, a considerably higher percentage is expected to display such an effect.

If exposed at the present PTWI, corresponding to 70 μg/day from food, approximately 7% of the population will be expected to develop tubular dysfunction; the figure for the at-risk groups will be higher.

Dietary intake of Cd has been estimated to be 10–50 μg/day (0.13–0.84 μg/kg body weight) on average in various countries (see Section 4.1.1) These intakes are all below the present PTWI of 7 μg/kg body weight, corresponding to 1 μg/kg body weight/day or 70 μg/day for a 70-kg person. Some people do, however, exceed a level of 30 μg/day for an adult person (discussed in the foregoing text), and it is likely that some adverse effects on the kidneys do occur in particularly sensitive people in some populations of the world. Recently reported data from the general populations cited in the foregoing text indicate effects at internal Cd doses lower than those used in this risk characterization. The present assessment was performed in relation to an increase in β_2-microglobulin excretion. Increases in more sensitive biomarkers of renal tubular effects (i.e., prot HC, NAG, and CC16) have been reported in some general population groups at Cd levels in urine <2.5 μg/g CR. In diabetics, people with increased levels of MT antibodies, or people coexposed to other nephrotoxic substances, tubular dysfunction has been reported at even lower Cd levels (Chen *et al*., 2006b; 2006c).

Recent reports indicate the possibility that renal dysfunction may occur in diabetics and in children at urinary Cd levels of 0.5–1.0 μg/g CR, but these observations require further confirmation. When there is simultaneous exposure to other nephrotoxic agents, renal dysfunction can occur at these, or lower, urinary Cd levels.

On the basis of renal effects in humans (Nogawa *et al*., 1989), the ATSDR (1999) derived a chronic-duration oral minimal risk level (MRL) of 0.0002 mg/kg/day (i.e., 0.2 μg/kg/day).

9 LIFE PROGNOSIS

In a study of English Cd workers, mortality rates for bronchitis and emphysema were clearly increased in those exposed to high concentrations or for prolonged periods (Armstrong and Kazantzis, 1983; 1985; Sorahan *et al*., 1995). No increased mortality rates were noted for hypertension, cerebrovascular disorders, or renal disorders. Excessive mortality rates for respiratory disorders have similarly been reported in Swedish Cd workers (Kjellstrom *et al*., 1979).

For the inhabitants of Japanese Cd-polluted regions, Cd-induced renal tubular dysfunction has been reported to adversely affect life prognosis. In a 15-year follow-up survey of 5725 inhabitants of the Jinzu River basin, Toyama Prefecture, persons with proteinuria and glucosuria, both markers of Cd-induced renal injury, exhibited significantly elevated mortality risk ratios for both genders of inhabitants in the polluted region (Matsuda *et al*., 2002). In addition, from a comparison of inhabitants of districts from which unpolished rice exhibited a Cd concentration ≥ 0.3 mg/kg (ppm) and those from districts with Cd concentrations in unpolished rice <0.3 mg/kg (ppm), as well as groups having lifetime Cd intakes of >2 g and <2 g, mortality risk ratios were found to be higher in the former group in both cases, with the differences being statistically significant in men (Ishihara *et al*., 2001; Matsuda *et al*., 2003). In a 9-year follow-up survey of 3178 inhabitants of the Cd-polluted Kakehashi River basin, Ishikawa Prefecture, the mortality risk ratio of the urinary β_2-mg positive group (≥1 mg/g CR) was significantly higher in both men (1.4-fold) and women (1.8-fold) than it was in the corresponding negative group (Nakagawa *et al*., 1993). In addition, when the mortality risk ratios were similarly compared after dividing the subjects into four groups according to their urinary β_2-mg levels (<300, 300–999, 1000–9999, and >10,000 μg/g CR, respectively), with <300 μg/g CR considered as 1, in each group the mortality risk ratios (Table 8) increased in parallel with increasing urinary β_2-mg concentration in both men (1.00, 1.27, 1.47, and 1.69, respectively) and women (1.00, 1.58 [$P < 0.01$], 2.04, and 2.43, respectively). Furthermore, when the follow-up period of this population was extended to 15 years, and the subjects were divided into the same four groups, according to their urinary β_2-mg concentrations, as in the 9-year survey, and mortality risk ratios calculated, the risk ratios of all of the groups increased even more markedly than at the time of the former survey (Nakagawa, 1999). The importance of this research lies in the fact that because the upper limit of normal urinary β_2-mg in Japanese adults is approximately 300 μg/g CR, these results prove that even small elevations of urinary β_2-mg above the normal range are associated with an increased risk of mortality. Significantly elevated mortality ratios were noted for renal, cardiovascular, and cerebrovascular disorders (Nakagawa *et al*., 1993). Moreover, by use of urinary RBP, proteinuria, glucosuria, and aminoaciduria as markers of renal tubular injury, similar findings were obtained: mortality risk ratios were elevated significantly in each of the

TABLE 8 Analysis of Urinary β_2-mg Concentration Difference, Related to Mortality, Using Proportional Hazard Model of Cox

	Regression coefficient	SE	Hazard ratio	*P* Value
Males				
Age	0.1192	0.0074	3.29 (10+x)/x	<0.001
β_2-MG(1)	0.2357	0.1582	1.27 (1)/(0)	—
β_2-MG(2)	0.3884	0.1572	1.47 (2)/(0)	<0.05
β_2-MG(3)	0.5273	0.2305	1.69 (3)/(0	<0.05
Females				
Age	0.1144	0.0076	3.14 (10+x)/x	<0.001
β_2-MG(1)	0.4600	0.1718	1.58 (1)/(0)	<0.01
β_2-MG(2)	0.7127	0.1665	2.04 (2)/(0)	<0.001
β_2-MG(3)	0.8879	0.1987	2.43 (3)/(0)	<0.001

β_2-MG concentration has been coded as follows: (0) <300 μ/g Cr; (1) 300–<1000 μ/g Cr; (2) ≥1000–<10,000 μ/g Cr; (3) ≥10,000 μ/g Cr.

Source: Nakagawa *et al*. (1993).

respective positive groups (Nishijo *et al*., 1994; 1995). In these populations, by investigating the relationship between the urinary Cd concentration and mortality during a 15-year follow-up study, it was demonstrated that the mortality risk ratios also increased on increasing the urinary Cd concentrations (Nishijo *et al*., 1999).

For the inhabitants of Cd-polluted Tsushima in the Nagasaki Prefecture, the mortality rates were also reported to be higher in the groups exhibiting elevated urinary β_2-mg levels (Iwata *et al*., 1991a, b).

In this manner, long-term follow-up surveys conducted in Japan have clarified that Cd-induced renal tubular injury adversely affects life prognosis and that, in particular, even slightly increased urinary β_2-mg levels provide an adverse risk factor for life prognosis.

10 DIAGNOSIS, TREATMENT, PROGNOSIS, AND PREVENTION

10.1 Acute Poisoning

10.1.1 Inhalation

Because the initial symptoms, such as irritation and dryness of the nose and throat and coughing, resemble those of metal fume fever, when a worker engaged in smelting or cutting metal complains of such symptoms, it is most important to suspect the possibility of Cd exposure. A definitive diagnosis can be made if Cd is detected in the occupational environment. Although the blood Cd concentration should be measured swiftly, a clear elevation is not inevitable. Treatment must focus especially on the prevention of death caused by pulmonary edema developing several days after exposure. General symptomatic treatment is provided as indicated for pneumonia. As indicated in animal experiments (McFarland, 1960), administration of BAL may be efficacious, but adequate attention must be paid to any renal injury. Although the symptoms will usually remit after approximately 10 days, in some cases respiratory symptoms may persist for years, and respiratory functional impairment may become permanent. Prevention relies on providing proper ventilation at sites of metal welding or cutting.

10.1.2 Ingestion

Because vomiting occurs immediately after the ingestion of Cd-contaminated foodstuffs or drinking water, the interval between ingestion and the onset of symptoms is short; this situation provides a clue to the possibility of Cd intoxication. A definitive diagnosis is made when a high concentration of Cd is detected in the food or water. Treatment is symptomatic, with recovery usually rapid and no apparent sequelae. Prevention is based on avoiding the use of Cd-containing materials in utensils coming into contact with food or water.

10.2 Chronic Intoxication

10.2.1 Diagnosis

With airborne exposure, pulmonary dysfunction, and renal tubular dysfunction are the major findings; renal tubular dysfunction predominates after oral exposure. In the case of airborne exposure, investigations of the inhaled substance and of the occupational environment, as well as measurements of Cd levels in the occupational environment, are performed, thereby confirming the presence of Cd exposure. When investigating the pulmonary influence, pulmonary function tests and chest radiography are performed, with special attention paid to the presence of any emphysematous changes. The earliest manifestation of the renal effects of Cd is proximal tubular dysfunction, of which the most extensively used biomarker is increased urinary β_2-mg excretion. Because β_2-mg is unstable at values of pH <5.5, at such times the levels of other low-molecular-weight proteins, such as α_1-microglobulin, retinol-binding protein (RBP), or NAG, should be measured instead. With progressive proximal tubular injury, the excretion into the urine of various other substances, such as total protein, glucose, amino acids (general), and NAG, also increases. With increasingly severe disease, the proximal tubular dysfunction worsens, followed by increasing glomerular dysfunction. Accordingly, the global picture of renal function must be grasped by evaluating various parameters of renal function, including serum creatinine and β_2-mg, as well as the %TRP, acid/base balance, and degree of creatinine clearance.

To determine the degree of Cd exposure, the levels of blood Cd and urinary Cd are measured (for the dose-response relationships, see Section 8.2). In general, the former reflects recent exposure, and the latter mainly the total amount accumulated in the body (see Section 5). With respect to the effects on bone, radiographic examinations focusing on the sites of pain are undertaken, and the degree of osteoporosis and presence/absence of osteomalacia are determined. In cases in which osteomalacia is suspected, with decreased serum inorganic phosphorus and serum calcium and elevated alkaline phosphatase, a bone biopsy is performed.

10.2.2 Treatment, Prognosis, and Prevention

No active therapy is available for pulmonary or renal damage. The most important intervention is cessation

of the Cd exposure. When osteomalacia complicates the clinical picture, vitamin D is prescribed. In patients with *Itai-Itai* disease, vitamin D_2 is administered orally (100,000 IU/day) in addition to 600,000 IU once per week through intramuscular injection. Once the subjective symptoms have ameliorated, oral administration of 50,000–100,000 IU/day is continued. Total doses exceeding 1 million IU have been administered to 34 of 83 patients with *Itai-Itai* disease, of whom 40–60% exhibited mitigation of bone pain and 50–80% improvement in bone radiographic findings. In recent years, 1α,25-dihydroxycholecalciferol has also been administered, although its effects have not been entirely satisfactory. In chronic intoxication, the use of chelating agents, such as Ca-EDTA, cannot be recommended. Even with cessation of Cd exposure, the manifestations of both pulmonary and renal injuries may progress, with the life prognosis also adversely affected (see Section 9).

Various organizations have recommended exposure limits considered to be safe for occupational exposure (see Section 4.2). The WHO recommends an ambient air level of 5ng/m^3 (WHO, 2000). The WHO/FAO (2003; 2005) JECFA has recommended an intake from food of 7μg/kg body weight/week (see Section 8.2). The reference doses (RfD) of 5×10^{-4} mg/kg/day in water and 1×10^{-3} mg/kg/day in food (IRIS, 1995) are similar to those suggested by JECFA. The WHO drinking water guideline is 3μg /L. IARC has classified Cd as a human carcinogen (cf. Section 7.3). The US EPA has a limit of 5μg/L of Cd in drinking water (5ppb); it does not allow Cd to be used in pesticides. The US Food and Drug Administration (FDA) limits the amount of Cd in food colors to 15mg/kg (15ppm; ATSDR, 2004). The US EPA classifies Cd as a probable human carcinogen (Group B1). The Swedish National Board of Occupational health lists Cd as a carcinogen.

Cadmium in food is regulated by the EU (EG nr 466/2001) to levels varying between 0.05mg/kg wet weight for some vegetables and fruits to 1mg/kg for the kidneys of cattle and pigs. For potatoes, a level of 0.1mg/kg applies; for wheat grain and rice, the level is 0.2mg/kg (see also Nordberg, M., 2004). The Swedish Food Administration recommends that liver and kidney intake be limited to 1–2 pieces/week, or preferably no consumption at all.

The allowable level of Cd in rice in China is 0.2mg/kg; in Japan, this level is 0.4mg/kg (Nogawa *et al*., 2004).

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CHAPTER

24

Chromium

SVERRE LANGÅRD AND MAX COSTA

ABSTRACT

Chromium (Cr) is found in nature primarily as chromite ore with Cr in the trivalent (Cr(III)) form. This ore is used for manufacturing monochromates, dichromates, chromic acid, and Cr pigments, as well as for Cr metal. Chromium chemicals are of great significance to the health of workers.

The uptake of Cr through the airways and the digestive tract is much quicker in the hexavalent (Cr(VI)) than in the Cr(III) state. Chromium is found in all human organs of adults and newborns. The Cr concentrations are generally highest in lung tissue, where the concentration tends to increase with age, most likely resulting from inhalation and retention of Cr compounds with low water solubility. Chromium is excreted through the urine and feces, predominantly through the urine. Chromium excretion in rats has been reported to take place exponentially and with half-times of 1.5, 5.9, and 83.4 days.

Trivalent Cr seems to be a nutritional supplement for humans and animals and may play a role in the in metabolism of glucose. However, the mechanism still remains to be further explained. The daily diet is the main source of Cr in humans, and minute amounts of the intake come from drinking water and inhalation, but any daily requirement is still not fully defined.

Exposure to Cr(VI) compounds is the main source of acute and long-term untoward effects of Cr. Allergic reaction of the skin is the adverse effect for Cr most frequently observed, and Cr(VI)-induced ulcerations of the mucosa of the nasal septum and ulcers of the skin are still seen frequently in developing countries.

Long-term inhalation exposure to various Cr(VI) compounds has been shown to result in a high risk of carcinomas of the respiratory organs. Some of the Cr(VI) compounds when inhaled seem to be among the most potent human carcinogens. Many animal experiments with installation and injection of different Cr compounds have resulted in local cancers in the skin and muscle tissue. Compounds of Cr(VI) are shown to induce mutations, chromosomal aberrations, DNA damage in the form of single-strand breaks, DNA–protein, and DNA–DNA crosslinks. The end reductant product of Cr(VI) in the cell is Cr(III), which is thought to be responsible for much of the DNA damage and mutations induced by Cr(VI).

The most significant exposure to Cr(III) compounds in the general population is through food, but no untoward effects of Cr have been reported on the basis of Cr in food.

Manufacture, exposure, and analyses of Cr compounds have been reviewed by IARC (IARC, 1990) and have been classified as carcinogenic to humans. Toxic and biological effects of Cr compounds are also covered in a number of reviews.

1 PHYSICAL AND CHEMICAL PROPERTIES

Chromium has the following characteristics: atomic weight, 52; atomic number, 24; density, 7.2; melting point, 1857 ± 20°C; boiling point, 2.672°C; crystalline form, steel-gray, cubic, very hard; oxidations states chiefly +2, +3, +6. Besides the naturally occurring ferrous chromite, the Cr compounds dealt with in this chapter include lead and barium chromates, and

sodium or potassium chromate or dichromates, Cr acetate, Cr citrate, chromic chloride, chromic acid mist, and chromates of zinc, calcium, and strontium.

The quite unstable divalent (chromous) ion is rapidly oxidized to the trivalent (Cr(III)) (chromic) form. Hexavalent Cr (Cr(VI)) compounds (chromates) are oxidized agents. Chromic acid is also a hexavalent compound. Reduction of Cr(VI) to the Cr(III) is of significance in the toxicity of Cr(VI) compounds.

Chromium in biological material is most likely always trivalent, except shortly after exposure to Cr(VI) compounds. Thus, it is unsafe for humans to become directly exposed to Cr(VI) compounds, but they can consume plants and animals that have been exposed to Cr(VI), because this form has been reduced to Cr(III) in these organisms. The trivalent form of Cr is 500–1000 times less toxic than the hexavalent form. Organic Cr-containing complexes of toxicological importance have not been reported.

2 METHODS OF CHEMICAL ANALYSIS

Several methods are available for the analytical determination of Cr in biological material in the range of interest (10–1000 µg/L), but it is not possible today to generalize as to what is the best method. The traditional colorimetric method that uses the violet complex of 1,5-diphenylcarbazide is still a valuable method for analyzing Cr in urine. The detection limit is 3.5 ng, but the method is subject to interference by other ions (Beyermann, 1962a,b). Methods for the analytical determination of Cr have been scrutinized by Beyermann (1962a,b), and a review of the literature on Cr analysis has been given in Cr (NAS, 1974), by the IARC (1990), and Torgrimsen (1982).

Flame photometry and X-ray emission spectrography are less sensitive than the diphenylcarbazide method. Polarographic methods with a detection limit as low as 1 ng are reported, but these are also subject to interference by other ions (Beyerman 1962a,b). Chromium analyses by neutron activation are currently being used for analyses of Cr in urine, serum, subcellular fractions, and other biological materials (Avelar *et al.*, 2002; Chen, 2004; Cornelis *et al.*, 1975; Feng *et al.*, 2003; Liu *et al.*, 2001). A gas–liquid chromatographic method with a detection limit of 0.01 ng for Cr has been described, but the practical experience with the method is limited to date (Sievers *et al.*, 1967). Atomic absorption spectrophotometry has previously given too high values when normal concentrations of Cr in plasma or in urine were estimated because of matrix influence (Gutherie *et al.*, 1978; Kayne *et al.*, 1978). By use of the most recent instrumentation, a detection limit of 0.2 ng/µL urine sample was reported (Routh, 1980). A "proposed selected method" is published (Veillon *et al.*, 1982a,b). Stable isotope dilution by combined gas chromatography-mass spectrometry with dual ion monitoring allows precise determination of Cr at sub ppb levels (Veillon *et al.*, 1982a,b). Atomic absorption with graphite furnace and mass spectrometry analysis are currently the preferred methods.

Separate determination (speciation) of trivalent and hexavalent Cr is possible by analyzing the sample before and after oxidizing the trivalent to the hexavalent form. Only the Cr(VI) form is determined both by the colorimetric 1,5-diphenylcarbazide method and by atomic absorption spectrometry method if organic extraction of the Cr-carbazide complex is used to concentrate the sample (Mertz, 1969). Variables not controlled in ordinary extraction and mineralization procedures are introduced when Cr is bound to organic compounds in the glucose tolerance factor (GTF). Because no one has been able to identify GTF, there is still some doubt about what the GTF really is. A more recent Cr (III) binding substance in mammals has been identified as chromodulin, and although the amino acid composition is known, the structure of the molecule with Cr (III) has not been determined (Vincent, 2000).

3 MANUFACTURE AND USES

3.1 Manufacture

In various nonscientific sources it is stated that the Hittites had used swords containing Cr when they came into Egypt approximately 1300–1200 BC. It is, however, well documented that swords used in the Qin Dynasty were plated with Cr (displayed at the Provincial Museum as well as at the terra cotta museum of Xian, China). In Europe, Cr is considered to be isolated as CrO_3 by Louis-Nicholas Vauquenlin in 1797–1798. The mineral Cr-containing crocoite ("red lead") was found in Siberia in the mid-1700s; chromite, $FeOCr_2O_3$, is the only significant Cr ore, however, not found in pure form. The mineral also contains silica in varying amounts and small quantities of other compounds. The highest grade of ore contains approximately 55% chromic oxide. Trivalent, as well as hexavalent, Cr is found in nature, but the trivalent is the more prevalent form.

The world production of chromite in 1976 was approximately 8.6 million tons, the U.S.S.R. (about 2.1 million tons), and the Republic of South Africa (approximately 2.4 million tons), being the main producers (Morning, 1978). As of 1999, the world production of chromite had risen to 12.8 million tons of which 5.6 million tons are produced in South Africa

(U.S. Geology Service). Ferrochromium is manufactured by direct reduction of the ore. Chromium metal is produced either electrolytically after chemical treatment of high-carbon ferrochromium or by reduction of Cr compounds.

Sodium chromate and dichromate are produced by roasting chromate ore with soda ash, or with soda ash and lime, followed by chemical treatment for removing impurities. Most other Cr compounds are produced from sodium chromate or dichromate.

3.2 Uses

Industries like metallurgical, refractory, and chemical industries are the principal industrial consumers of Cr. The U.S. figures for consumption by these industries were 60%, 20%, and 20%, respectively, of the total consumption. Ferrochromium and Cr metal are the most significant classes of Cr used in the alloy industry. After Cr was discovered in 1797–1798, Cr and its salts found a wide range of applications in the chemical industry, graphics industry, artistic paints, anticorrosion paints, electroplating, steel alloys, stainless steel (SS) welding, and a multitude of other uses. The tanning industry was for many years an important consumer of Cr. There are millions of SS welders worldwide (Stern, 1981; 1982) and SS welding may, at present, be the most common sources of human exposure to Cr in the workplace. The wide range of uses of Cr has resulted in exposure to Cr compounds for numerous workers.

4 CONCENTRATIONS IN THE ENVIRONMENT

4.1 Occurrence in Natural Environments and Soil

Chromium occurs in nature primarily as ferrous chromite, where Cr is in the chromic state, which is the most stabile state of oxidation. Chromium also occurs in the divalent state, the hexavalent state, and the very unstable four- and five-valent states. Except during reduction from Cr(VI) to Cr(III) in a biological environment, during which five- and four-valent Cr may occur, Cr(III) and the Cr(VI) states only are of interest in relation to human exposure. Cr(III)) compounds are generally quite insoluble in water at physiological pH, probably because they can form polymeric species (Zhitkovich, 2005). Some Cr(VI) compounds (i.e., Cr_2O_3), are readily soluble in water, whereas calcium chromate and lead chromate are characterized by very low water solubility, and some are intermediate soluble (i.e., zinc chromate).

4.2 Concentrations in Food

The intake of Cr through the daily diet has been estimated to range from 0.03–0.1 mg (Schlettwein-Gsell and Mommsen-Straub, 1973). Fishbein (1976) estimated the mean daily intake to be 280, 4, and 0.28 µg from food, water, and air, respectively. From Belgium, Deelstra *et al*. (1988) reported that human milk contained on average 0.18 ng Cr/mL. Because other sources contribute minor amounts only compared with these figures, the figures also represent an estimate of the total daily intake of Cr for the general population. Food items vary considerably in the concentration of Cr. Among major sources are meat and vegetables. Unrefined sugar, white fish, vegetable oil, and fruit contain smaller amounts. Concentrations are reported from nondetectable to approximately 0.5 mg/kg wet weight for various food items.

4.3 Water and Ambient Air

The Cr concentration in rivers and lakes is usually between 1 and 10 µg/L—a fraction of which may be in the hexavalent form, that in seawater being considerably less: from <0.1 to approximately 5 µg/L (NAS, 1974). Municipal drinking water has been reported to contain higher amounts of Cr than river water.

Soil concentrations range from trace to 250 mg/kg, occasionally higher. The average Cr concentration in the earth's crust is 125 mg/kg. Chromium in phosphates applied in fertilizers could be a significant source of Cr in soil, in water, and in some food.

Urban air concentrations of Cr have been reported from <10 ng/m^3 up to approximately 50 ng/m^3. Annual mean values for rural stations seldom reached 10 ng/m^3 (NAS, 1974). Average levels of total Cr in the urban air of Turin in 1986–1988 were found to be 28.6 ng/m^3 (Pavan *et al.*, 1988).

4.4 Tobacco

Cigarettes have been reported to contain 390 µg/kg of Cr (Schroeder *et al.*, 1962), but there have been no published estimates of the inhaled amount of Cr from smoking. Later values of 0.24–14.6 mg/kg have been reported (Al Badri *et al*., 1979), but no new systematic data are available.

4.5 Daily Intake in Humans

The daily intake of Cr through food has been estimated to be in the range of 0.03–0.1 mg (Schlettwein-Gsell and Mommsen-Straub, 1973). Fishbein (1976) estimated the mean daily intake to be 280, 4, and 0.28 µg from food, water, and air, respectively. In

reports from Europe and Mexico, the daily intake of Cr in adults is measured to be ±30–35 μg/day (Grijalva *et al*., 2001; Wilpinger *et al*., 1996). Although some regulatory authorities recommend daily intake of Cr(III) at 100–200 35 μg/day, there is no scientific evidence giving support to such a specific daily requirement.

5 WORK-RELATED EXPOSURE

Potential hazardous exposures are incurred in the production of dichromates, in the use of chromates in the chemical industry, in the SS industry, in the manufacture of alloys, in refractory work, and in Cr-electroplating. In the latter industry, health hazards are related to the Cr-containing mist. Chromium inhaled as Cr(VI) is partially reduced to Cr(III) after being deposited in the airways. A fraction of the Cr may be transported in the Cr(VI) form by the mucous escalator to the pharynx and subsequently being swallowed—the size of this fraction depending on the inhaled aerosols particle distribution and the efficacy of the escalator. Hence, inhalation of Cr(VI) may lead to ingestion of Cr(VI).

Exposure to Cr during welding of SS may constitute a health hazard, both because Cr is a significant constituent in SS and acid-stable steel (i.e., 18–21% Cr) and because Cr-containing electrodes are used. Whenever mild steel is covered by Cr-containing anticorrosive paints, welding on mild steel may also entail a Cr(VI)-related health hazard to welders (Gylseth *et al*., 1977; Ruf, 1970; Stern, 1981; 1982). When using the manual metal arc (MMA) method for welding on ship sections, Karlsen *et al*. (1994) found average Cr(VI) levels in the work atmosphere at 140 μg/m^3, Cr(VI) comprising approximately 50% of the total Cr. Conversely, Karlsen *et al*. (1996) measured average air levels of total Cr and Cr(VI) at 230 and 3.3 μg/m^3, respectively, during SS welding when applying the MIG/MAG method.

Air levels of Cr in industry have been reported only to a limited degree. Mancuso (1951) reported air concentrations up to 1 mg/m^3 of Cr in a chromate plant. Most concentrations are reported in the range of 0.26–0.51 mg/m^3, but up to approximately 20 mg/m^3 have been reported (IARC, 1990). A 5-day, 8-hour value of 1.35 mg/m^3 of chromates in air for sack-filling operation in an old chromate plant was reported (Langård and Norseth, 1975), whereas modern plants showed levels <0.1 mg/m^3. Most Cr concentrations recorded after personal sampling during 8 hours in a ferrochromium manufacturing plant were in the range of 0.02–0.05 mg/m^3, but occasional values were up to 0.4 mg/m^3 (Gylseth and Øien, 1975). In a review, Cr exposure up to 5 mg/m^3 in the Cr-plating industry was mentioned, but most exposure levels reported were in the range of 0.1–0.2 mg/m^3 (NIOSH, 1973). In modern plants, values are often <10 μg/m^3 (IARC, 1990). Until recently, the OSHA permissible limit of exposure to Cr(VI) was 100 μg/m^3, which in a recently conducted epidemiological study was higher than the levels that gave a statistically significant elevated incidence of lung cancer (Gibb *et al*., 2000). This study motivated OSHA to lower the standard to 51 μg/m^3 and now they are considering a permissible exposure level (PEL) of 1 μg/m^3. Some other countries introduced PEL for Cr(VI) of 1 μg/m^3 in the 1980s.

6 UPTAKE AND METABOLISM

6.1 Dietary Intake

Animal experiments indicate that Cr(III) is poorly absorbed from the digestive tract; values less than 1% of an oral dose have been reported (Mertz, 1969). Chromates are absorbed at 3–6% in rats (Mertz *et al*., 1965) and approximately 2% in humans (Donaldson and Barreras, 1966). Recent studies have shown that human absorption of Cr(VI) can be higher and are quite variable not only between individuals but also in the same individual at various times (Costa, 1997). There is evidence that not only the valence state of Cr in the diet, but also the functional state of the intestines, has a bearing on absorption, because the balance between Cr(III) and Cr(VI) may be altered (Donaldson and Barreras, 1966). Enhanced absorption of Cr(VI) has been observed in achylic patients, probably because of the absence of reduction of the hexavalent state by gastric juice. These absorption values, which are extrapolated from urinary excretion after oral administration, may be underestimated, because the gastrointestinal tract also takes part in Cr excretion (Hopkins, 1963).

De Flora *et al*. (1984; 1997; 2000) have shown that the mucosa lining of the esophagus and stomach possess a large capacity to reduce Cr(VI) to Cr(III). Hence, this reducing capacity seems likely to be the prime explanation that even quite heavy, long-term inhalation exposure is unable to induce stomach cancer at a risk level that can be revealed by the currently available methods in epidemiology. Testing the reductive capacity of various cells of Wistar rats, Shrivastava *et al*. (2003) found that upper villus cells of the of the intestinal tract reduced 100% of 10 μg/mL Cr(VI) in the form of $K_2Cr_2O_7$ in the presence of 5% CO_2, being the strongest reducer of the cell types tested. Assuming that approximately 50% of Cr_2O_3 deposited in the

airways in the study by Sorahan *et al*. (1987) at air levels up to 8.0 mg/m^3 as Cr_2O_3, which was not documented to cause digestive cancer, it may be assumed that up to approximately 7 mg of Cr_2O_3 ingested per hour could be reduced to Cr(III). However, Zhitkovich (2005) has shed some doubt as to the preceding, suggesting that the use of diphenylcarbazide in the presence of 8% sulfuric acid with biological materials present in the acid (DNA, protein) could have enhanced the Cr(VI) reduction (De Flora, 1984; 2000; De Flora *et al*., 1997). In a study by Paustenbach *et al*. (2003), the authors estimated, on the basis of results from nine publications, that exposure by ingestion to concentrations of Cr(VI) in water up to 10 mg/L did not overwhelm the reductive capacity of the stomach and blood and that the available data indicate that Cr(VI) ingested in tap water at concentrations <2 mg/L is rapidly reduced to Cr(III). However, as shown by Kanojia *et al*. (1998), feeding female rats before gestation at different doses of $K_2Cr_2O_7$ in drinking water resulted in significant elevation of the blood Cr-level of the mother, placenta, and fetuses, showing that uptake had taken place.

6.2 Inhalation

There are only a few reports on the uptake of Cr compounds after exposure by inhalation. The high concentration of Cr found in the lung tissue of humans exposed to Cr indicates that at least part of the Cr is deposited in the pulmonary tissue in an insoluble form. Because Cr(VI) compounds are more soluble, it may be assumed that these compounds are more easily absorbed than Cr(III) compounds, but high uptake of the Cr(VI) form as a lignosulfate has been demonstrated (Kiilunen *et al*., 1983).

Laskin and Isloa (1972) studied the retention of Cr in the lungs of animals for 48 hours after 6 hours of inhalation; however, neither dose levels, Cr compounds, nor animal species were reported. Urinary excretion of Cr increased for the first 6 hours, and then gradually decreased. Fecal excretion of Cr also increased. Experiments with rats inhaling zinc-chromate dust have shown that water-soluble chromates may be taken up in the lungs in the hexavalent state and transferred into the red blood cells before reduction to the trivalent state takes place (Langård *et al*., 1978). The results in this study also suggest that after inhalation of water-soluble chromates, the urine is the major route of Cr excretion. A correlation between exposure to Cr and the urinary excretion of Cr was demonstrated; results that have been reported also by other authors (Mutti *et al*., 1979; Stridsklev *et al*., 1993; Tola *et al*., 1977; Tossavainen *et al*., 1980).

6.3 Distribution

The distribution of Cr in the rat depends on the valency and compound of Cr (Hopkins, 1965; Kraintz and Talmage, 1952; Visek *et al*., 1953). The relative impermeability of biological membranes to Cr(III) compared with the Cr(VI) compounds, and the rapid reduction of the Cr(VI) compounds with subsequent binding of the Cr(III) to macromolecules has made it challenging to understand the uptake and distribution of Cr in the body (Alexander *et al*., 1982; Connett and Wetterhahn, 1983; Jennette, 1980; Langård, 1979; 1982; Levis and Bianchi, 1982). Trivalent Cr acetate or citrate is also rapidly excreted, whereas the tissue concentrations after corresponding doses of chromic chloride or chromates are higher at the same time interval. Organs that specifically retained Cr in experiments with dose levels of 60–250 µg/animal were the reticuloendothelial system, the liver, the spleen, and the bone marrow (Visek *et al*., 1953). Hopkins (1965) reported retention of Cr after injection of the trivalent form to the rat (i.e., in the bone marrow, the spleen, the testes, and in the epididymidis) at dose levels were 10 and 1 µg/kg rat weight, respectively. The organ retention of Cr after chromate injection to mice decreases with the age of the animal (Vittorio *et al*., 1962). However, animals dosed with Cr(VI) compared with Cr(III) by ingestion absorb considerably more Cr in tissues such as brain, liver, and kidney (Costa, 1997).

The distribution appeared to be different after administration of Cr as GTF, with the highest concentration in the liver, followed by the uterus and the kidney (Mertz, 1975; Mertz and Roginski, 1971). Chromium is found in the newborn and in the fetus. High natural Cr content in the mother's diet enhances the Cr content in the fetus (Mertz, 1975). Transfer of Cr as the GTF over the placental membrane was reported to take place (Mertz *et al*., 1969; Visek *et al*., 1953), and Diab and Södermark (1972) demonstrated transplacental transfer of Cr chloride in mice using a radiographic method. The embryonic and fetal uptake of Cr after exposing pregnant rats to chromate was 10 times that found after exposure to corresponding doses of Cr(III) (Danielsson *et al*., 1982).

6.4 Excretion and Biological Half-Life

Once absorbed and bound in biological tissues, Cr compounds are in the trivalent form (Mertz, 1969). When the reducing capacity of liver cells is compromised, however, the hexavalent form may be excreted in the bile (Norseth *et al*., 1982). All excretion mechanism and results are related to the valency state.

After parenteral administration to rats, Cr is excreted predominantly in the urine. Hopkins (1965) found less than 2% of the intravenous dose in the feces 8 hours after injection. Visek *et al*. (1953) found less than 20% after 4 days. Studies on the mechanism of Cr excretion by the kidneys indicate glomerular filtration followed by tubular reabsorption of up to 60% of the filtered amount (Collins *et al*., 1961).

The rate of excretion of Cr in the gastrointestinal tract is not known. Biliary excretion of Cr has been demonstrated in the rat (Cikrt and Bencho, 1979; Norseth *et al*., 1982); less than 1% of an intravenously injected dose of the trivalent form was excreted in 5 hours, 6–8% of corresponding doses of the hexavalent form was found (Norseth *et al*., 1982). The fecal content of Cr may vary considerably and is mainly a consequence of ingested unabsorbed Cr compounds.

The elimination curve for Cr, as measured by whole-body counting, has an exponential form. In rats, three different compartments of the curve have been identified with half-times of 0.5, 5.9, and 83.4 days, respectively (Mertz *et al*., 1965).

In kinetic studies in humans to evaluate red cell lifetime, a rapid excretion component related to nonerythrocyte Cr has been identified. Chromium used for labeling of erythrocytes is almost exclusively excreted in the urine. Kinetic studies of this rapid component have not been reported, and half-time is only given for Cr representing the red cell compartment (Shih *et al*., 1972). Tossavainen *et al*. (1980) calculated a half–life of 15–41 hours for Cr in urine from four welders using a linear one-compartment kinetic model. The kinetics of excretion of Cr as the GTF or chromomodulin is not known.

6.5 Concentrations in Biological Fluids and Tissues

The highest concentration of Cr in humans is found in hair, values from 200–2,000 µg/kg as reported (Mertz, 1969). Schroeder *et al*. (1962) reported on 700 µg/kg of Cr in the lung for persons in the New York/Chicago area; other organs had lower concentration: liver 270 µg/kg and kidney 90 µg/kg. There are geographical variations in Cr concentrations: the values from the Denver area were 140 µg/kg for the lung, 30 µg/kg for the liver, and 40 µg/kg for the kidney. In a case-referent study on lung cancer in Finland, Antilla *et al*. (1989) found that average lung tissue levels of Cr in smokers was 6.4 µg/g versus 2.2 µg/g in nonsmoking referents. A high concentration of Cr in the lung and a lower concentration in other organs were confirmed by Tipton and Cook (1963, 1965) both in the United States and other parts of the world. Values up to approximately 300 µg/kg for the lung and 40 µg/kg for the liver and approximately 15 µg/kg for the kidneys were reported from Japan (Hyodo *et al*., 1980). All values are on a wet-weight basis. Gerhardsson and Nordberg (1993) reported on a median lung tissue concentration of 390 µg Cr/kg wet weight in smelter workers in Northern Sweden having attracted lung cancer compared with 110 µg Cr/kg in deceased rural referents.

Previously reported normal levels of Cr in blood of 3–30 µg/L (Feldman *et al*., 1968) and urinary excretion of up to 10 µg/day (Cornelius *et al*., 1975; Mertz, 1975) were probably too high. Later the concentration of Cr in plasma was reported to be 0.14 µg/L (Nomiyama *et al*., 1980; Veillon *et al*., 1979). Paakka *et al*. (1989) determined the lung tissue level of Cr in 45 deceased subjects in Northern Finland using plasma emission spectroscopy and found the average level at 1.3 and 4.8 µg/g in dry and wet tissue, respectively. For a review, see Guthrie (1982).

The Cr concentration in all tissues decreases from the moment of birth up to the age of approximately 10. After this time, there is a slight increase in lung concentration, but a continuing fall in all other organs (Schroeder *et al*., 1962). This indicates that Cr in the lungs is a result of deposition from inhaled air, whereas Cr in food is the main source of Cr in other organs.

7 DOSE AND OUTCOME EFFECTS

Trivalent Cr has been suggested to be essential for the maintenance of normal glucose tolerance in animals and humans, and the factor of the group of factors containing Cr(III), called GTF (chromodulin) has been suggested to be responsible for the favorable action of Cr (Mertz, 1969; 1975). The Cr status in diabetes subjects seems to be abnormal, and Cr is capable of potentiating insulin, but so far the exact structure(s) of the biological active Cr complex(es) has (have) not been identified (Mertz, 1982). Therefore, Cr supplementation still cannot be considered as a therapeutic agent in established diabetes, however, it may delay or prevent the appearance of diabetes in some cases (Guthrie, 1982). In reviewing the biochemistry of Cr, Vincent (2000) indicated that recent studies had shed some light on how Cr contributes to maintaining a proper carbohydrate metabolism on a molecular level, where it seems that the oligopeptide chromodulin seems to play a role in binding chromic ions in response to a chromic ion flux, resulting in stimulation of the receptor's tyrosine kinase activity. In this, the chromodulin may play a role in an autoamplification mechanism in insulin signaling.

Chromium deficiency has been described both in humans and in animals, but a quantitative definition of the daily requirement of Cr in human nutrition has not been formulated (Mertz, 1969).

7.1 Local Effects

7.1.1 Animals

Numerous publications reporting on local effects of Cr compounds in humans are presented, but few experiments have verified such effects in animals. Samitz and Epstein (1962) succeeded in inducing ulcers by applying various Cr(VI) compounds to animal's skin. They also demonstrated that a local skin defect might be considered a prerequisite for the development of chrome ulcers. Mosinger and Fiorentini (1954) induced skin ulcers in different animal species through application of potassium chromate to the skin.

7.1.2 Humans

7.1.2.1 Chrome Ulcers

Generally, chrome ulcers of the skin are induced by the corrosive action of Cr(VI) compounds. When chromic acid, dichromate compounds, or other Cr(VI) compounds are deposited on broken skin surface, a deeply penetrating round hole may develop (Dewirtz, 1929). Favored sites for ulcer development are the nail root areas, over the knuckles and finger webs, on the back of the hands, and on the forearm (Maloof, 1955). The ulcer may persist for months and slowly heals. These ulcers are not related to allergic sensitization to Cr compounds (Edmunson, 1951).

7.1.2.2 Reactions in Upper Airways

7.1.2.2.1 Corrosive Reaction in the Nasal Septum Ulcerations on and perforations of the nasal septum in chromate workers were frequently reported on in the 20th century, but few have been reported recently in the Western world. The site of the ulceration is usually approximately 1.5–2 cm from the anterior and lower margin of the septum (Kleinfeld and Rosso, 1965). Data by Lindberg and Hedenstierna (1983) indicated that inhalation of 20 µg Cr(VI)/m^3 could result in septum ulcers. Generally, the ulcerations seem to result from deposition of relatively large chromate-containing particles on the mucous membrane. Ulceration on both sides of the septum may result in necrosis of the cartilage, leading to septum perforation (Leineberg, 1955).

7.1.2.3 Irritative Dermatitis

7.1.2.3.1 Acute Irritative Dermatitis This disease may be observed in the Cr industry, particularly among workers coming in contact with Cr(VI) compounds. This dermatitic reaction seems to become less prominent by renewed contact. It must be differentiated from allergic eczematous dermatitis.

7.1.2.4 Allergic Dermatitis

7.1.2.4.1 Allergic Eczematous Dermatitis This type of skin reaction was most likely observed years before evidence of Cr sensitivity was reported by Engelhart and Mayer (1931). A large proportion (84%) of chromate workers with dermatitis reacted positively when tested with 0.5% ammonium dichromate, but none reacted to Cr(III) compounds. Samitz *et al*. (1969) found Cr(VI) compounds to be strong sensitizers, whereas Cr(III) compounds were poor sensitizers and elicitors. It is concluded in different reviews that Cr(VI) compounds (Pedersen, 1982; Polak *et al*., 1973; Proctor *et al*., 1998) are responsible for most cases. These reviews conclude that the difference in the sensitizing property of the two valence states results from the fact that Cr(VI) is capable of penetrating undamaged skin where it is reduced to Cr(III) that binds covalently to proteins or other skin components to form allergens. When sensitization has developed, even the small amounts of Cr(III) compounds that penetrate the skin may be capable of eliciting reactions.

Allergic eczematous dermatitis is reported in a variety of workers (i.e., housewives, cement workers, woodworkers, limestone workers, welders, painters, printers, leather workers, polishers, and others) (Pedersen, 1982; Polak *et al*., 1973; Proctor *et al*., 1998). Cement eczema has been shown to be induced by traces of chromate compounds in the cement (Engebrigtsen, 1952; Freget, 1981).

The prevalence of Cr-induced eczema has been shown to be higher in males than in females. In males living in industrialized countries, Cr sensitivity seems to be the most significant skin sensitizer, the skin patch test being positive in 8–15% of all male patients from eczematous dermatitis (Proctor *et al*., 1998), whereas the prevalence in female patients is approximately 3–5%. This difference between genders could be because men are more likely to be exposed to Cr(VI) in their work than are women (Freget *et al*., 1969; Langård and Hensten-Pettersen, 1981). Estimates of the prevalence of Cr sensitivity in the general population varies from less 1/1000 (Proctor *et al*., 1998) to 17/1000 (Peltonen and Fraki. 1983).

For many years, potassium dichromate has been the most commonly used substance for allergy testing to Cr (Freget and Bandmann, 1975). Most frequently, a concentration of 0.5% of the substance dissolved in water or petrolatum is used. Test procedures for performing different skin patch tests are reviewed by Polak *et al*. (1973) and Pedersen (1982) and surveyed by Proctor *et al*. (1998).

7.1.2.5 Local Effects in the Lungs

7.1.2.5.1 Local Effects in the Lungs Mancuso and Hueper (1951) reported on a spotty, moderately severe not nodular pneumoconiosis in chromate workers. Sluis-Cremer and du Toit (1968) reported on fine nodular pneumoconiosis—somewhat more radiopaque than in simple coal miner's pneumoconiosis—in a few chromite workers in South Africa, who might also have been exposed to crystalline quartz. The results in these reports have never been confirmed in other studies.

7.2 Systemic Effects and Dose-Effect and Dose-Response

7.2.1 Animals

Although some toxic effects of chromates in humans have been known for more than 100 years (Brieger, 1920; Major, 1922; Reischer and Glesinger, 1922), relatively few experiments were carried out in animals. Most of the reports based on animal experiments have considered untoward effects in more than one organ, and some of these effects may be secondary to effects in other organs. Tandon (1982) reviewed systemic effects in animals resulting from Cr compounds.

7.2.1.1 Effects in the Kidneys

Hunter and Roberts (1933) administered potassium dichromate in high doses subcutaneously to rhesus monkeys and found necrosis of the proximal and the distal tubules, as well as diminution in the number of glomerular epithelial and endothelial cells, and some effect on the capillary basement membrane. Later, a large number of studies demonstrated necrosis of the proximal tubular epithelium after intravenous administration of chromate doses ranging from 0.5–30 mg/kg (Tandon, 1982). In an extensive study, Evan and Dail (1974) administered 10 and 20 mg of aqueous sodium chromate/kg intraperitoneally to rats and found lysozymuria and proteinuria 6 hours after the 20 mg/kg dose and 24 hours after the 10 mg/kg dose, respectively. By use of electron microscopy techniques, they showed that sodium chromate at these dose levels selectively caused damage in the convoluted portion of the proximal tubuli, with no evidence of damage to glomeruli, most of the nephrons being affected. The sequential changes were (1) sealing and loss of microvilli; (2) formation of intracellular vacuoles of varied sizes; (3) mitochondria in the orthodox configuration; (4) appearance of cytosegresomes; (5) pyknosis of the nuclei; (6) mitochondrial swelling; (7) cytoplasmic liquefaction; and (8) rupture of the cell membrane. Evan and Dail (1974) suggested that many of the observed effects are related to an induced impairment of the electron-transport system and the phosphorylation in the mitochondria. Schubert *et al*. (1971) demonstrated similar changes in the proximal tubuli after subcutaneous administration of 15 mg/kg potassium dichromate to rats, and Langård (1979) showed perivascular edema and focal necrosis of the proximal tubuli 4 days after intravenous administration of as low a dose as 0.56 mg/kg sodium chromate. No dose-effect or dose-response relationship has been established for tubular damage after administration of Cr(VI).

7.2.1.2 Effects on the Liver

Few studies have focused on hepatotoxic effects of Cr compounds. Tandon *et al*. (1978) compared the hepatotoxic effects of Cr(III) and Cr(VI) by intraperitoneal administration of 1.3 mg Cr/ kg to rabbits for 6 weeks as Cr nitrate and potassium dichromate, respectively. The Cr(VI) compound resulted in thickening of the liver capsule, congestion in the central vein, and adjacent sinusoids and coagulation necrosis of hepatic cells throughout the parenchyma. The Cr(III) compound also resulted in congestion in the central vein and necrosis of the hepatocytes, but less extensive. The mechanisms of hepatotoxicity were not clarified in this study, and some untoward effects may be secondary to intravascular microthrombi. Dinu and Boghianu (1973) suggested that potassium chromate given to rats in the diet at 100 ppm concentration inhibits the activity of glucose-6-phosphatase. The biochemical mechanisms involved in hepatocellular necrosis are not fully explained, but inhibition of this enzyme may result in hypoglycemia that may bear some relation to the toxicity. No dose-response relationship has been established for the hepatotoxic effects of Cr(VI) or for Cr(III) compounds.

7.2.1.3 Effects on the Airways

Akatsuka and Fairhall (1934) exposed cats to 80–115 mg/m^3 Cr(III) salts 1 hour daily for 4 months and fed another group a diet containing high amounts of chromic salts. No adverse effects were observed in the airways. Nettesheim *et al*. (1971) observed enhance subepithelial connective tissue and flattened epithelium in the large bronchi when exposing mice to calcium chromates by inhalation. Also, they observed morphological changes in tracheal and submandibular lymph nodes. To examine whether observations in humans could be confirmed, indicating that inhalation of chromite dust may induce pneumoconiosis, Swensson (1977) introduced 40 mg chromite particulates suspended in Ringer's solution intratracheally to rats, but did not observe pneumoconiosis.

7.2.1.4 Effects in the Digestive Tract

After administering potassium chromate to small animals by various routes, the total dose varying from

2–10 mg, Mosinger and Fiorentini (1954) observed acute gastritis and enteritis, necrosis of the proximal tubuli, and hepatocellular necrosis. After long-term inhalation of calcium chromate in mice, Nettesheim *et al*. (1971) observed small ulcerations in the stomach and the intestinal mucosa. Whether these effects observed in the digestive tract are systemic effects or local, resulting from particles of chromate transported to the site, cannot be concluded from these studies.

7.2.1.5 Effects on the Blood and Blood Forming Organs

Exposure to significant doses of chromates result in erythrocytes glutathione reductase inhibition (Koutras *et al*., 1965), possibly explaining the lowered capacity to reduce methemoglobin to hemoglobin, as observed in humans (Brieger, 1920; Schlatter and Kissling, 1973).

7.2.2 Humans

7.2.2.1 Effects on the Airways

Inhalation of dust containing chromate or chromic acid fumes is reported by a number of authors to result in bronchial asthma, most authors referring to a lag time of 4–8 hours between exposure to Cr(VI) compounds and the asthmatic attack (Card, 1935; Joules, 1932; Williams, 1969). Once sensitized, the patient is likely to react with a new attack on renewed exposure to chromate-containing aerosols and possibly on subcutaneous provocation. Asthmatic reactions were also reported in workers of the ferrochromium industry where they are exposed to chromate-containing mixed dust (Broch, 1949; Langård, 1980).

7.2.2.2. Effects on the Kidneys

Tubular necrosis has been reported to contribute to death in fatal intoxications with chromates, but these effects are more predominant in cases in which the ingested chromate dose is not fatal (ingested dose, 1.5–2 g or less). Ingestion of approximately 5 g of chromate may result in toxic effects within 12 hours. Ingestion of smaller amounts (i.e., 1 or 2 g in adults) may result in tubular necrosis 1–4 days after ingestion (Ellis *et al.,* 1982; Fristedt *et al*., 1965; Kaufman *et al*., 1970). Nephrotoxic effects are also reported after uptake of chromate acid through the skin (Schiffl *et al*., 1982). Dartsch *et al*. (1998), exposed epithelial cells from the kidneys to Cr(III) and Cr(VI) compounds at concentrations raging from 0.01–1.0 mmol/L observed that 5 mmol/L of Cr(VI) resulted in 50% cell death. None of various chloride channel blockers applied were able to prevent Cr(VI)-induced cell damage to occur.

7.2.2.3 Effects on the Liver

In some fatal cases after ingestion of chromates, death may in part be attributed to diffuse necrosis of the liver. These untoward effects seem to be most predominant in cases where the ingested dose is 1.5–2 g or less. Subsequent to ingestion of ±5g of chromate, these toxic effects may occur within 12 hours. After ingestion of smaller amounts (i.e., 1–2 g for adults), these effects may occur within 1–4 days (Ellis *et al*., 1982; Fristedt *et al*., 1965; Kaufman *et al*., 1970). One author (Pascale *et al.,* 1952) reported on centrolobular liver necrosis after inhalation of approximately 2.5 mg Cr(VI)/m^3 of mist from chromic acid baths, but no other authors have reported similar observations.

7.2.2.4 Effects on the Cardiovascular System

Subsequent to ingestion of 5 g or more of chromates, bleeding from the digestive tract and massive loss of fluid may take place, and death may occur in a clinical picture of cardiovascular shock and a neurological picture with cramps, convulsions, or generalized seizures. In nonfatal cases, *hemorrhagic diathesis* may be a predominant symptom. It is not clear whether chromates may exert direct effects on the heart or if the cardiovascular symptoms are secondary to untoward effects in other organs (Brieger, 1920; Ellis *et al*., 1982; Fristedt *et al*., 1965; Kaufman *et al*., 1970; Partington, 1950; Schiffl *et al*., 1982; Schlatter and Kissling, 1973).

7.3 Carcinogenic, Mutagenic, and Teratogenic Effects

7.3.1 Animals

7.3.1.1 Carcinogenic

Although well documented in workers exposed to chromates, experiments in animals have not been clearly successful in identifying the Cr compounds that may enhance the risk of bronchial cancer. Hueper (1958) reported a small number of malignant tumors in rats after intramuscular and intraperitoneal administration of roasted chromite ore. Baetjer *et al*. (1959) were not able to induce bronchial cancers in mice and rats by daily chamber exposure to 2 mg/m^3 mixed chromate particulates, the animals being exposed 4 hours per day, 5 days a week until death or scarification. These authors also reported on four cases of lymphosarcomas, which has not been observed by other authors. Payne (1960) demonstrated that calcium chromate was capable of inducing injection-site sarcomas in mice, and Hueper and Payne (1962) found three fibrosarcomas after 2 years subsequent to intratracheal administration of 10–12.5 mg calcium and strontium chromate to 218 rats. Nettesheim *et al*. (1971) found adenocarcinomas and adenomas induced in the bronchial tree of some of 1090 mice exposed to calcium chromate aerosol at 13 mg/m^3 in inhalation

chambers for 35 hours/week lifetime. That study is the only one in the literature showing a carcinogenic effect of chromates after inhalation. A recent study examined the carcinogenic activity of Cr(VI) by drinking water exposure in hairless female mice that were also exposed to solar UV radiation. (Davidson *et al*., 2004) The doses of potassium chromate used in the drinking water were in the range of what humans have been exposed to (1–5 ppm), and neither the chromate nor UV alone induced tumors, but the combination of the two greatly enhanced the incidence of malignant squamous cell carcinomas. The effect of chromate on skin cancer was linearly dose related, but the formation of malignant squamous cell carcinomas required exposure to solar UV (Davidson *et al*., 2004). Confirmation in other studies that use the addition of sex and strains of animals are needed.

7.3.1.2 Teratogenic Effects

Diab and Södemark (1972) demonstrated, by using a whole-body radioautography, Cr(III) in the skin and the vertebral bone of the fetuses 1 hour after intravenous dosage of isotonic $^{51}CrCl_3$ to pregnant mice. They also found that the later in the pregnancy the dose was administered, the more Cr could be demonstrated in the fetuses. Visek *et al*. (1953) found minute amounts of Cr only in the fetuses of rats after intravenous administration of both Cr(VI) and Cr(III). As of today, the mechanisms of Cr transport through the placental membrane are still not clarified. It is, however, demonstrated that intravenous administration of 8 mg CrO_3/kg to pregnant golden hamsters may induce cleft plate in the fetuses (Gale and Bunch, 1979). Furthermore, Gilani and Maranco (1979) injected CrO_3 in saline into embryonating chicken eggs at doses ranging 0.002–0.05 mg per egg and demonstrated a high incidence of malformations (i.e., reduced body weight, microphthalmia, short and twisted limbs, ectopic heart, and everted viscera). The relative numbers of these malformations was lower in the controls, but statistical significance was not analyzed.

7.3.2 Humans

Newman (1990) at the Glasgow Royal Infirmary reported on the very first Cr-related cancer in humans in a 47-year-old worker exposed to chrome pigment, a case of adenocarcinoma of the left turbinate body. Lehmann (1932) reported on two lung cancer cases among workers in an alkali-chromate manufacturing plant with approximately 200 employees, and Pfeil (1935) reported on five additional lung cancer cases among senior workers from the same plant and expressed the view that occurrence of seven cases in that small group of workers was unlikely a result of chance.

After these reports, numerous epidemiological studies were performed confirming that some Cr(VI) compounds have the potency to enhance the risk of cancer in the respiratory organs among the exposed (Costa, 1997; De Flora, 2000; IARC, 1990; Langård, 1990). Only a limited number of Cr(VI) compounds have been scrutinized in epidemiological studies, basically because groups of humans are exposed to a limited number of Cr(VI) compounds. These studies confirm that groups of workers exposed to calcium chromate, zinc chromate, and chromic acid are subject to enhanced risk of cancers in the respiratory organs. For other Cr(VI) compounds, only scanty information from epidemiological studies is available on possible carcinogenic effects, most likely because too small groups are exposed to constitute cohorts of sufficient size to be studied in cohort and case-control studies. However, it should be considered likely that exposure to those Cr(VI) compounds that have not been confirmed in studies to enhance cancer risk is associated with enhanced risk of cancer of the respiratory organs.

Gross and Kölsch (1943) reported eight lung cancer cases in workers from three other Cr pigment–manufacturing plants in Germany, five of which occurred among workers in a plant producing zinc chromate, the remaining cases were among workers in plants producing both zinc chromates and lead chromates. Four of the eight patients were only 33–37 years old. The authors suggested that zinc chromate should be considered as the prime causative agent and that zinc chromate should be considered as a hazardous compound. At the First International Congress on Diseases of the Chest in Rome, Imprescia (1952) presented seven primary lung cancer deaths (age, 45–62 years) among workers at a Kentucky chromate plant of 400 employees.

7.3.2.1 Manufacturing Chromium Compounds

Early epidemiological studies on the occurrence of cancer among workers with exposure to Cr(VI) compounds were presented by Machle and Gregorius (1948), Baetjer (1950), Mancuso and Hueper (1951), and Gafafer (1953). In the latter study, data were compiled on death claims submitted to seven U.S. plants manufacturing Cr compounds and included active male workers employed during 1940–1950, who were members of sick-benefit associations. The reference entity was age-specific and race-specific U.S. male mortality rates during 1940–1948. The study cohort included 5522 person-years. Ten deaths from lung cancer were observed among white workers, versus 0.7 expected (RR = 14.3). Sixteen deaths from lung cancer were observed among black workers versus 0.2 expected (RR = 80.0). The study by Bidstrup and Case (1956) is also included,

reporting on 12 lung cancer deaths, versus 3.3 expected. Smoking and other possible confounders could explain the increased death rates.

Ohsaki *et al*. (1978) reported on 14 cases of lung cancer among 133 male workers with 10 years' experience at a Japanese chromate manufacturing plant between 1936–1973 stated to have been exposed to Cr trioxide, sodium dichromate, potassium dichromate, and water-soluble Cr(III) compounds. Duration of employment ranged from 10–36 years and age at diagnosis ranged from 27–64 years (mean, 50.3 years). Twelve of the case subjects were confirmed to be smokers. Eleven of 17 cases reported by Zober (1979) had worked in alkali-chromate manufacturing, only three of whom were reported to have undergone exposure to Cr(III); however, there was no documentation on absence of exposure to Cr(VI).

Twenty cases of lung cancer among chromate manufacturing workers were reported on by Abe *et al*. (1982), of which all but five had occurred in heavy smokers. Tsuneta (1982) reported on 25 lung cancer cases that had occurred in male workers in a chromate plant, and the abstract reported that the lung cancer incidence was 413×10^{-5}/year, 16 times in excess of that of the general population. The average employment time at a chromate plant was 21.5 years, whereas the average time between first exposure to chromates and cancer was 25.4 years.

A number of studies have reported on the content of Cr in lung tissue in previous chromate workers with lung cancer (Hyodo *et al*., 1980; Kim *et al*., 1985; Kishi *et al*., 1987). None of these studies succeeded in explaining exposure-response relationships.

7.3.2.2 Chromate Manufacture

The studies by Taylor (1966) and Enterline (1974) presented strong evidence on the association between Cr(VI) exposure and lung cancer. Mancuso (1975) reported on the mortality of a cohort recruited from a chromate-manufacturing plant that he had studied before (Mancuso and Hueper, 1951). Industrial hygiene data available from 1949 were used to calculate weighted average air concentrations of water-soluble, insoluble, and total Cr, respectively. The mortality rates for the subcohort recruited between the years 1931and 1937 were calculated for dose/time categories, and analysis was limited to 41 lung cancer deaths that occurred in this cohort during 1931–1974. The standardized mortality ratios (SMRs) were compared with those of the entire plant population. Deaths from lung cancer were clustered in workers with 27–36 years since first employment. Death rates for lung cancer increased from SMR 144.6–649.6 in five exposure categories ranging from 0.25–0.49 to 4.0+ mg/m^3, and from 80.2–998.7 at exposures ranging from <0.25 to 2.0+ mg/m^3 for insoluble Cr(III) and soluble Cr(VI), respectively. The corresponding SMR for assumed exposure to total Cr concentrations increased from 225.7 in the 0.50–0.99 mg Cr/m^3 category to 741.5 in the 6.0+ mg/m^3 category. At a given air concentration of Cr, increased levels of total Cr resulted in an enhanced death rate from lung cancer in four of the five exposure categories. For exposure to total Cr, increasing air levels of insoluble Cr resulted in a declining death rate from lung cancer in three of the five categories. No corresponding analysis was presented for the possible relationship between a given exposure to total Cr and increasing concentrations of soluble Cr.

Mancuso (1997) revisited the preceding cohort using limited exposure data from an earlier analysis (Mancuso, 1975). A study was later carried out (Luippold *et al.,* 2003), with follow up to 1997, which was based on the same recruitment cohort (Mancuso, 1975) but with altered inclusion criteria, studying only those with more than 12 months of employment after 1940. These authors stated that no exposure data for trivalent chromium were identified in the historical files. They found that the excess lung cancer was linked to the cohort first employed before 1960, when the plant used a high-lime mixture in their process, assumed to result in exposure to calcium chromates.

Hayes *et al*. (1979) examined whether workers employed in sections of the plant constructed to reduce hazardous exposures revealed a declining risk of respiratory cancer compared with workers employed in an old facility. The cohort comprised 2101 workers, all first employed between 1945 and 1974 and with 90 days+ employment. Subjects for whom vital status was ascertained as of July 1977 (i.e., 88% of the cohort; n = 1803) were included in the analyses. As reference, entity, age-, race-, and time-specific male mortality rates of the city of Baltimore were applied. On the basis of 59 observed deaths, the SMR for cancer of the trachea, bronchus, and lung (ICD 162) was 202 (95% CI = 155–263), versus 29.2 expected. SMR for lung cancer was 180 (95% CI = 110–270) on the basis of 20 deaths for employees hired between 1945 and 1949 with less than 3 years work. Those with 3 years+ of work, hired between 1945 and 1949, presented an SMR of 300 (95% CI = 160–520) on the basis of 13 deaths. The SMR for lung cancer on the basis of two deaths was 70 in workers hired 1950–1959 with less than 3 years work at the new facility. Workers hired between 1950 and 1959 and employed less than 3 years at the older facility presented an SMR of 180 (95% CI = 90–310) on the basis

of 12 deaths. Workers first employed between 1950 and 1959, with 3 years+ in the new facility had SMR for lung cancer of 400, on the basis of three deaths (95% CI = 80–1170), whereas their counterparts in the older facility presented an SMR of 340 (95% CI = 260–650) on the basis of nine deaths. Hence, the lung cancer hazard for workers persisted at the newer plant unit constructed in 1950–1951.

Alderson *et al*. (1981) studied a cohort comprising 2715 chromate-manufacturing workers with 1 year+ of continuous work between 1948 and 1977, from whom 298 were lost to follow-up. Production ceased at one factory in 1966 and at another one in 1967. Reduced chromate exposure took place in these two factories from 1954 onward. The "low-lime" process had been introduced from 1957 to 1959 at the plant closing in 1967. The one factory still in operation as of 1981 was modified in 1957 to 1961 when the "non-lime" process was introduced. The third plant used the "high-lime" process until closure in 1966. In the "high-lime" process, insoluble Cr_2O_3 is converted into sodium chromate and some low water-soluble calcium chromate is formed. Data were analyzed in 1981 on subjects employed from 1948 onward who participated in lung X-ray screening from 1956+. Heavy smoking was reported to be less prevalent than in the general population. The authors observed 602 deaths against 445.3 expected, including 116 from lung cancer versus 48.0 expected ($P < 0.001$). There were 75 deaths from lung cancer in one plant versus 26.3 expected. Two deaths from cancer of the nasal sinuses were observed in this group, versus 0.15 expected ($P = 0.03$). In the plant still in operation in 1981, workers employed after modifying production presented a RR of lung cancer at 1.8 versus 3.0 among those previously employed. The total number of deaths from lung cancers in that plant was 36 versus 16.7 expected ($P < 0.001$). The authors considered the results to suggest that duration of exposure to chromates could be a stronger determinant for lung cancer than the level of exposure.

A cohort study among 896 males manufacturing Cr compounds in the Tokyo area in 1918–1975 was reported by Satoh *et al*. (1981), observing the workers from 1918 to 1978 or to death, and age- and cause-specific mortality among Japanese males was used as reference. Exposure to Cr(VI) and Cr(III), respectively, was based on the relative production volume between 1934 and 1975; 84% being compounds of Cr(VI) and 16% Cr(III). Among the 31 deaths from respiratory cancer in the entire cohort, 26 were due to lung cancer (RR = 9.5). No other cancers sites were in excess of expected. There were five (expected, 1.18) deaths from respiratory cancer in the subgroup with 1–10 years of work when grouping the cohort by duration of employment, respectively 9 versus 1.2 in the 11–20 year group, and 17 versus 0.97 expected in the group with 21+ years of work.

Korallus *et al*. (1982) attempted to study whether reduction in Cr exposure in German (FRG) chromate manufacturing plants had resulted in a reduced mortality from lung cancer. A group of 1140 chromate workers in North Reine-Westphalen employed 1 year+ between 1934 and 1979 was compiled, excluding those deceased before 1948. The cohort was observed from January 1948 through March 1979, and the SMRs in the province North Reine-Westphalen were used as a reference entity. The death rate from lung cancer of males of North Reine-Westphalen was 80×10^{-5}/year as of 1976 compared with 60×10^{-5} in FRG, and the corresponding rates in 1950 had been 32×10^{-5} and 35×10^{-5}. The average age at first employment was 38 and 36 years in factory "A" and "B," respectively. Fifty-one deaths from lung cancer had occurred before the end of the observation period and an additional six lung cancer cases in live workers. Average time from first employment to diagnosis of lung cancer was 28 and 26 years in factory A and B, respectively. The SMR for all deaths was 77 and 80 in factory A and B, respectively; the SMRs for all noncancer deaths were 62 and 56; and the SMRs for stomach cancer were 17 and 160, respectively.

An Italian study on 981 chromate manufacturing workers, employed 1 year+ from 1948 to 1985, presented 20 deaths from respiratory cancer (De Marco *et al*., 1988); 14 deaths from lung cancers, 3 pleural tumors, and 3 laryngeal cancers. Cause-specific SMRs for Italians were used as reference. The SMR for lung cancer was 212, whereas that of a subgroup employed more than 10 years was 420.

Watanabe and Fukuchi (1984) studied the mortality experience in a group of 276 Japanese chromate-manufacturing workers employed in 1947 or later. They observed from January 1960 through December 1982 and used the mortality rates in the entire Japanese male population as reference. Sixty deaths from all causes were observed, 33 were from cancers, of which 25 were from lung cancer versus 1.36 expected (RR = 18.3) ($P < 0.01$).

7.3.2.3 Chromate Pigments

Reports on case subjects from Germany in the 1930s and 1940s indicated that heavy exposure to some Cr pigments could result in cancer. In a study performed among workers in a Norwegian primarily zinc chromate manufacturing cohort of only 133 male workers observed from January 1953 through 1972, Langård and Norseth (1975) seemed to confirm that asso ciation. A small subset of 24 workers with 3 years+

employment before December 1972 had experienced three cases of lung cancer between 1951 and 1972 versus 0.079 expected ($P < 0.1$), corresponding to an absolute risk of $\pm 1500 \times 10^{-5}$/year. The levels of exposure to Cr(VI) in the past were not known, but work air concentrations of Cr(VI) in 1973 ranged from 0.01–1.35 mg/m^3. The authors suggested that the lung cancer case subjects most likely had been exposed to air levels of 0.5–1.5 mg Cr(VI)/m^3 over their 6–8 years of employment. In a follow-up through 1980, Langård and Vigander (1983) found six (99% CI = 1.5–15.7) cases of lung cancer (absolute risk still approximately 1.500×10^{-5}/year) in the subgroup of 24 workers versus 0.135 expected on the basis of national rates, and 0.08 expected on the basis of county-specific reference level. Five of the six case subjects were confirmed to have smoked. One lung cancer case had occurred in the 109 workers with <3 years of employment before 1972.

Davies (1979) studied the mortality experience through mid-1977 among workers at three plants in the U.K. manufacturing chromate pigments. Excess lung cancer deaths were found in male workers in two plants with exposure to "high" and "medium" air concentrations. Workers in two facilities (A and B) were also exposed to zinc chromate. In a later follow-up, the population was observed from the 1930s or 1940s through December 1981 (Davies, 1984a). Seven lung cancer deaths were found (E = 6.45) among the workers of factory C, probably exposed to compounds occurring in lead chromate manufacture only. A high RR of lung cancer deaths was observed in a group of 33 heavily exposed workers first employed before 1946, in which three deaths were observed versus 0.84 expected. Among heavily- and medium-exposed workers in factory A and B, observed for less than 30 years, the RR varied from unity to 5.5, and in 31 heavily exposed workers first employed between 1932 and 1945, corresponding to absolute risk of 390×10^{-5}/year. Three lung cancer deaths (E = 2.32) in medium exposed workers engaged from 1932 to 1945 and observed for more than 30 years, gave the highest absolute risk of 575×10^{-5}/year.

Davies (1984b) also observed a group of 57 lead chromate workers from the same plants; subjects having been notified as lead poisoning cases between 1930 and 1945. Causes of death from the time of lead poisoning were obtained through 1981. Four deaths from bronchial carcinomas were found versus 2.8 expected, all occurring in workers with lead poisoning before age 40. The number of observation years was 1585, giving an absolute risk of lung cancer deaths of 252×10^{-5}/year.

Haguenoer *et al*. (1981) reported on deaths and cancers in a poorly defined cohort with 251 zinc and lead chromate manufacturers who were employed at a French plant 6 months+ from January 1958 through December 1977. There were 50 deceased, for whom cause of death was known in only 30. There were 11 deaths from lung cancer (2.38 expected; CI = 5.5–19.7) on the basis of northern France as reference. The mean time from first employment until diagnosis of cancer was 17 years, mean age was 54.1 years, and duration of employment for the case subjects was 15.3 years. Smoking data were not presented in sufficient detail to evaluate interaction.

In a New Jersey plant manufacturing lead and zinc chromates, Sheffet *et al*. (1982) studied 1296 white and 650 nonwhite males employed 1 month+ from January 1940 to December 1969. Expected figures for deaths were based on cause-, age-, and time-specific SRMs for U.S. males. Estimates suggested approximately nine times more lead chromate than zinc chromate in the work environment. Sixteen deaths from lung cancers were observed among whites (RR = 1.6; $P < 0.05$) and seven (RR = 1.6; nonsignificant) among nonwhites. Among whites with 10 years+ employment, RR was 1.7 ($P < 0.05$) and for nonwhites 2.6 (nonsignificant). The same recruitment cohort was observed between 1940 and 1982 by Hayes *et al*. (1989), who included 1879 male workers employed for 1 month+ during 1940–1969, and they used the same reference entity. The vital status was ascertained for 1737 workers (92%) to whom analyses were limited. "Air-borne" Cr was measured in late observation years, resulting in estimates of 0.5 mg/m^3 for exposed jobs and levels >2 mg/m^3 for heavily exposed jobs. They found the SMR at 93 (O/E = 101/108.8) for all cancer deaths. Forty-one deaths from lung cancer (E = 35.3) were observed in workers from jobs with exposure to Cr(VI). Lung cancer deaths in the entire cohort corresponded to absolute risk 81×10^{-5}/year (E = 70×10^{-5}). SMRs for lung cancer among those unexposed and those with less than 1 year Cr exposure were 92 and 93, respectively. For workers with cumulative work periods 1–9 and 10+ years, SMRs were found to be 176 and 174, respectively. When excluding those with less than 30 years since first employment among those with 10+ years' employment, SMR rose to 321 based on six cases.

This same cohort was also followed up by Gibb *et al*. (2000) through 1992 including all workers ($n = 2357$) employed until closure in 1985. A total of 122 deaths from lung cancer gave an overall excess of 180 (SMR). Unsuccessful attempts were made to identify possible dose-response between cumulated exposure to Cr(VI) and lung cancer.

A PMR study was performed by Dalager *et al*. (1980) in a group of spray painters who used zinc chromate primer paints, a cohort included 977 workers who had painted 3 months+ and terminated employment within the 10-year period before July 1959. The expected

"relative frequency" of the cause of specific death rates in the white male U.S. population was used as reference. Total number of deaths was 202, which was expected, of which 50 were from cancers versus 36.9 expected ($P = 0.05$), of whom 21 were from respiratory cancer (ICD 160–164) versus 11.4 expected ($P = 0.01$).

Other authors (Boice *et al*., 1999) performed a study among 3634 airplane spray painters who had been exposed to concentrations of Cr(VI) encountered at the workplace in the 1950s and 1960s. This study revealed no excesses of cancer that could be linked to Cr(VI) exposure, and the estimated air concentrations of Cr(VI) seemed to have been very low compared with other groups of workers exposed to Cr(VI) in the 1940s–1960s.

7.3.2.4 Ferrochromium Manufacture

A study among workers in a Swedish plant manufacturing ferrochromium alloys from chromite, was carried out by Axelsson *et al*. (1980). Males employed for 1 year+ from January 1930 to December 1975 were included and were categorized by work duration and site. Deaths from cancer were obtained from the national Central Bureau of Statistics, and cancer incidences were collected manually from the Cancer Registry of Sweden. The expected deaths and cancer incidence were computed by a life-table method. A 15-year latent period from start of employment was accounted for. In all, 1876 workers were included; 380 were deceased (383.3 expected) of whom 69 were from cancer (76.7 expected). Five lung cancer cases (ICD 162–163) were observed versus 7.2 expected. The observed/expected ratio was 18/20 for cancer of the digestive tract (ICD, 151–153). Maintenance workers were the only group with excess cancer of the respiratory organs; four cases (E = 1.0), of which two were mesotheliomas. One mesothelioma case also occurred among arc furnace workers where asbestos was used for insulation. Air levels of Cr(III) ranged from 0.0–2.5 mg/m^3 and for Cr(VI) levels from 0.0–0.25 mg/m^3. No information on smoking was given.

A parallel study in Norway among ferrochromium and ferrosilicon furnace workers (Langård *et al*., 1980) included all workers employed at the plant for 1 year+ from 1928 and onward, allowing for latency through exclusion of entries after 1960. Observed and expected cancers and deaths for the 976 males were both derived from national data. There were 182 deaths versus 228.2 expected, and the observed/expected figures for all cancers were 64/79.4. Nine lung cancer cases were observed (E = 9.45) of which 7 (E = 3.1) were in the ferrochromium group (absolute risk = 92×10^{-5}/year) ($P = 0.08$). The expected figure for lung cancers was reduced to 1.8 when using local reference rates ($P = 0.06$). Air measurements in the plant in 1975 documented the presence of both Cr(III) and Cr(VI) in the work environment, containing 0.01–0.29 mg/m^3 Cr, of which 11–33% was water-soluble Cr(VI). In a subsequent study, 8 more years of observation was added to the same cohort (Langård *et al*., 1990) by unchanged criteria for inclusion, except that entries between January 1960 and December 1964 were added, which expanded the cohort to 1235 subjects. Results were presented separately for the 976 workers studied earlier, in which observed cancers from all sites had increased from 64 (Exp = 79.4) to 124 (Exp = 144.9), of which 17 were lung cancers (Exp = 17.8). The incidence for lung cancer was high in the subgroup of ferrochromium workers; 10 cases observed versus 5.7 expected (95% CI = 4.8–18.4). The RR for cancer of the prostate (31/26.6) had declined from the first study. In the population that included recruits from 1960 to 1964 ($n = 1235$), 357 deaths had occurred (E = 440.6; SMR = 91), and SIR for all cancers was 84 (O/E = 132/157.3). Enhanced risk of lung cancer was observed in the ferrochromium subgroup ($n = 379$), O/E-ratio = 10/6.5 (SIR = 154; absolute risk = 95×10^{-5}/year). The observed/expected (O/E) ratio for lung cancer in the ferrochromium group was 3.0 (99% CI = 1.1–6.4) when applying all nonferrochromium workers as an internal reference. Five of a total of nine cases of kidney cancer had occurred in the ferrochromium group (SIR = 273), with a mean of 39 years from first employment, whereas the mean latency time for all nine cases was 37.3 years.

7.3.2.5 Uses of Chromium Compounds

7.3.2.5.1 Chromium Electroplating A mortality study was carried out among previous and current Cr electroplaters with 3 months+ employment in 54 Cr electroplating plants in Yorkshire, U.K. (Royle, 1975a,b), including 1056 male and 182 female platers 142 of whom were deceased as of May 1974. A reference group matched for age, gender, and smoking habits was recruited, comprising non-Cr-plating past and current workers of two industrial companies located in the same area. Work and smoking histories were compiled through a questionnaire among 997 live platers and 1117 live subjects from the reference group. The platers had experienced a significant excess of deaths from all cancers; 51 observed versus 24 in the reference group ($P < 0.01$), of which there were 24 lung cancer deaths in male platers versus 13 expected (not significant). The mean development time from first exposure to death for lung cancer was 13.6 years compared with 16.4 years for all cancer deaths. Cancer of the digestive tract in males was also in excess; 8 observed versus 4 expected (not significant). A larger proportion of the reference group than the study group

had worked in asbestos processing. Smoking habits were reported to be similar in platers and referents.

Results were reported on the mortality among 952 Cr platers in Tokyo (Okubo and Tsuchiya, 1979) where workers had been recruited from 1970 to 1976 records of the Tokyo Health Insurance Society of the Plating Industry and comprised workers born before May 31, 1937, with 6 months or more experience with Cr plating. Expected deaths were based on SMRs of the general Tokyo population. No lung cancer deaths had occurred in males versus 1.2 expected (not significant).

A study on cancer mortality among 178 Italian Cr platers was reported on by Franchini *et al*. (1983), 62 of whom were "bright" and 116 were "hard" platers. Seven cancer deaths had occurred in hard platers versus 2.7 expected on the basis of national rates. Three deaths were from lung cancer (E = 0.7) ($P = 0.03$); absolute risk = 182×10^{-5}/year. When excluding 10 years presumed latency, six cancer deaths (E = 2.2) and three lung cancer deaths (E = 0.6) ($P = 0.02$) remained. Exposure to Cr(VI) in hard plating in 1980 averaged 7 µg/m^3, but it was assumed that exposure had been higher in the early exposure years.

Sorahan *et al*. (1987) reported on the mortality experience among 2689 (males/females = 1288/1401) Cr electroplaters from the period 1946–1983 performing primarily bright (thin) plating of bumpers and overriders. The cohort included workers first employed during 1946–1975 with 6 months+ experience as Cr platers. Air measurements of Cr had been taken before 1973, measuring the concentration of Cr_2O_3 as high as 8.0 mg/m^3, 1.6 mg/m^3, and 0.4 mg/m^3, respectively. From 1973 onward, the air levels were generally <50 µg Cr/m^3. All participants had at least some period of work associated with Cr exposure, but concomitant exposure to nickel chloride and nickel sulfate had taken place. Death rates were compared with that of the general population of England and Wales, and there were 213 cancer deaths from all sites versus 164.2 expected, when combining genders. Sixty-three of 72 lung cancer deaths were in males (E = 40; $P < 0.001$), and nine in females (E = 8.1). The highest O/E ratios were 13/3.8 ($P < 0.001$) after 10–14 years and 12/4.9 ($P < 0.01$) after 15–19 years of work at chrome baths in combined genders. There were 25 deaths from stomach cancer (E = 16.2), but excess in males only.

In a study among 176 consecutive hospital-based male lung cancer cases in Norway, Kjuus *et al*., (1986) found that seven cases and six referents had been exposed for 3 years or more to Cr and nickel compounds (welding excluded). The odds ratio adjusted for smoking was 1.4(CI = 0.4–4.4).

7.3.2.5.2 Stainless Steel Welding Stainless steel (SS) generally contains 8–10% nickel (Ni) and 18–20% Cr, and the electrodes applied during welding on SS generally contain more Ni and Cr than the base material. The fume generated during welding on SS contains Cr and Ni along with other toxic gases and fumes (i.e., CO, NO_x and O_3). Although the Cr in the base materials is present in the inert 0 valency form, it oxidizes to Cr(VI) resulting from the high temperatures (i.e., 6000°C+) during the welding process. Consequently, unprotected welders may be exposed to Cr(VI) at various air levels; the exposure levels depend largely on which method of welding is being used, generally with highest levels during manual metal arc (MMA) welding. Because welders frequently weld on different materials, they may also be exposed to oxides of other metals (i.e., oxides of Mn, Fe, Al, and others) (Karlsen *et al*., 1996; Stern, 1981).

Given the mixed exposure among welders, epidemiological studies on SS welders are difficult to perform, but few studies are carried out among welders who have welded SS only. Some studies indicate that there is an excess cancer of the respiratory organs among SS welders. Sjögren (1980) studied a group of 234 subjects who had welded SS only for 5 years+ between 1950 and 1965. Dilution was avoided by excluding subjects from the study with <5 years of welding. Sjögren found that smoking habits of the study group were similar to the general male population used as reference entity. There were three cases of lung cancer in the group versus 0.8 expected. Sjögren *et al*. (1987) subsequently studied the same cohort through an additional period until 1984 and observed five cases of lung cancer versus 2.0 expected. Compared with other studies, exposure to welding fumes was well characterized, and latencies for possible exposure-related cancer were accounted.

Becker *et al*. (1985) performed a cohort study in 1224 SS welders who had used different methods. The only subgroup with excess lung cancers was that performing MMA with 20–29 years since first exposure; five observed versus 2.1 expected, whereas the entire MMA subgroup showed five cases versus 5.1 expected. A short observation time was an inherent weakness of the study. In a later follow-up through 1995 (Becker, 1999), it seemed that the excess lung cancer was confounded by previous exposure to asbestos (i.e., there were 7 deaths from mesothelioma versus 0 expected), indicating that a significant number of the previously observed excess lung cancer deaths are likely to be due to exposure to asbestos fibers.

A major study was carried out by the IARC including both mild steel (MS) as well as SS welding in 11,092 welders (Simonato *et al*., 1991). Welders from nine

European countries participated, recruited mainly during the 1960s and the 1970s. There were 116 lung cancer deaths versus 86.8 expected. In the subgroup of primarily SS welders, there were 39 lung cancer deaths versus 30.45 expected, whereas in the subgroup of SS welders with more than 10 years of welding and accounting or 20 years of "latency" the figures were 13 deaths versus 9.1 expected.

Danielsen *et al*. (1996) studied a recruitment cohort of 2957 boiler welders recruited in 1942–1981 in a historical, prospective cohort study with observation from 1953 through 1992, including a subcohort of 606 certified SS welders. Observed and expected outcomes were derived from the Norwegian cancer register, and there was a 4.9% loss at follow-up.

There were 50 lung cancers versus 37.5 expected in the entire cohort (SIR = 133; CI, 99–176) and six lung cancers versus 5.8 expected in the SS steel subgroup. When accounting for the 15 years presumed latent period, the slight excess was reduced to a deficiency of 2 observed versus 3.4 expected, suggesting that there was no excess. Hence, this study's results did not lend support to the view that SS welding results in a higher risk of lung cancer than MS welding.

7.3.2.5.3 Chromium Compounds in the Environment At Tokushima University in Japan (Kondo *et al*., 1971), a study was carried out among residents close to a newly started chromate manufacturing plant. The conclusion was: "no definite signs exist for health injury caused by the chromate."

Environmental exposure to Cr(VI) and its possible relationship to the development of cancer was examined in Sweden (Axelsson and Rylander, 1980) in a population living near a ferrochromium smelter. No difference was observed between cancer mortality among the local population living close to the smelter compared with that of the population living farther away. In this study, the exposure levels to Cr compounds was not measured and must have been quite low compared with levels experienced by Cr manufacturing workers.

A study carried out in the People's Republic of China (Ziang and Li, 1997) was hampered by insufficient characterization of the alleged exposure to Cr(VI) compounds, poorly defined exposed study population, and a too short observation period to reveal a possible relationship that might be present between Cr(VI) exposure and occurrence of cancers of the respiratory organs. Therefore, the nonpositive result from the study permits conclusions either on a possible relationship or on absence of relationship between Cr(VI) exposure and the cancer outcome.

A study was performed that included residents of the villages: Ketleman, Hinkley, and Topack in the southern part of San Bernadino County in California (Fryzek *et al*., 2001). In these villages, soluble Cr(VI) was added to water used for cooling of pipes from gas compressor plants. Wastewater from these towers was transferred to ponds possibly leaking into the groundwater. The presumed exposed population was observed for 10 years only (1989–1998). The control of migration over the decades of interest, 1950s and onward, was not fully controlled, which may have contributed to the significant deficit of the total cancer mortality and lung cancer mortality over the study period. Despite the low rates of cancer, the results of the study may be considered nonconclusive.

7.3.2.5.4 Cancer at Other Sites Teleky (1936) drew attention to a possible connection between exposure to chromates and enhanced risk of cancer of the intestines after observing five digestive tract cancer cases among 44 deceased chromate workers. Results in some epidemiological studies suggest elevated risks of cancer of the stomach and digestive tract. In the large body of literature published on the relationship between exposure to Cr(VI) compounds and occurrence of cancer (Costa, 1997; De Flora, 2000; IARC, 1990; Langård, 1990), studies that may help resolve this question are likely to be found in well-designed epidemiological studies with high accesses of lung cancer, in particular in studies showing a dose-response relationship between Cr(VI) exposure and lung cancer. The heavier the exposure to Cr(VI) by inhalation, the more Cr(VI) is likely to be ingested. Thus, epidemiological studies that do not demonstrate a strong association between heavy exposure to Cr(VI) compounds and cancer of the respiratory organs are unlikely to present valid information on the possible relationship between exposure to Cr(VI) compounds and cancer for the digestive tract.

Most epidemiological studies of cancers are done in cohorts inhaling Cr(VI). There were some hints that Cr by inhalation exposure could enhance cancers at other sites such as stomach (Costa, 1997; Langård, 1990). There are no epidemiological studies on the ingestion of Cr(VI) compounds. However, exposure to chromates by inhalation also results in ingestion of some Cr(VI). Therefore, information on cancer possibly resulting from ingestion of Cr(VI) could be derived from some epidemiological studies on populations exposed to Cr(VI) by inhalation.

Enterline (1974) reanalyzed the mortality data reported by Taylor (1966) and derived from workers employed between 1930 and 1947 in three U.S. plants manufacturing Cr(VI)-compounds. The study covered

the period 1941–1960. Enterline observed a significant excess of deaths from bronchial cancer, as well as an excess of cancer of the stomach and the digestive tract, 16 deaths observed versus 10.4 expected. There were no data on the intensity of exposure to Cr(VI), and he did not account for the time required for cancer to develop ("latency"), tobacco smoking, use of alcohol-containing beverages, or dietary habits. When excluding the subgroups first employed from 1960 onward from his study cohort, to account for "latency," the excess cancers of the digestive tract seem to be limited to a small subgroup first employed in 1941–1945. That subgroup accounted for five digestive cancer deaths versus 1.4 expected (RR = 2.9). This RR is the highest observed for digestive cancer in any reported Cr(VI) study. Confounders were, however, not accounted for; this is why the results cannot be used to scrutinize the possible exposure/response relationship between Cr(VI) and cancer of the digestive tract.

Sorahan *et al*. (1987) reported on the mortality experience among 2689 (males/females = 1288/1401) Cr electroplaters from the period 1946–1983. However, there were 25 deaths from stomach cancer (E = 16.2) in males only.

Royle (1975b) observed a nonstatistical significant excess of deaths from cancer of the digestive tract among 1056 male and 182 female Cr electroplaters with 3 months+ of work experience (e.g., O/E ratio of 9/4). The data on the personnel were obtained as completely as possible from the management, but information was not given on the completeness of the study group, and only the endpoint (1972) of the observation period was defined. There were six deaths from cancer of the digestive tract in males versus 4 in the referent group, correspondingly 3 and 0 in females, but figures for stomach cancer were not given. Attempting to reduce confounding, the author excluded workers who might have been extensively exposed during nickel or cadmium plating. Smoking habits at the time of the study were stated to be quite similar in the platers and in the referent group, but information on previous smoking was not available. Other possible confounders and latent periods were not accounted for, but the authors stated that a larger proportion of the reference group than of the study group had worked in asbestos processing. Information on levels of exposure to Cr(VI) was scanty. The results of the study could seem to lend support to the view that there is causality between exposure to Cr(VI) and digestive tract cancer. However, there were weaknesses in the methods applied by Royle (1975a); neither the recruitment period nor the observation period was defined, and information on the completeness of the recruitment from the study base to the study population was lacking. The author stated that "definitive Cr platers" with more than 3 months experience were included in the cohort, but the number of eligible subjects was not defined. Possible confounding from previous smoking and other exposure factors was not ruled out.

Hayes *et al*. (1989) based exposure to Cr(VI) on measurements of "airborne Cr" and estimated the average level of exposure to 0.5 mgCr/m^3 for exposed jobs and levels higher than 2 mg/m^3 for heavily exposed jobs. The SMR for digestive cancers was 83 (O/E = 26/31.3) in the entire cohort. The authors found that the risk for digestive cancer was only weakly associated with exposure to chromate dust, and no clear pattern was seen when analyzing by time period since first exposure or for duration of employment.

Case reports and small numbers of cases occurring within cohort studies, too small to show statistical significance, suggest a relationship between exposure to Cr(VI) compounds and occurrence of cancer of the nose and paranasal sinuses. In a follow-up study in ferrochromium workers (Langård *et al*., 1990), the mean latency period for cancer of the kidneys was 2 years longer than for all kidney cancer cases in the study cohort, suggesting that exposure to Cr as the cause of the unexplained excess was less likely than the presence of other causes.

7.3.2.5.5 Significance of Valency State Evidence from numerous studies reviewed indicates that previous chromate workers had high rates of lung cancer (Alderson, 1981; Baetjer, 1950; Bidstrup and Case, 1956; Gafafer, 1953; Hayes, 1979; Machle and Gregorius, 1948; Mancuso and Hueper, 1951). The evidence is strong that the lung cancer hazard is associated primarily with manufacturing hexavalent monochromates and dichromates. No conclusive evidence for cancer hazard among humans exposed to aerosols formed by Cr metal or Cr(III) compounds have been presented to date. No case reports or epidemiological data lend support to the view that exposure to Cr(III) compounds constitutes a cancer hazard in humans.

7.3.2.5.6 Significant Water Solubility Evidence presented by Ohsaki *et al*. (1978) suggesting that exposure to relatively water-soluble chromates of sodium and potassium may result in enhanced lung cancer risk. Zober's (1979) study presented some support to the view that exposure in the high-lime process could be a major factor in the causation of the lung cancer cases that he observed. Evidence was also presented by Nishiyama *et al*. (1988) that sodium chromate and potassium chromate could be causative agents in 4 of the 11 lung cancer cases discussed.

There is some evidence (Alderson, 1981; Baetjer, 1950; Hayes, 1979) that work in the high-lime process could be associated with a greater lung cancer hazard than the low-lime process. The high-lime process seems to generate more calcium chromate than the low-lime process. However, the evidence as of today is insufficient to confirm that exposure to calcium chromate poses a higher lung cancer hazard than other Cr(VI) compounds present in the work atmospheres. Mancuso (1975) suggested that "carcinogenic potential extends to all forms of chromium." Although the results from his study suggest that both soluble and insoluble Cr(VI) compounds are involved in enhanced risk of Cr(VI)-related cancers, the results also seem to suggest a stronger relationship between exposure to soluble Cr(VI) and lung cancer than between insoluble Cr(VI) and lung cancer. Hence, the results seem to support the view that soluble Cr compounds are more potent lung carcinogens than are the insoluble ones. Because exposure to Cr(VI) seems to have occurred in all subgroups in the study and could thus have accounted for the enhanced cancer risk, it seems unjustified to infer that compounds other than Cr(VI) be regarded to cause lung cancer in the study.

There are case reports (Brochard *et al*., 1983; Kim *et al*., 1985; Korallus *et al*., 1974; Michel-Briand and Simonin, 1977; Pfeil, 1935; Takemoto *et al*., 1977; Zober, 1979) presenting some evidence in support of an association between Cr(VI) exposure in electroplating and lung cancer, possibly also strengthened by the fact that some of these case subjects were very young at diagnosis.

Studies among Cr electroplaters could lend support to the view that fumes from Cr_2O_3 exert a carcinogenic potency. The results presented by Sorahan (1987) may be considered weak but seem to be in favor of a relationship between work at the Cr electroplating baths and enhanced risk of lung cancer. Air measurements suggested that the participants' exposure to Cr had been intensive, even later than the time period when enhanced cancer risk must have been accumulated. The study presented no association between work in nickel electroplating and lung cancer. In Royle's study (1975a,b) an unusual short latent period for lung cancers was observed (mean 13.6 years), suggesting that work exposures before plating and smoking could have influenced the results. In a small Italian study (Franchini *et al*., 1983), exposure to Cr(VI) was well documented, and later follow-up may provide additional information.

Hence, epidemiological studies present some evidence in favor of a relationship between exposure to fumes from the quite water-soluble Cr_2O_3 and cancer of the respiratory organs. The exposure-response relationship is, however, shown to be significantly weaker than among workers in dichromate and Cr pigment manufacture.

7.3.2.5.7 Carcinogenic Potency Some reports (Gross and Kölsch, 1943; Letterer *et al*., 1944; Newman, 1890) strongly suggest zinc chromate as the prime causative agent among some young workers with lung cancer. Among studies presenting data necessary to calculate absolute risk, the study by Langård and Vigander (1983) presents the highest absolute risk, approximately 1500 cases per 100,000 observation years in a subgroup of 24 workers primarily exposed to zinc chromate. Five case subjects had been exposed solely to zinc chromate, whereas one case subject had also been in contact with some lead chromate. In Davies' study (1984a), excess lung cancer deaths were observed primarily in workers of the study plants where workers had been exposed both to zinc and lead chromate, whereas excess lung cancer deaths were nonsignificant in those who were stated to manufacture lead chromate only, making the author suggest that zinc chromate was the most likely cause of lung cancer among workers exposed to both compounds. A nonsignificant excess of lung cancer deaths was also observed in a subgroup exposed only to lead chromate (Davies, 1984a) and in a study of a small group of previously lead-intoxicated chromate workers (Davies, 1984b). These results suggest that zinc chromate is a more potent human carcinogen when inhaled than are other chromate pigments like lead chromate.

In the study by Hayes *et al*. (1989), much lower relative risks were reported despite higher air concentrations than in the study group by Langård and Vigander (1983). Given that the measurements are comparable in the two studies and the airborne Cr in the U.S. study was primarily lead chromate, the results are not conflicting. No epidemiological studies have included exposure to strontium chromate.

The current evidence from epidemiological data seems to point out chromates of zinc and calcium as the most potent carcinogenic chromates. However, there are only few data on other Cr(VI) compounds.

7.3.2.5.8 Levels of Cancer Risk Absolute risk of lung cancer experienced by workers in some of the early study cohorts seems to be lower than in some of the Cr pigment studies. However, the reference risks in the 1940s and 1950s were much lower than in the 1970s and 1980s. Therefore, although the absolute risk was lower in the early study by Gafafer (1953), the RRs varied between 14 and 80, reflecting an even higher cancer hazard than in more recent studies with highest absolute risks. Imprescia (1952) gave no

information on the "frequency" of lung cancer in his study, but assuming that six lung cancer deaths in 1947 were "average" incidence for the cohort, the absolute risk might have been approximately 1.500×10^{-5}/year, which is equivalent to the highest absolute risk reported recently.

7.3.3 Interaction with Other Carcinogenic Factors

7.3.3.1 Animals

Nettesheim *et al*. (1971) induced 13 lung adenomas in 136 virus-infected mice exposed to 13 mg calcium chromate/m^3 5 hours a day, 5 days a week for a lifetime. In rodents, Balansky *et al*. (2000) failed to reveal any additive interaction between cigarette smoking and Cr(VI) in inducing clastogenic damage.

7.3.3.2 Humans

In the first article by Bidstrup (1955), there was some suggestion on possible potentiating interaction between inhalation exposure to Cr(VI) and smoking in inducing lung cancer. However, no subsequent epidemiological study has confirmed such an interaction between the two exposure factors. It is still possible that interaction may take place between the two factors, but few epidemiological studies among Cr(VI)-exposed workers have reported smoking habits in sufficient details to permit studying such a possible interaction. In the study by Tsuneta (1982), 25 lung cancer cases were reported. Smoking was confirmed among 20 case subjects, and four were reported to be nonsmokers. Moulin *et al*. (2000) studied lung cancer in workers manufacturing stainless steel and metallic alloys but did not unravel confounding of tobacco smoking when adjusting for that exposure factor. Antilla *et al*. (1989) found that average lung tissue levels of Cr in smokers was 6.4 µg/g versus 2.2 µg/g in nonsmoking referents, which is likely to result from Cr in tobacco smoke.

Many workers exposed to Cr(VI) have undoubtedly also been exposed to asbestos fibers. However, no author has reported specifically on the possible interaction between exposure to Cr(VI) and asbestos. Numerous Cr electroplaters have also carried out Ni electroplating, having been exposed to both Cr(VI) and Ni compounds. Also in SS welding, the welders are exposed to both Cr and Ni compounds. However, no author has attempted to differentiate between the two carcinogenic factors to scrutinize possible interaction between the two.

7.3.4 Mutagenic and Genotoxic Effects

Chromium compounds are mutagenic in bacterial and mammalian systems (IARC, 1990). Early studies examined the clastogenic effects of chromium compounds in cell cultures and *in vivo* and found that chromate was able to induce chromosomal damage in cell culture and *in vivo* in bone marrow after drinking water exposure (Ginter, *et al*., 1989; Sen and Costa 1986; Sen *et al*., 1987). Vitamin C was found to inhibit the ability of chromate compounds to induce micronuclei in bone marrow of guinea pigs given chromate by ingestion (Ginter *et al*., 1989). Chromate mutagenesis in tissue culture may have been difficult to study because of the absence of ascorbic acid in cells grown in culture. This vitamin is the major reducer of hexavalent Cr *in vivo,* and because it is not added to tissue culture media, the only source of it comes from the fetal bovine serum that is added to cultured media. Thus, the levels of this vitamin are low, and, in general, chromate compounds are not potent mutagens in tissue culture, although they induce chromosomal damage and form DNA adducts. A recent study has suggested that the actual Cr adducts on DNA may not be very mutagenic, because they bind to the phosphate backbone of DNA and do not distort the DNA helix very much (Blankert, 2003). This finding may explain the discrepancy between the DNA damage observed by chromate compounds and their low mutagenicity (Aiyar, 1991; Cupo, 1985a,b; Tsapakos, 1981).

Chromate compounds have been found to induce DNA damage *in vivo* in experimental animals and in lymphocytes of chromate-exposed workers (Cheng *et al*., 2000; Costa *et al*., 1993; Taioli, 1994; Toniolo, 1993). Thus, chromate exposure by inhalation is able to induce DNA damage in blood cells or in other organs that may be distant from the exposure site (Cheng, 2000).

7.3.5 Teratogenic Effects

By use of a whole-body radioautographic method, Diab and Södermark (1972) demonstrated Cr in the skin and the vertebral bone of the fetuses 1 hour after intravenous administration of isotonic $^{51}CrCl_3$ to pregnant mice. The later in the pregnancy the dose was administered, the more Cr could be demonstrated in the fetuses. Visek *et al*. (1953) detected only minute amounts of Cr in the fetuses of rats after intravenous administration of both the Cr(VI) and the Cr(III) form. Many details on the mechanisms of Cr transport through the placental membranes are still unknown. It has, however, been demonstrated that intravenous administration of 8 mg CrO_3/kg to pregnant golden hamsters induced cleft plates in a great number of the fetuses (Gale and Buch, 1979). Gilani and Marano (1979) injected CrO_3 in saline into embryonating chicken eggs at a dose ranging from 0.002–0.05 mg per egg. They demonstrated a high incidence of malformations, such as reduced body weight, microphthalmia, short and twisted limbs, ectopic heart, and everted viscera.

The occurrence of these malformations was low in the controls, but statistical significance was not tested. As shown by Kanojia *et al*. (1998), when feeding female rats before gestation at doses 250, 500, and 700 ppm of $K_2Cr_2O_7$ in drinking water, a significant reduction in the number of implantations and the number of fetuses was observed. Also, skeletal abnormalities (i.e., reduced ossification in parietal, interparietal, and caudal bones) were observed in the fetuses.

8 BIOLOGICAL MONITORING

8.1 Biomarkers of Exposure

There are considerable background levels of Cr(III) in foods, and many individuals supplement their diet with Cr picolinate and other trivalent Cr nutritional supplements, thus contributing to the high body burden of Cr(III). This background results in a high level of urinary Cr, making it difficult to use urine as a biomarker of exposure to Cr(VI). However, as mentioned earlier in this chapter, Cr(III) is barely (not) able to enter the cell, and thus, measurement of intracellular Cr levels will yield an excellent measure of human exposure to Cr(VI). Any Cr inside the red blood cell (RBC) is likely derived from Cr(VI) that survives extracellular reduction (Lukanova *et al*., 1996), but the short lifespan of RBCs limits the use of the RBC Cr-level as a marker. However, measurement of Cr in RBCs may be used at least as biomarker for high exposure to Cr(VI) shortly after exposure (1–3 months).

8.2 Biomarkers of Effects

At high levels of exposure, perforation of the nasal septum may occur after only 2–3 weeks of exposure and may be considered a warning symptom of more serious outcome effects. Lung X-ray screening among workers previously exposed to Cr(VI)—at high risk of cancer—has been shown to have some success (Bidstrup, 1983) and seems even more promising when combined with frequent sputum cytology, as shown by Nishiyama *et al*., (1988) who found that nine of nine screening-detected and subsequently treated lung cancer cases were alive 20 months to 8 years after treatment. Such secondary screening may prove useful among groups having accumulated very high cancer risk (i.e., an *a priori* risk of lung cancer exceeding 800×10^{-5}/year) provided supervision by experienced personnel.

A number of small pilot studies examined DNA–protein crosslinks in peripheral blood lymphocytes of chrome electroplaters and as a function of air pollution in Bulgaria as a biomarker of chromate exposure and effect (Costa *et al.*, 1996). DNA–protein crosslink levels correlated well with Cr RBC levels and with external measurements of exposure. The induction of DNA–protein crosslinks represents DNA damage occurring in the lymphocytes of humans exposed to chromate, and, thus, is a biomarker of chromate exposure and effect.

9 CELLULAR MECHANISM OF TOXICITY AND CARCINOGENICITY

9.1 Molecular Toxicology of Cr

As mentioned previously, Cr(III) compounds enter cells poorly because they do not resemble essential molecules that we need in the body, as does chromate. At physiological pH, Cr(VI) resembles phosphate and sulfate because it exists as an oxyanion with an overall –2 charge (Boyd, 1986). Thus CrO_4^{-2} is taken up into the cells by the sulfate and phosphate transport system and accumulates into cells through this molecular mimicry mechanism (Tsapakos, 1983). The uptake of all Cr(III) compounds is probably not negligible if the trivalent Cr is stably complexed with a ligand as is the case of Cr picolinate (Zhitkovich, 2005). However, if the Cr(III) complex is maintained in water solution (e.g., $CrCl_3$), it can form polymeric species that may eventually be too large to enter the cell (Zhitkovich, 2005). Regardless of whether the Cr(III) enters the cell or not, if it is stably complexed to a ligand, it will generally be nonreactive. Thus, the most important toxicological species is the hexavalent form of Cr that enters the cell and is immediately subject to reduction by means of one or two electron reducers. The reduction of hexavalent Cr depends on the pH and will generally occur much faster at acidic pH than at higher pH values (Sugden, 2002). It also depends on the level of the reducing agent. Inside the cell there are three major reducers of Cr(VI): ascorbic acid, glutathione, and cysteine (O'Brien, 2003; Zhitkovich, 1996; 2000). If the level of the reducing agent is very high relative to the concentration of Cr(VI), then reduction to Cr(III) will proceed quickly. However, if the level of the reducing agents are low compared with the amount of Cr(VI), then reduction may proceed by means of Cr(V) and Cr(IV) intermediates (Zhitkovich, 2005). The major toxic effect of hexavalent Cr in the cell is due to the reaction of these intermediates with protein and DNA (Zhitkovich, 1996). Attack can occur by means of the intermediates of Cr(III) (Zhitkovich, 1996). The reduction of hexavalent Cr by these reducing agents also generates oxygen radicals in the cell, which has also been implicated in the cellular injury induced by hexavalent Cr (Kawanishi, *et al*., 1994; Shi, 1999a,b).

Hexavalent Cr results in the induction of DNA adducts that involve not only Cr(III) but also the agents that were involved in the reduction of Cr(VI) such as ascorbic acid, glutathione, and cysteine (Quievryn, 2003). Ternary complexes of DNA, Cr(III), and these reducers have been found, and these complexes constitute most of the Cr that is bound to DNA (Zhitkovich, 2001;2002). These ternary complexes have mutagenic activity, and the most mutagenic activity is observed with larger molecular weight ternary complexes (Voitkun, 1998; Zhitkovich, 1996). A small percentage of the Cr bound to DNA represents DNA–protein and DNA–DNA crosslinks (Zhitkovich, 2005).

Hexavalent Cr compounds are among the only metal compounds that actually form strong adducts with DNA. They are also among the only carcinogenic metal compounds that have mutagenic activity, but this activity may have been underestimated in tissue culture because of the absence of ascorbic acid in most tissue culture systems (Zhitkovich, 2005). Ascorbic acid is the most active reducer of hexavalent Cr, and it yields trivalent Cr, which can be bound to DNA and induce mutagenesis (Capellmann, *et al*., 1995).

10 DIAGNOSIS, TREATMENT, PROGNOSIS, AND PREVENTION

Comparing the very high cancer rates in German studies in the 1930s and 1940s (Gross and Kölsch, 1943; Pfeil, 1935) and in the United States in the 1940s (Machle and Gregorius, 1948) and 1950s (Gafafer, 1953; Imprescia, 1952), and the much lower rates in studies 2–3 decades later (Alderson *et al*., 1981; Hayes *et al*., 1979; Langård *et al*., 1990) strongly suggest that reduced exposure levels over the past 4–5 decades has had a significant preventive impact. Even in the latter studies, lung cancers related to Cr(VI) exposure most likely resulted from exposure in the 1940s, 1950s, and 1960s. Thus, primary prevention may have been even more successful than what is reflected in these study cohorts. Sometimes low rates of Cr(VI)-related lung cancer may be explained by a shortened observation period, a phenomenon that may explain the low rates in the study by Korallus *et al*. (1974), where low rates of lung cancer may have resulted from the fact that sections for the population were quite young, and not as a result of prevention.

In the early studies, lung cancer incidence occurred in quite young Cr(VI)-exposed workers than in recent studies, a shift that is likely to result from reduced levels of exposure.

As illustrated previously, death rates of lung cancer among workers exposed to Cr(VI) in the 1920s to the 1960s may have been as high as 50–80 times that of the reference populations. Recent cohort studies generally present RRs of lung cancer two times the reference levels. The reference levels over these eight decades have increased significantly, possibly to some extent distorting the declined lung cancer rates among the Cr(VI)-exposed over these decades.

Active primary prevention should be investigated among workers still exposed to Cr(VI), and secondary prevention should be initiated among those already at high risk of respiratory cancer. Exposure to Cr(VI) still takes place among SS welders and workers in metal industries, together constituting a large population in developed and developing countries. A significant contribution to the prevention of future Cr(VI)-related cancers could be accomplished by conveying information on Cr(VI)-related health hazards and means of control to developing countries.

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CHAPTER

25

Cobalt

DOMINIQUE LISON

ABSTRACT

Cobalt is a relatively rare element of the earth's crust, which is essential to mammals in the form of cobalamin (vitamin B_{12}). The adult human body contains approximately 1 mg of cobalt, 85% of which is in the form of vitamin B_{12}. Human dietary intake of cobalt varies between 5 and 50 μg/day, and most of the cobalt ingested by humans is inorganic, vitamin B_{12} representing only a small fraction. The oral bioavailability of inorganic cobalt varies with the solubility of the cobalt compound (5–45%). In occupational settings, workers are exposed to cobalt compounds by inhalation of dusts. The main industrial use of cobalt is for the manufacture of alloys and hard metals. The absorption rate of inhaled cobalt also varies with the species considered. Cobalt does not accumulate in the organism and is rapidly excreted in urine. The concentration of cobalt in urine or in blood is proposed as a biomarker of recent exposure to soluble cobalt species. The respiratory system is the main target organ (asthma, fibrosing alveolitis, lung cancer) on inhalation exposure to cobalt, with a higher risk of fibrosing alveolitis (hard metal disease) and lung cancer in the hard metal industry, where workers are exposed to cobalt metal mixed with tungsten carbide particles. A physicochemical interaction leading to the formation of reactive oxygen species may account for the increased toxicity of this mixture of particles. Other target organs include the hematopoietic system, the myocardium, the thyroid gland, and possibly the reproductive system.

1 PHYSICAL AND CHEMICAL PROPERTIES

Cobalt (Co): atomic weight, 58.9; atomic number, 27; density, 8.9; melting point, 1495°C; boiling point, 2870°C; crystalline form, silver-grey metal, cubic. The main radioactive isotopes ^{57}Co, ^{58}Co, and ^{60}Co decay with respective half-lives of 272 days, 71 days, and 5.271 years, and emit γ-rays. This document only deals with the stable isotope. Pure cobalt metal is steel-grey, shiny, hard, ductile, brittle, and has magnetic properties. Cobalt compounds have oxidation states (II) or (III), the former being the most stable. The main compounds of toxicological interest are the metallic form (cobalt metal and its alloys and composite materials), the oxides (cobalt oxide and tetroxide), and the salts (cobalt(II) chloride, sulfide and sulfate). The most important Co(II) salts of carboxylic acids include formate, acetate, citrate, naphtenate, linoleate, oleate, oxalate, resinate, stearate, succinate, sulfamate, 2-ethylhexanoate, and cobalt carbonyl. A biologically important cobalt compound is vitamin B_{12}, or cyanocobalamin, in which cobalt is complexed with four pyrrole nuclei joined in a ring called corrin, similar to porphyrins.

The main characteristics of the most common cobalt compounds are summarized in Table 1.

Physicochemical studies have shown that cobalt metal, and not its Co(II) ionic species, is thermodynamically able to reduce ambient oxygen in reactive oxygen species (ROS); the kinetics of this process is, however, slow as a result of the poor oxygen-binding capacity at the surface of cobalt metal particles. In the presence of tungsten

TABLE 1 Some Physicochemical Properties of Cobalt Compounds

	CAS No.	Formulae	MW	Melting point (°C)	Density	Water solubility (g/L)
Cobalt(II,III) oxide	1308-06-1	Co_3O_4	240.80	895	6.07	Insoluble (0.00084)
Cobalt(II) oxide	1307-96-6	Co	74.93	1795, 1935	6.45	Insoluble (0.000313)
Cobalt(II) acetate (tetrahydrate)	71-48-7 (6147-53-1)	$Co(CH_3CO_2)_2$	177.03	—	—	Readily soluble
Cobalt(II) carbonate	513-79-1	$CoCO_3$	118.94	Decomposes	4.13	Practically insoluble
Cobalt(II) chloride (hexahydrate)	7646-79-9 (7791-13-1)	$CoCl_2$	129.84	Decomposes	3.36	Soluble (529 @ 20°C)
Cobalt(II) hydroxide	21041-93-0	$Co(OH)_2$	92.95	Decomposes	3.60	Very slightly soluble (0.0032)
Cobalt(II) nitrate (hexahydrate)	10141-05-6 (10026-22-9)	$Co(NO_3)_2$	182.96	Decomposes (100)	2.49	Soluble
Cobalt(II) sulfate (heptahydrate)	10124-43-3 ()	$Co(SO_4)_2$	154.99	Decomposes (735)	3.71	Soluble (362 @ 20°C)
Cobalt(II) sulfide	1317-426	CoS	90.99	>1100	5.45	Insoluble (0.0038 @ 18°C) α- and ß-forms

carbide (WC) particles (i.e., in hard metal powders), the reduction of oxygen in ROS by cobalt metal is catalyzed at the surface of WC particles (Keane *et al.*, 2002; Lison *et al.*, 1995), and soluble cobalt cations are produced in larger amounts. In this system, cobalt (II) ions are produced during the critical reaction but they do not drive it. This mechanism is illustrated in Figure 1.

In the presence of H_2O_2, Co(II) ions are able to produce hydroxyl radicals through a Fenton-like mechanism (Kadiiska *et al.*, 1989), and the oxidation potential of Co(II) ions can be modulated by chelating agents (e.g., 1,10-phenanthroline) to facilitate the generation of these radicals (Kadiiska *et al*., 1989; Mao *et al.*, 1996).

In vivo, the bioavailability of Co(II) ions is relatively limited, because these cations precipitate in the presence of physiological concentrations of phosphates (K_{sp}: 2.5×10^{-35} at 25°C) and nonspecifically bind to proteins such as albumin.

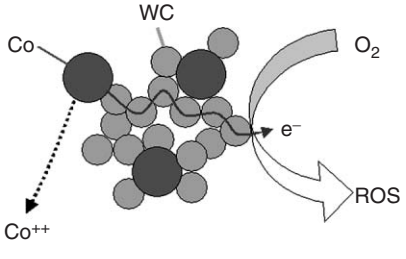

FIGURE 1 Physicochemical mechanism of interaction between cobalt metal (Co) and tungsten carbide particles (WC) producing amounts of reactive oxygen species (ROS) in aqueous solution and leading to the ionisation of cobalt (Co^{++}). Adapted with permission from Lison *et al*. (1995). Copyright (1995) American Chemical Society.

2 ANALYTICAL METHODS

Colorimetric methods were introduced during the 1940s for analyzing cobalt in biological materials. Detection limits of approximately 0.1 µg Co in a 20-mL sample of blood have been reported with these methods (Hubbard *et al.*, 1966; Stone, 1965). During the 1960s, emission spectrographs, polarography, X-ray fluorescence, and atomic absorption spectrophotometry (AAS) were introduced. By use of flame AAS, detection limits of 0.05 and 0.03 mg/L were reported in water (Fishman and Midgett, 1967; Sachdev *et al*., 1967). Saari and Paaso (1980) used a flameless AAS method for the measurement of cobalt in various foodstuffs after digestion of the samples in acid and extraction into methyl isobutylketone (MIBK). The authors were able to detect cobalt in foodstuffs down to concentrations in the order of 1 to 10 µg/kg. Lidums (1979) also used flameless AAS, but by use of an ion-exchange technique to concentrate cobalt, was able to detect cobalt in urine and in blood at concentrations of approximately 0.15 µg/L. A neutron activation method described by Cornelis *et al*. (1975) detected cobalt in urine at concentrations <0.5 µg/L.

Since then, a variety of modern techniques have been developed, and lower detection limits have been achieved.

Baruthio and Pierre (1993) have reported a method for the determination of cobalt in blood and urine by use of electrothermal atomic absorption spectrometry after mineralization and extraction with MIBK. The detection limit of this method was 1.90 nmol/L (approximately 0.12 µg/L) in serum and urine.

By use of ICP atomic emission spectroscopy (AES) coupled to online injection and preconcentration (Amberlite XAD-7), Farias *et al*. (2002) reported a detection limit of 0.025 µg/L in human urine. With the same technology but a concentration on a DPTH-gel chelating microcolumn, Zougagh *et al*. (2004) reported a detection limit in human urine of 0.0085 µg/L.

By use of adsorptive stripping voltammetry, the detection limit in human serum was 0.03 µg/L (Kajic *et al.*, 2003).

3 PRODUCTION AND USES

3.1 Production

Cobalt is a relatively rare element, composing approximately 0.001% of the earth's crust. The most important minerals are Cobalite ((Co, Fe)AsS), smaltite ($CoAs_2$), and erythrite ($Co_3(AsO_4)_2.8H_2O$). Cobalt often occurs in association with nickel, silver, lead, copper, and iron ores. In 2003, approximately 43,000 tons of cobalt were refined; the main producers are China, Russia, Zambia, and Nordic countries (Cobalt Development Institute, 2004).

3.2 Uses

The use of cobalt compounds for the manufacture of blue-colored pottery and glass antedates the Christian era, but the main consumption of cobalt is now in the production of steel and alloys. The CAS Registry lists approximately 5000 alloys of cobalt with other metals, of which cobalt is the base metal for approximately 2000. Advantages of cobalt alloys are high melting point, strength, and resistance to oxidation. So-called superalloys are made of cobalt, chromium, and other metals (tungsten in Stellite, but also molybdenum, tantalum, niobium, zirconium, hafnium) and are used to make very hard cutting tools and surfaces subject to heavy wear (e.g., in turbines or space vehicles). The so-called hard metals (also known as cemented carbides in the United States) are not genuine alloys but composite materials manufactured by a powder metallurgy process from tungsten carbide (approximately 90% in weight) and cobalt metal (up to 10 %) particles. Hard metals possess extraordinary properties of hardness (close to that of diamond) and remarkably resist heat and wear, and, unlike other metals, their hardness increases with temperature. Other alloys such as Vittalium (CoCrMo) form the basis of the prosthetic alloys used to produce hip and knee replacement joints.

Cobalt metal and cobalt oxides are also used for the manufacture of NiCd and Li-ion rechargeable batteries. Cobalt compounds are also used in nanotechnology for the synthesis of carbon nanotubes (Delpeux *et al*., 2002), and nanometric cobalt oxide particles have been produced at the laboratory level (Zao *et al*., 2004). The remainder of the cobalt use goes to different types of salts used in inks, plastics, and petroleum industry. A marginal part of refined cobalt is used in fertilizers, because a low cobalt concentration in soil may cause cobalt deficiency in sheep and cattle. Cobalt has also been used in human medicine in the treatment of certain iron-resistant anemias (Coles, 1955; Schirrmacher, 1967; Shuttleworth *et al*., 1961).

4 ENVIRONMENTAL LEVELS AND EXPOSURES

4.1 General Environment

4.1.1 Food and Daily Intake

Human dietary intake of cobalt is highly variable, with reported values generally between 5 and 50 µg/day. Most of the cobalt ingested by humans is inorganic, vitamin B_{12} representing only a small fraction. The main sources of vitamin B_{12} in food are beef liver and molluscs (USDA Nutrient Database).

In a survey conducted during the period 1986–1988 in Canada, the mean cobalt concentration in food items prepared for consumption was 9.4 ng/g (<0.3–75.7), with the highest concentration in waffles (76 ng/g), corn cereal (74 ng/g), and potato chips (70 ng/g). The estimated dietary intake was 11 µg/day (Dabeka and McKenzie 1995). In the United States, much higher values have, however, been reported in a survey on the metal content in more than 230 food items collected in 1984 by the Food and Drug Administration; Co intakes were estimated at 493–1489 µg/day.

In 15 commercial beers analyzed by Stone (1965) by a colorimetric method, cobalt generally measured well below 0.1 mg/L. If cobalt sulfate had been added in processing, values of up to 1.1 mg/L were recorded.

4.1.2 Soil, Ambient Air, and Water

Through the process of weathering, cobalt is leached from its parent earth crust material and enters the soil sediment system from which it is partially taken up by plants and animals. Elevated levels of cobalt in soil may also result from anthropogenic activities such as the application of cobalt-containing sludge or phosphate fertilizers, the disposal of cobalt-containing wastes, and atmospheric deposition from activities such as mining, smelting, refining, or combustion. Total cobalt in most soils ranges from 0.1–50 µg/kg.

Natural and anthropogenic sources contribute almost equally to the emission of cobalt in the atmosphere. Natural sources of cobalt in the atmosphere include

windblown continental dust, seawater sprays, volcanoes, forest fires, and continental and marine biogenic emissions. The primary anthropogenic sources of cobalt in the atmosphere are the burning of fossil fuels and sewage sludge, phosphate fertilizers, mining, transformation of cobalt-containing ores, and industries that produce or process cobalt. The natural background air concentration of cobalt is in the order of 1 pg/m^3, but concentrations up to 40 ng/m^3 have been reported in urban areas (Hamilton, 1994).

An average concentration of 0.3 µg Co/L has been reported in seawater, cobalt ranking as the 44th element in abundance, but there is a large regional variability. A few micrograms of Co/L can be present in river water, lakes, spring water, ground, or well water. Drinking water has a low-cobalt content, usually between 0.1 and 5 µg/L (Punsar *et al*., 1975; Schroeder *et al*., 1967).

4.2 Occupational Environment

Cobalt-containing dust may be released into the workroom air during the production of cobalt compounds and in the processing of hard metals. Typical concentrations in the working environment have ranged from 0.01–1.7 mg/m^3 (Fairhall *et al*., 1947; Kraus *et al*., 2001; Kusaka *et al*., 1986; Meyer-Bisch *et al*., 1989; Scansetti *et al*., 1998; Sprince *et al*., 1984, Swennen *et al*., 1993). In the hard metal industry, powder preparation, pressing, and shaping were found to represent the most polluting activities (Kumagai *et al*., 1996). Cobalt exposure has also been recorded, although to a lower extent, among diamond polishers (Lahaye *et al*., 1984) and dental technicians (Andersson *et al*., 1984; Leghissa *et al*., 1994). The importance of cobalt dissolution in cutting liquids used as coolants and working fluid aerosols for the processing of hard metals has also been emphasized (Sjögren *et al*., 1980). Present occupational exposure limits in different countries throughout the world range from 0.02–0.5 mg/m^3.

There is some experimental and clinical evidence that cobalt can be absorbed through intact human skin, in particular in hard-metal workers (Filon *et al.,* 2004; Linnainmaa and Kiilunen, 1997; Scansetti *et al*., 1994).

5 METABOLISM

As a constituent of vitamin B_{12}, cobalt is an essential element and, so far, another physiological role of cobalt has not been demonstrated in humans. The human body (adult, 70 kg) contains on the average 1.1 mg cobalt, 85% of which is in the form of vitamin B_{12}. The normal range of cobalt and vitamin B_{12} plasma concentrations are <0.2 ng/mL and 200–900 pg/mL, respectively (Nève, 1991). The daily requirements of vitamin B_{12} are of approximately 1 µg (0.1–2.5 according to different authors), which corresponds to 0.0434 µg of cobalt per day (4.34% of vitamin B_{12} weight). Considering that only 50% of vitamin B_{12} is absorbed by the gastrointestinal tract, the recommended daily allowance of vitamin B_{12} is 2.4 µg (or 0.10 µg cobalt). Because 10–30% of older people may malabsorb vitamin B_{12} from the food, it is recommended that individuals older than 50 years in the United States add a supplement of vitamin B_{12} to their diet (e.g., vitamin B_{12} fortified food) (Food and Nutrition Board, 2004).

In the following paragraphs, forms of cobalt other than vitamin B_{12} will be discussed.

5.1 Absorption

5.1.1 Animal Studies

5.1.1.1 Inhalation

Although limited in number, available studies indicate a substantial uptake of cobalt by inhalation. The absorption rate probably depends on the solubility of the cobalt compounds in biological fluids and in pulmonary macrophages, but data are only available for cobalt oxides and metallic cobalt particles. Studies in hamsters indicate that approximately 30% of inhaled CoO (particle size, 1–2.5 µm) is absorbed (Wehner and Craig, 1972). Of the inhaled amount, 60% of the CoO was recovered in the gastrointestinal tract, possibly reflecting particle clearance from the lung. In rats, the pulmonary absorption of the same compound (CoO) may be considerably less than in hamsters, because intratracheally instilled CoO (1.5 µg) was retained in the lung for a relatively long period (elimination half-time of approximately 15 days), indicating that the pulmonary absorption of cobalt oxide in this species is a slow process (Rhoas and Sanders, 1985).

Kreyling *et al*. (1993) performed clearance studies that used inhalation of monodisperse, porous cobalt oxide particles (1.4- and 2.7-µm mass median diameter) in Long–Evans rats. Of these particles, approximately 35% were eliminated by mechanical clearance to feces within 3 days. The half-life for long-term thorax retention was between 25 and 53 days. After 6 months, cobalt was still distributed mainly in the lung (91–52%), skeleton, and in soft tissue. Kyono *et al*. (1992) exposed rats to ultrafine metallic particles (primary diameter, 20 nm) by use of a nebulizer producing droplets with a mass median aerodynamic diameter of 0.76 µm (GSD, 2.1) at an arithmetic mean concentration of 2.12 ± 0.55 mg/m^3 for 5 hours per day for 4 days and induced reversible lung lesions (see Section 7.2.3.1). Clearance from the lung followed two phases: 75% of the cobalt was

cleared within 3 days with a biological half-time of 53 hours; the second phase cleared with a half-time of 156 hours.

Clearance from the lung depends on solubility. Kreyling *et al*. (1990) studied *in vitro* dissolution of cobalt oxide particles by human and canine alveolar macrophages. Smaller particles had faster dissolution rates. *In vitro* dissolution rates were found to be similar to *in vivo* translocation rates previously found for human and canine lung. Dissolution of ultrafine cobalt powder in simulated body fluid was six times higher than that of standard cobalt powder (Kyono *et al*., 1992).

In vivo, nontoxic doses of cobalt metal were administered intratracheally to rats either alone (0.03 mg cobalt /100 g body weight [bw]) or mixed with tungsten carbide (0.5 mg WC-Co containing 6% of cobalt metal particles/100 g bw). Sequential measurements of cobalt in the lung and urine demonstrated that the retention of the metal in the lung was longer in cobalt than in WC-Co–treated animals (12 vs 5 µg cobalt/g lung after 24 hours) (Lasfargues *et al*., 1992). This increased bioavailability of cobalt from hard-metal particles has been interpreted as the result of the physicochemical interaction between cobalt metal and tungsten carbide particles (Lison *et al*., 1995; Section 1).

By use of autoradiography and gamma-spectrometry, Persson *et al*. (2003) examined the transport of radiolabeled Co(II) in the olfactory system after intranasal administration as cobalt chloride in rats. The results showed an uptake of the metal in the olfactory mucosa and a transport to the olfactory bulbs of the brain.

5.1.1.2 Digestive

Gastrointestinal absorption of cobalt depends on the type of compound given, dose, and nutritional status. Less than 0.5% of CoO given orally to hamsters at a dose of 5 mg is absorbed (Wehner and Craig, 1972), whereas gastrointestinal absorption of cobalt chloride given to rats is estimated to be approximately 30% (Carlberger, 1961; Comar and Davis, 1947; Taylor, 1962). The gastrointestinal absorption of radiolabeled cobalt chloride in rats was found to vary between 11 and 34%, depending inversely on the administered dose (0.01–1000 µg/rat) (Taylor, 1962). Both amino acids and sulfhydryl groups complex with cobalt ions and reduce absorption. Iron deficiency increases the absorption of cobalt, and simultaneous administration of iron and cobalt reduces cobalt absorption (Schade, 1970). Some data also indicate that the gastrointestinal absorption is more efficient in young rats (98 and 85% at days 1 and 20, respectively) (Taylor and Harrison, 1995). After oral administration, the largest concentrations are found in liver, kidney, and spleen (Domingo, 1989).

5.1.2 Humans

5.1.2.1 Inhalation

There are few data on the respiratory absorption of inhaled cobalt-containing materials in humans. Increased excretion of the element in postshift urine of workers exposed to soluble cobalt-containing particles (cobalt metal and salts, hard-metal particles) has been interpreted as an indirect indication of rapid absorption in the lung; in contrast, when workers were exposed to less-soluble cobalt oxide particles, the pattern of urinary excretion indicated a lower absorption rate and probably a longer retention time in the lung (Lison *et al*., 1994). The importance of speciation and solubility for respiratory absorption has also been highlighted by Christensen and Poulsen (1994), who found that cobalt in blood and urine was increased in pottery plate painters who used a soluble pigment, whereas only slightly increased values were found in those using an insoluble pigment (see Section 6).

5.1.2.2 Digestive

The gastrointestinal absorption of cobalt compounds is reported to vary from 5–45% in different individuals. Ripak (1961) studied cobalt balance in three school children, and their daily intake was calculated to be 41 µg. Of this figure, 92–95% was found in feces and 4–5% in urine. When ^{60}Co as $CoCl_2$ was given orally to humans, the average absorption was calculated at 5–44%; these figures are based on the amount of unabsorbed cobalt remaining in the feces (Smith *et al*., 1972; Valberg *et al*., 1969). On the basis of 24-hour urinary excretion studies after oral administration of ^{60}Co, absorption has been estimated to be on average 18%, individual values ranging from 9–42% (Sorbie *et al*., 1971). Absorption and/or excretion is influenced by the amount of cobalt given and by nutritional factors. For example, the 48-hour urinary excretion of labeled cobalt chloride given orally ranged between 1 and 16%, depending on the amount of cobalt given, the fasting or nonfasting state of subjects, or the addition of albumin to the diet (Paley and Sussman, 1963). In a short-term crossover study in volunteers, the gastrointestinal uptake of soluble cobalt chloride was found to be considerably higher than that of insoluble cobalt oxide (urine ranges, <0.17–4373 and <0.17–14.6 nmol/mmol creatinine, respectively). It was also shown that ingestion of controlled amounts of soluble cobalt compounds resulted in significantly higher urinary cobalt levels ($P < 0.01$) in women (median, 109.7 nmol/mmol creatinine) than in men (median, 38.4 nmol/mmol creatinine), suggesting that the gastrointestinal uptake of cobalt is higher for women than men (Christensen *et al*., 1993).

Inverse relationships between iron status (serum ferritin, soluble transferrin receptor in serum) and cobalt in blood have been reported in adolescents, suggesting that the gastrointestinal absorption of the element depends on transport mechanisms similar to that of iron and is, therefore, influenced by the iron status that regulates the expression of these transporters in the gut (Barany *et al.*, 2005).

5.2 Distribution

5.2.1 Animal Studies

Although cobalt metal particles are practically insoluble in water, the solubilization of these particles is greatly enhanced in biological fluids because of extensive binding of Co(II) ions to proteins (0.003 mg/L in physiological saline, but 152.5 mg/L in human plasma at 37°C) (Harding, 1950). Moreover, the solubilization of cobalt metal particles is increased up to sevenfold in the presence of WC particles (in oxygenated phosphate buffer at 37°C) (Lison *et al.*, 1995).

Cobalt given to animals parenterally or in food is found at the highest concentrations in liver, kidneys, adrenals, and thyroid (Comar and Davis, 1947; Comar *et al.*, 1946; Copp and Greenberg, 1941; Taylor and Marks, 1978). Twelve to 36 hours after the administration of a small dose of radiolabeled cobalt chloride to pregnant mice, Söremark *et al*. (1979), using an autoradiographic technique, found the highest activity of radioactive cobalt in liver, kidney, fetus, and placenta. Taylor and Marks (1978) studied rats 1 week after they had received 10 daily subcutaneous injections of cobalt chloride (40 mg/kg) and found the highest amount of cobalt in the liver, which on average contained 11% of the administered dose. In dogs exposed to ^{60}Co oxides from inhalation (Co_3O_4 and CoO), the highest cobalt concentrations were recorded in the lung after 140 days, followed by kidney and liver (Barnes *et al.*, 1976).

5.2.2 Humans

After absorption, cobalt is distributed systemically, but there is no specific organ that accumulates the element, except the lung in case of inhalation of insoluble particles. Normal cobalt content in human lung has been reported to be 0.27 ± 0.40 (mean ± SD) µg/g dried lung on the basis of tissue samples taken from 2274 autopsies (mainly neoplasms) in Japan (Takemoto *et al.*, 1991). There was no increase in concentration with age, no gender difference, and no association with degree of emphysema or degree of contamination (the grade of particle deposition in the lung). In human beings exposed to cobalt, the liver and the kidney contain the highest concentration of cobalt. After intravenous administration of radiolabeled $CoC1_2$, Smith *et al*. (1972) estimated the liver to hold approximately one fifth of the total body burden of cobalt, a finding in accordance with data from experimentally exposed animals (see Section 5.2.1). In the human body, there is no indication of any accumulation of cobalt with age (see Section 67).

5.3 Excretion

5.3.1 Animal Studies

The major proportion of systemically distributed cobalt is cleared rapidly (within days) from the body, mainly through urine, but a certain proportion (10%) has a longer biological half-time in the range of 2–15 years. After intravenous administration of cobalt chloride to rats, 10% of the dose was excreted in feces, indicating that cobalt can also be secreted in the bile.

5.3.2 Humans

As in animals, the main excreted cobalt will be found in urine. Kent and McCance (1941) gave daily cobalt injections of 2.6 mg for 5 days to human volunteers. Two days after the last injection, 15% of the given dose had been recovered in urine and 5% in feces. A similar study on human volunteers was performed by Smith *et al*. (1972). After 8 days, 56 and 11% of the given dose had been excreted in urine and feces, respectively. Six to eight percent of an oral dose of cobaltous chloride was eliminated within 1 week in normal healthy persons. The elimination of cobalt is considerably slower in hemodialysis patients, which further supports the importance of renal clearance (Curtis *et al.*, 1976).

6 BIOLOGICAL MONITORING

In nonoccupationally exposed subjects, a wide range of cobalt concentrations in urine and blood have been reported, and normal values are probably <2µg/L (or µg/ g creatinine) for urine and 0.5µg/L for blood (Lauwerys and Hoet, 2001). Increased urinary cobalt concentrations may be observed in individuals taking multivitamins and patients with a Co-containing prosthesis (Hennig *et al.*, 1992; Schaffer *et al.*, 1999). Increased urinary cobalt values have been reported in smokers, but blood values were not similarly increased (Alexandersson, 1988).

In subjects occupationally exposed to cobalt compounds, the urinary excretion and the blood or serum concentration of the metal has been proposed as biological indicator of exposure. Most biological monitoring studies have been carried out in hard metal workers.

Alexandersson and Lidums (1979) measured the urinary excretion of cobalt in workers occupationally exposed to hard metal dusts. During one working week, these workers displayed urinary excretion of cobalt more than 100 times higher than that found in controls. Urinary levels of cobalt increased during the working week and were approximately twice as high in the afternoon after an 8-hour working shift as morning values. The workers were also given a follow-up for a period of 4 weeks after vacation (i.e., during a nonexposure period). During this time, the average urinary concentration decreased from approximately 60–5 μg/L. This latter value was, however, approximately 10 times higher than that found in urine obtained from men without previous cobalt exposure. In a later study, Alexandersson (1988) compared blood and urinary values in 70 cobalt-exposed workers from the hard metal industry. He found that variations of cobalt in blood were smaller than for urine, but both parameters were shown to follow ambient exposure (5–150 μg/m^3). On the Friday afternoon samples, the average exposure to cobalt for the entire work week was correlated ($n = 10$), with the concentration of the element in urine (0.79) and in blood (0.87).

Studying 10 groups of hard metal workers (airborne cobalt concentration, 28–367 μg/m^3), Ichikawa *et al*. (1985) found a good correlation between cobalt concentration in blood and cobalt in air on the basis of the mean values observed in the different groups. On the basis of the correlation analyses, an airborne exposure to 100 μg/m^3 was associated with a cobalt concentration of 5.7–7.9 μg/L in blood and 59–78 μg/L in urine (95% confidence intervals). In a survey involving similar groups of workers (airborne cobalt concentration, 120–284 μg/m^3), Perdrix *et al*. (1983) suggested that the difference between the end and the beginning of the shift urinary cobalt concentration reflected the day exposure. The concentration in the Friday evening urine was indicative of the cumulative exposure during the week, and the level of cobalt in urine collected on Monday morning mainly reflected long-term exposure. In another group of hard metal workers exposed to cobalt airborne concentrations <100 μg/m^3, it has been shown that there was an increase of urinary cobalt concentration as the workweek proceeded (Scansetti *et al*. 1985). In a study in diamond polishers who used cobalt-containing disks, the measurement of urinary cobalt concentration (0.03–75 μg/g creatinine), when considered on a workshop basis, was found to reflect the level of exposure to the metal (<50 μg/m^3) (Nemery *et al*., 1992).

Limited comparative data are available for other forms of cobalt to which workers may be exposed. Their absorption rate depends on their solubility in biological media, which may also be influenced by the concomitant presence of other substances, and this is likely to affect the interpretation of biomarkers of exposure. The importance of the chemical nature of the exposure has been pointed out by Christensen and Poulsen (1994), who observed increased concentrations of cobalt in blood (0.2–24 μg/L) and urine (0.4– 848 μg/L) of pottery plate painters who used a soluble cobalt pigment, whereas only slightly increased values were measured in those using an insoluble cobalt pigment (0.05–0.6 and 0.05–7.7 μg/L in blood and urine, respectively).

The relationships between environmental and biological (blood and urine) parameters of exposure for different chemical forms of cobalt have been investigated in a cross-sectional study in workers exposed to cobalt metal, oxides, and salts in a refinery or to a mixture of cobalt and tungsten carbide in a hard metal producing plant (Lison *et al*., 1994). The main conclusion of this study was that although biological monitoring of workers exposed to cobalt oxides revealed increased blood and urine levels compared with nonexposed subjects, these parameters poorly reflected recent exposure level (23–7772 μg/m^3). In contrast, when exposure was to soluble cobalt compounds (metal, 17–10,767 μg/m^3; salts, 1–4690 μg/m^3; and hard metals, 1–203 μg/m^3), the measurement of urine and/or blood cobalt at the end of the workweek could be recommended for the monitoring of workers. It was calculated that an 8-hour exposure to 20 or 50 μg/m^3 of a soluble form of cobalt would lead to an average urinary concentration of 18.2 and 32.4 μg of cobalt/g creatinine, respectively (postshift urine sample collected at the end of the workweek).

In workers from the hard metal industry, it has been shown that concentrations of cobalt in urine rapidly increase in the hours that follow cessation of exposure, with a peak of elimination approximately 2–4 hours after exposure, and a subsequent decrease (more rapid for the first 24 hours) in the following days (Apostoli *et al*., 1994).

There is no biological marker of effect that would be specific for cobalt or hard metal exposure.

The determination of the HLA-DP genotype may have some relevance in terms of individual susceptibility to hard metal disease (see Section 7.2.3.2).

7 EFFECTS AND DOSE-RESPONSE RELATIONSHIPS

7.1 Local Effects

The existence of cobalt allergy is well documented; it generally causes an erythematous and/or papular

dermatitis (Camarasa, 1967; Marcussen, 1963; Raben *et al*., 1966; Schwartz *et al*., 1945). The differentiation with an irritant reaction is difficult because both reactions can coexist. Allergic dermatitis caused by cobalt is apparently much more common in Europe than in the United States, possibly because of the presence of the element in European detergents and cement and because cobalt allergy is not routinely tested in the United States. Cobalt dermatitis may result from exposure to substances containing the element, such as hair dyes, flypaper, shell splinters, antiperspirant creams, crayons, and fertilizers. Occupations in which cobalt allergy may occur are not only found in the cobalt industry, but also among polyester resin industry, offset printers, and building workers handling cement. Photosensitization to cobalt in cement has also been described by Spanish investigators (Camarasa and Alomar, 1981; Romaguera *et al.*, 1982). Cobalt skin allergy is usually found in connection with other types of contact allergies (nickel, chromium) but does not represent genuine cross-reactions (Jung and Zimmermann, 1976; Rystedt, 1979). Among 853 patch-tested hard metal workers in Sweden, 39 (4.6%; 9 males and 30 females) had a confirmed allergic reaction to 1% cobalt chloride. In 24 cases, the reaction was isolated to cobalt, and most (92%) of the workers with a positive patch test to cobalt had a history of eczema. Most of these lesions developed within the first year of employment, and risk factors were prior sensitization to nickel and irritative dermatitis (Fischer and Rystedt, 1983). In a cross-sectional study in the Swedish hard metal industry, the prevalence of hand eczema and irritant reaction was 10 and 15%, respectively. The cumulative incidence of hand eczema over a period of 10 years was estimated to be 17% (Fischer and Rystedt, 1983). Dissolution of cobalt in cutting fluids used during industrial operations represents an aggravating factor, because appreciable amounts of cobalt can solubilize in these fluids (Einarsson *et al*., 1979).

7.2 Respiratory Effects

The respiratory system is the critical target organ when exposure is by inhalation, and toxic manifestations have been reported at different levels, inculding the upper respiratory tract, the trachea and bronchi, and the alveolar parenchyma.

7.2.1 Upper Respiratory Tract

7.2.1.1 Animal Data

In experimental systems, soluble cobalt salts have been shown to cause severe toxicity to the upper respiratory tract. Degeneration of the olfactory epithelium, squamous metaplasia of the respiratory epithelium, and inflammation in the nose and larynx have been observed in rats and mice exposed by inhalation to cobalt sulfate heptahydrate (Bucher *et al*., 1990). The most sensitive tissue was the larynx, with squamous metaplasia observed in rats and mice at the lowest exposure concentration of 0.3 mg/m3. The effect of other forms of cobalt on the upper respiratory tract has not been examined.

7.2.1.2 Human Data

In workers exposed to cobalt-containing dusts, inflammation of the nasopharynx seems to be particularly frequent in the hard metal industry (Fairhall *et al*., 1947; Tolot *et al*., 1970), and it is unclear whether this results from a nonspecific irritation by cobalt-containing particles or from an immunologically mediated reaction (allergic rhinitis).

7.2.2 Bronchial Tree

7.2.2.1 Human Data

Coates and Watson (1971) described, in hard metal workers, a respiratory disease characterized by cough, wheezing, and shortness of breath that was relieved by removal from the work environment. This type of disease was suggested to have an allergic background. Similar symptoms related to occupational exposure to cobalt were studied by Bech (1974) and by Scherrer and Maillard (1982). The subsequent disorder was named hard metal asthma. Exposure to other cobalt species such as salts (Roto, 1980) or cobalt metal released from diamond polishing activities (Gheysens *et al*., 1985) may also cause typical bronchial asthma in a small proportion of workers (generally <5%). Most cases of cobalt-induced asthma have, however, been reported from the hard metal industry (Shirakawa *et al*., 1989). In some patients, a type I allergic reaction has been suspected, because IgE antibodies against a complex of Co(II) with albumin could be identified. For the remaining patients, the mechanism of cobalt-induced asthmatic reaction still remains to be explained (Shirakawa *et al.*, 1988). A specific inhalation challenge test with a cobalt salt, hard metal, or cobalt metal powder may be indicative to confirm the diagnosis of cobalt-induced asthma. A positive lymphocyte transformation test with cobalt(II) ions has also been reported in hard metal asthma, suggesting a role for cellular immunity (Kusaka *et al*., 1991).

Several cross-sectional studies have also indicated that workers exposed to cobalt-containing dusts in hard metal plants (Alexandersson and Lidmus, 1979), in diamond-polishing tools (Nemery *et al*., 1992), and

in cobalt producing plants (Linna *et al*., 2003; Swennen *et al*., 1993) had an increased prevalence of symptoms reflecting irritation of the airways and/or showed altered lung function tests, reflecting the existence of obstructive disease (decreased FEV_1 and/or FEV_1/VC). In a longitudinal study (13 years follow-up) conducted in workers from a cobalt production plant (Verougstraete *et al*., 2004), the main finding was that cobalt exposure contributed to a decline in FEV_1 over time only in smokers. On the basis of the estimates provided by the modeling of their data, these authors calculated that an exposure entailing a cobalturia of 10, 20, or 40 µg/g creatinine (which is roughly equivalent to a time-weighted average exposure at 10, 20, or 40 µg/ m^3) would cause, for a 30-year-old smoking worker, an additional decrement of 64, 84, or 103 mL of FEV_1 after 10 years of work at this plant (compared with a decrement of 518 mL caused by smoking alone).

7.2.3 Lung Parenchyma

7.2.3.1 Animal Data

Harding (1950) was probably the first to describe severe and fatal pulmonary edema and hemorrhage in rats treated intratracheally with cobalt metal powder (500 µg/rat). In guinea pigs, a single intratracheal instillation of cobalt metal (10–50 mg) led to the development of acute pneumonia with diffuse cellular infiltration (eosinophils) and bronchiolitis obliterans. The subchronic response assessed 8–12 months after the acute dosing was characterized by the presence of multinucleated cells and a lack of cellular reaction within the alveolar walls. Instillation of cobalt metal mixed with tungsten carbide (150 mg in a 9:91 ratio, or 13.5 mg cobalt metal) in guinea pigs induced a transient inflammatory reaction with residual papillary hypertrophy of bronchial mucosa and peribronchial and periarterial fibrosis in the vicinity of retained particles; multinucleated giant cells were present in some instances (Schepers, 1955). Kaplun (1963) found that cobalt metal particles instilled intratracheally in rats (5 or 10 mg/animal) produced, after 4–8 months, diffuse sclerosis and sclerosis of the peribronchial and perivascular tissue. Kerfoot *et al*. (1975) exposed mini pigs for 3 months (6 hours/day, 5 days/week) to aerosols of cobalt metal at concentrations of 0.1 and 1.0 mg/m^3. The animals were first submitted to a sensitization period of 5 days followed by a 10-day removal from exposure. Postexposure lung function studies demonstrated a dose-dependent and reversible reduction in lung compliance but no radiographic or histological sign of fibrosis, except some increased collagen deposition that could only be seen under the electron microscope. Kyono *et al*. (1992) examined the effect of ultrafine cobalt-metal particles (20 nm) on the lung of rats exposed by inhalation (2 mg/m^3) during 5 hours per day for 4 days. Focal hypertrophy and proliferation of the lower airway epithelium, damaged macrophages, and type I pneumocytes, as well as proliferation of type II cells, fibroblasts, or myofibroblasts, were observed. The morphological lesions were reversible after 1 month.

In a study designed to compare the acute toxicity of hard metal particles (WC-Co mixture containing 6% of cobalt metal particles, median particle size d_{50}, 4 µm) with that of an equivalent dose of cobalt metal particles alone, the lung response was first examined 48 hours after intratracheal instillation in rats. After treatment with a high dose of cobalt metal particles (1 mg/100 g bw), a significantly increased lung weight was noted, but the changes were much more severe and even fatal in animals treated with cobalt mixed with tungsten carbide particles. A second series of experiments using a lower dose of cobalt metal (0.06 mg/100 g bw) was performed to analyze the cellular fraction of the bronchoalveolar lavage (BAL) and histology 24 hours after dosing. This dose of cobalt metal alone did not produce any significant biochemical or cellular modification in BAL, and histological sections were almost normal. Increased mortality was observed in the group of animals dosed with WC-Co but not in those treated with cobalt metal or WC alone. The analysis of the cellular fraction of the BAL from animals treated 24 hours previously with hard metal particles (1 mg/100 g bw) showed a marked increase in the total cell number. Similarly, biochemical analyses of the BAL showed an increase in LDH activity, total protein, and albumin in the hard metal-treated group, whereas the individual components of the mixture (i.e., Co and WC) did not produce any significant modification of these parameters when administered alone. Although histological lung sections from rats instilled with cobalt alone (0.06 mg/100 g bw) or tungsten carbide (1 mg/100 g bw) particles were almost normal, an intense alveolitis was observed with the hard metal mixture (1 mg/100 g bw) (Lasfargues *et al*., 1992).

Wehner *et al*. (1977) examined the influence of lifetime inhalation of cobalt oxide (10 µg/L, 7 hours/day, 5 days/week) alone or in combination with cigarette smoke in hamsters with the aim of detecting a carcinogenic effect (see Section 7.5.1). In cobalt oxide-exposed animals, some pulmonary changes consisting of focal interstitial fibrosis, granulomas, hyperplasia of alveolar cells, and emphysema were observed. In rabbits (Johansson *et al*., 1983, 1986), exposure to 0.4 and 2.0 mg/m^3 cobalt chloride for 1 and 4 months increased the number of alveolar macrophages and their oxidative metabolic activity. Accumulation of alveolar

macrophages and hyperplasia of type II pneumocytes were found after inhalation of cobalt chloride (0.5 mg/m^3 for 1 month). Exposure to 0.4 and 2 mg/m^3 cobalt chloride for 14–16 weeks (6 hours/day, 5 days/week) induced a combination of lesions characterized by nodular aggregation of type II cells; abnormal accumulation of enlarged, vacuolated alveolar macrophages; and interstitial inflammation (Johansson *et al*., 1987). Camner *et al*. (1993) reported that the inflammatory reaction (BAL neutrophils and eosinophils) induced by the inhalation of cobalt chloride (2.4 mg/m^3, 6 hours/day during 2 weeks) was more pronounced in guinea pigs that had been presensitized to cobalt by repeated application of cobalt chloride.

In Fischer 344/N rats and B6C3F_1 mice exposed to cobalt sulfate heptahydrate aerosols of 0, 0.3, 1.0, 3.0, 10, or 30 mg/m^3 for 6 hours/day on 5 days/week for 13 weeks, the investigators found, in addition to lesions to the upper respiratory tract (see earlier), histiocytic infiltrates, bronchiolar epithelial regeneration, and epithelial hyperplasia in the lung alveoli. In rats, proteinosis, alveolar epithelial metaplasia, granulomatous alveolar inflammation, and interstitial fibrosis were observed at all dose levels. The nonneoplastic lesions were less severe in mice and mainly consisted of cytoplasmic vacuolization of the bronchi (Bucher *et al*. 1990; 1999).

The intratracheal instillation of cobalt chloride (1–1000 µg/kg) into hamster lungs induced biochemical changes compatible with the development of oxidative stress (decreased levels of reduced glutathione, increased levels of oxidized glutathione, as well as stimulation of the pentose phosphate pathway). Similar changes were also observed *in vitro* after incubation of lung slices with cobalt chloride (0.1–10 mmol/L); these manifestations preceded the detection of cellular toxicity, indicating their possible early involvement in the pulmonary toxicity of cobalt (II) ions (Lewis *et al*., 1991).

7.2.3.2 Human Data (Hard Metal Disease)

Interstitial (or parenchymal) lung disease caused by cobalt-containing particles is a rare occupational lung disease, generally called *hard metal lung disease* (Balmes, 1987; Bech *et al*., 1962; Cugell, 1992; Hartung, 1986; Lison, 1996). This disease affects a small percent of the workforce at most (Lison, 1996). This lung disease generally presents as a giant-cell interstitial pneumonia that is now accepted as being pathognomonic of hard metal lung disease (Ohori *et al*., 1989). It occurs mainly in workers from the hard metal industry but has also been reported in diamond polishers exposed to cobalt metal powder associated with iron and diamond dust in the apparent absence of tungsten carbide (Demedts *et al*., 1984). There is no available evidence that exposure to other cobalt species (cobalt metal alone, salts, oxide, or other alloys) may cause interstitial disease (Linna *et al*., 2003, Lison *et al*., 1996; Swennen *et al*., 1993).

The clinical presentation of hard metal lung disease is variable, with some patients presenting with a picture of subacute alveolitis and others with that of chronic interstitial fibrosis, and most studies have found no relation between disease occurrence and length of occupational exposure (Balmes, 1987; Cugell, 1992; Cugell *et al*., 1990). In some cases, the outcome of hard metal disease may be fatal (Ruokonen *et al*., 1996). The high-resolution computed tomography (HRCT) appearance of HM-ILD includes reticulation, traction bronchiectasis, and large peripheral cystic spaces in a mid and upper lung distribution (Gotway *et al*., 2002). Some aspects of this pathology such as the lack of correlation with the intensity or duration of exposure, the low frequency of the disease, and the presence of T cells at the inflammation site suggest the existence of a genetic susceptibility. It has, indeed, been reported that hard metal disease was associated with a Glu-69 polymorphism of the HLA-DP beta chain (Potolicchio *et al*., 1997) but the strength of this association was less than in berylliosis (Richeldi *et al*., 1993).

7.2.3.3 Mechanisms of Toxicity

There is little doubt that cobalt plays a critical role in the pathogenesis of hard metal lung disease. As mentioned previously, experimental studies have demonstrated that the mixture of Co and WC particles, which compose the hard metal dust, exhibits a unique pulmonary toxicity compared with cobalt particles alone. The toxicity can be explained, at least in part, by the production of toxic oxygen species at the surface of WC particles (Keane *et al*., 2002; Lison *et al*., 1995; Section 1).

The basis for individual susceptibility for the development of hard metal lung disease is not known. Cobalt is known to elicit allergic reactions in the skin, but the relationship, if any, between allergy and hard metal disease is unknown. Occasionally, patients have been found to have both cobalt dermatitis and interstitial lung disease (Cassina *et al*., 1987; Demedts *et al*., 1984; Sjögren *et al*., 1980). Immunological studies (Kusaka *et al*., 1989; 1991; Shirakawa *et al*., 1988; 1989; 1990; 1992) have found specific antibodies and/or positive lymphocyte transformation tests against cobalt (as well as nickel) in some patients with hard metal asthma. The association of hard metal disease with the presence of glutamate at position 69 in the HLA-DP beta chain (Potolicchio *et al*., 1997) probably reflects a higher affinity of the mutated HLA-DP molecule for cobalt, which may interfere with antigen presentation (Potolicchio *et al*., 1999).

7.3 Other Systemic Effects

7.3.1 Blood

Cobalt has an erythropoietic action, increasing blood volume and total erythrocyte number in several animal species (Brewer, 1940; Liotti *et al*., 1971; Taylor *et al*., 1977; Tribukait, 1963). Doses necessary for eliciting an erythropoietic effect have been in the order of 1 mg/kg given daily. This effect is probably the result of the capacity of cobalt to mimic the pathophysiological response to hypoxia that involves various genes, including those coding for erythropoiesis and growth factors for angiogenesis (Gleadle *et al*., 1995; Steinbrech *et al*., 2000). Up-regulation of erythropoietin (EPO) gene expression has been observed *in vivo* after a single intraperitoneal injection of 60 mg/kg bw cobalt chloride into rats (Gopfert *et al*., 1995).

Cobalt has been used in the medical treatment of anemias. Duckham and Lee (1976) gave 12 anephric patients daily doses of cobalt chloride orally in amounts corresponding to 6.2 and 12.4 mg cobalt per day for a period of 12–30 weeks. This treatment gave rise to an average increase in the hemoglobin concentration of 46%. After cessation of cobalt treatment, the hemoglobin levels decreased. Polycythemia has also been reported in heavy drinkers of cobalt-contaminated beer (Alexander, 1972; Kesteloot *et al*., 1968; Morin and Daniel, 1967).

The possibility that polycythemia may result from industrial exposure to cobalt compounds has been raised, but it is difficult to differentiate between a direct effect of cobalt on the bone marrow and a response to arterial desaturation resulting from the lung disease caused by the inhalation of cobalt-containing particles. A trend toward reduced erythropoietic indices has been reported in workers exposed to cobalt metal powder (Swennen *et al.,* 1993). Injections of cobalt chloride in doses of several mg/kg also resulted in increases of serum lipids, mainly the triglyceride fraction (Caplan and Block, 1963; Taylor *et al*., 1977; Zarafonetis *et al*., 1965). Fiedler *et al*. (1971) demonstrated that cobalt compounds might also cause blood hypocoagulability.

7.3.2 Myocardium

Subsequent to the reports of cardiac failure in drinkers of beer in which cobalt was used as an additive (see later), a large number of animal studies have shown that repeated intramuscular injection of cobalt in the order of 5 mg/kg to rats produced myocardial degeneration. In an experiment that used electron microscopy, slight changes were seen after only one injection (Hall and Smith, 1968; Lin and Duffy, 1970). Electrocardiographic changes were also reported among exposed animals (Mohiuddin *et al*., 1970). Peroral administration of cobalt (as $CoSO_4$ and $CoCl_2$) to rats and rabbits in doses ranging from 10–100 mg/kg body weight, for periods exceeding two weeks, produced the same cardiotoxic effects as those observed after parenteral dosing (Desselberger and Vegener, 1971; Grice *et al*., 1969; Rona and Chappel, 1973).

According to Kerfoot *et al*. (1975), long-term inhalation exposure to cobalt (0.1 and 1 mg/m^3) produces ECG changes in miniature swine.

Sandusky *et al*. (1981) used repeated infusions of cobalt, approximately 30 mg/kg bw in dogs, as a model for investigating alcoholic cardiomyopathy in humans. Typical results of the cobalt-induced cardiomyopathy were dyspnea, exercise intolerance, abnormal ECG, and enlargement of the left ventricle. Microscopical examination revealed severe myocardial degeneration.

Endemic outbreaks of cardiomyopathy with mortality rates up to 50% have been described among heavy beer drinkers, who consumed beer containing high cobalt concentrations. Common findings were heart failure, polycythemia, and thyroid lesions. These outbreaks have been reported in Quebec, Canada, in Minneapolis, and in Belgium (Alexander, 1972; Kesteloot *et al*., 1968; Morin and Daniel, 1967). The syndrome first appeared approximately 1 month after a new brewing process had been introduced whereby cobalt chloride was added to beer to improve the stability of the froth. It was assumed that a really heavy beer drinker consuming up to 10 L of beer consumed 10 mg cobalt per day. Although this figure is excessively high compared with nutritional standards (see Section 4.1.1), it is not as large as doses given in the treatment of anemias (see Section 7.3.1). It has been suggested that the syndrome was multicausal, other factors probably having been malnutrition and excessive consumption of alcohol (Balazs and Herman, 1976). Morin *et al*. (1971) studied some of these beer-drinking patients and reported typical signs of heart failure with shortness of breath, ankle edema, and, in some cases, cyanosis and ECG changes. On autopsy, Bonenfant *et al*. (1969) found striking myocardial fiber degeneration when heart tissue was examined histologically. In a retrospective study, hearts of victims were shown to have approximately 10 times the cobalt concentrations of controls, 0.5 mg/kg wet weight and 0.04 mg/kg wet weight, respectively (Sullivan *et al*., 1968).

Three cases of myocardial toxicity have been associated with industrial exposure (Alusik *et al*., 1982; Barborik and Dusek, 1972; Kennedy *et al*., 1981). In a group of 30 hard metal workers, reduced right ventricular contractility was associated with radiographic pulmonary involvement, suggesting occult cor pulmonale in some of these workers. A subtle, but significant, inverse relationship between duration of exposure

and left ventricular function assessed by radionuclide ventriculography was, however, also reported (Horowitz *et al*., 1988). In workers from a cobalt production plant exposed to various cobalt compounds, Doppler echocardiography revealed an association between cumulative cobalt exposure and both left ventricular isovolumic relaxation time and deceleration time of the velocity of the early rapid filling wave, indicating altered left ventricular diastole (Linna *et al*., 2004).

It has also been suggested that cobalt retention plays a role in the development of uremic myocardiopathy (Lins and Pehrsson, 1976).

7.3.3 Thyroid Gland

Goiter is a well-known side effect of cobalt therapy in the medical treatment of certain anemias. A reduced ^{131}iodine concentration capacity has been seen in patients given 20–30 mg Co per day (Kriss *et al*., 1955; Roche and Layrisse, 1956; Schirrmacher, 1967). Usually, these adverse effects are regarded as reversible and probably reflect inhibition of tyrosine iodinase by cobalt (II) ions. Thyroid changes were also noted among the Quebec beer drinkers (Roy *et al*., 1968). On autopsy, 11 of 14 examined glands showed significant changes, with reduced follicle size. Of 12 patients treated for anemia with cobalt by Duckham and Lee (1976), one had hypothyroidism, but it was uncertain to what extent this side effect could be related to the cobalt treatment. In a cobalt production plant, a subclinical hypothyroid status has been reported (Swennen *et al*., 1993) and, inversely, in Danish plate painters exposed to cobalt-zinc silicate, increased concentrations of total and free serum T4 have been found (Prescott *et al.,* 1992).

Animal studies have confirmed the observation made in humans that cobalt is goitrogenic. Novikova (1964) noted a decreased ability of the thyroid to concentrate iodine among rats given a total of 150 µg Co per day as $CoC1_2$ in food.

7.4 Mutagenic Effects

7.4.1 Experimental Data

The genetic toxicology of cobalt compounds has been reviewed by Léonard and Lauwerys (1990), Beyersmann and Hartwig (1992), Lison *et al*. (2001), De Boeck *et al*. (2003), and IARC (2003).

Like other metallic compounds, cobalt compounds are known to be relatively inactive in prokaryotic systems (Rossman, 1981; Swierenga *et al*., 1987).

In mammalian cells, the results of the assays conducted with soluble cobalt(II) salts clearly demonstrate their mutagenic potential (reviewed by De Boeck *et al*. [2003]). Two molecular mechanisms seem to apply: (1) a direct effect of cobalt(II) ions to damage DNA through a Fenton-like mechanism (Mao *et al*., 1996) and (2) an indirect effect through inhibition of the repair of DNA damage caused by endogenous events or other agents (Kasten *et al.,* 1997). The capacity of Co(II) ions to compete with other species in the zinc finger domains of proteins involved in cell cycle control and/or DNA repair may contribute to explain the latter effects (Hartwig *et al*., 2002). These molecular mechanisms apply to soluble cobalt salts but also to cobalt metal or hard metal particles that are readily solubilized in biological media.

Extensive data exist demonstrating that the association of cobalt metal particles with tungsten carbide significantly increases the mutagenic activity. This activity results from the capacity of these particles to produce reactive oxygen species and is, at least in part, independent of cobalt(II) ions (see Section 1). The clastogenic activity of a WC-Co particle suspension has been evaluated by measuring DNA breaks and/or alkali labile sites in the alkaline elution and the Comet assays. When tested in the same experiment over a range of cobalt-equivalent concentrations, a WC-Co mixture (94 and 6% in weight, respectively), caused on average three times more DNA breaks than cobalt metal particles alone, both in isolated DNA (Anard *et al*., 1997) and in cultured human lymphocytes (Anard *et al.,* 1997, De Boeck *et al*., 1998, Van Goethem *et al*., 1997). Dose dependency and time dependency of DNA breakage or alkali labile sites were characterized with the Comet assay; significant DNA damage (increased tail DNA %) was noted after 15 minutes of incubation with 10 µg WC-Co/mL (De Boeck *et al*., 1998). The clastogenic activity of WC-Co particles in the alkaline elution assay was inhibited in part by scavenging of ROS with formate (1M) (Anard *et al*., 1997), but no significant increase in Fpg-sensitive sites (mainly oxidized bases) was detected in the Comet assay, suggesting the formation of DNA adducts (e.g., related to lipid peroxidation products) rather than direct DNA oxidation by ROS (De Boeck *et al*., 1998). In the same assays run concurrently, cobalt chloride (up to 1 mmol/) or cobalt metal particles alone produced less or no DNA breaks and/or alkali labile sites, and WC alone was completely inactive (Anard *et al*., 1997; De Boeck *et al*., 1998).

A similarly greater genotoxic activity of WC-Co compared with cobalt metal particles has been found with the cytokinesis-blocked micronucleus test applied *in vitro* to human lymphocytes (De Boeck *et al*., 1998; Van Goethem *et al*., 1997). Micronuclei induced by WC-Co were mainly identified as centromere positive by the fluorescence *in situ* hybridization (FISH) technique,

suggesting the involvement of an aneugenic activity of these particles. This activity is probably mediated by Co(II) ions, because, contrary to DNA breaks, it could be reproduced by $CoCl_2$ (De Boeck *et al*., 1998). This *in vitro* mutagenic activity has been extended to the mixture of cobalt metal with other carbides (NbC and Cr_3C_2). Although the metal carbides alone did not increase the micronucleus frequency in lymphocytes, cobalt alone and, to a greater extent, the carbide-cobalt mixtures induced a statistically significant and concentration-dependent increase in micronucleated binucleates. Mo_2C particles, which had a much smaller specific surface area than NbC and Cr_3C_2, did not interact with Co particles in this test, consistent with the contention that the mutagenic effect results, at least in part, from a surface chemistry reaction (De Boeck *et al*., 2003). Evidence of the *in vivo* mutagenic potential of WC-Co particles was obtained in type II pneumocytes of rats (De Boeck, 2003). DNA breaks/alkali-labile sites (alkaline Comet assay) and chromosome/genome mutations (micronucleus test) were documented after a single intratracheal instillation of WC-Co, including dose-effect and time trend relationships. In addition, the alkaline Comet assay was performed on peripheral blood mononucleated cells (PBMC). In type II pneumocytes, WC-Co induced a statistically significant increase in tail DNA (12-hour time point) and in micronuclei (72 hour) at a dose that produced mild pulmonary toxicity (16.6 mg/kg). The authors discussed two possible mechanisms to account for these mutagenic effects: the direct mutagenic activity of WC-Co particles demonstrated *in vitro* and/or the production of ROS by inflammatory cells recruited in the lung. In PBMC, no increase in DNA damage or micronuclei was observed after WC-Co administration, indicating that circulating lymphocytes are poor reporter cells for monitoring the genotoxic effects of inhaled particles.

IARC has reviewed the mutagenic effects of hard metals and cobalt compounds and concluded that "there is strong evidence that the WC-Co mixture is mutagenic *in vitro*" … and …"*in vivo* in rat lung cells" (IARC 2003).

7.4.2 Human Data

The possible genotoxic effects of occupational exposure to hard metal dust (WC-Co) has been explored in a study that used several endpoints for mutations (De Boeck *et al*., 2001). Authors aimed at assessing genotoxic effects in workers with an average exposure equivalent to the threshold limit value/time-weighted average (TLV/TWA) of cobalt-containing dust (i.e., 20 μg/m^3) (ACGIH, 2003). The study comprised three groups of male workers: 35 workers (mean age, 38.5 ± 7.7 years; range, 27.7–55.3) exposed to cobalt dust from three refineries, 29 workers (mean age, 40.7 ± 12.4 years; range, 20.7–63.6) exposed to hard metal particles from two production plants, and 27 matched control subjects (mean age, 38.0 ± 8.8 years; range, 23.3–56.4) recruited from the administrative departments of the respective plants. Smoking habits were identical in the three groups. In these groups, the (geometric) mean levels of urinary cobalt (μg/g creatinine) were consistent with an average exposure at the TLV-TWA in cobalt and hard metal workers (21.5; range, 5.0–82.5; and 19.9; range, 4.0–129.9, respectively) and the absence of occupational exposure in controls (1.7; range, 0.6–5.5). The study design integrated complementary biomarkers of DNA damage: 8-hydroxydeoxyguanosine (8-OHdG) in urine, DNA single-strand breaks and formamido-pyrimidine DNA glycosylase (Fpg)-sensitive sites with the alkaline Comet assay and the *in vitro* cytochalasin-B micronucleus test in blood lymphocytes. Micronuclei were scored both in binucleates and in mononucleates to discriminate between micronuclei accumulated *in vivo* (mononucleates) and additional micronuclei expressed *in vitro* (binucleates). No significant increase in genotoxic effects was detected in workers exposed to cobalt-containing dust compared with controls. No difference in any genotoxicity biomarker was found between workers exposed to cobalt and hard metal dusts. The only statistically significant difference observed was a higher frequency of cytokinesis-blocked micronuclei in workers exposed to cobalt compared with workers exposed to hard metal dusts but not in comparison with their concurrent controls. Multiple regression analysis indicated that workers who smoked and were exposed to hard metal dusts had elevated 8-OHdG and micronucleus values. The authors concluded that workers exposed solely to cobalt-containing dust at the TLV/TWA did not show increased genotoxic effects, but that workers who smoked and were exposed to hard metal dusts form a specific occupational group that needs closer medical surveillance. It has been concluded from a study of workers coexposed to low levels of cadmium (0.05–138 μg/m^3), cobalt (0–10 μg/m^3), lead (0–125 μg/m^3), and other heavy metals, that such mixed exposure may cause genotoxic effects (Hengstler *et al*., 2003), but these conclusions have been questioned (Kirsch-Volders and Lison, 2003).

7.5 Carcinogenic Effects

The carcinogenic effects of cobalt and its compounds have been reviewed by Léonard and Lauwerys (1990), Jensen and Tuchsen (1990), IARC (2003), Lison *et al*. (2001), and De Boeck *et al*. (2003).

7.5.1 Animal Data

In experimental animals, early studies performed with cobalt oxides found local tumors at injection sites and lung tumors after intratracheal instillation. No increased incidence of tumors was recorded by Wehner *et al*. (1977) when hamsters were exposed by inhalation to cobalt oxide at a concentration of 10 mg/m^3 7 hours/day for 5 days a week in a lifelong experiment. Early studies conducted with cobalt metal alone or alloyed with chromium and molybdenum (Vitallium) did not provide evidence of carcinogenicity except local site tumors induced after injection. Other soluble cobalt compounds (chloride, naphtenate) also produced local tumors at injection sites. In a 2-year inhalation study, cobalt sulfate heptahydrate (0.3, 1.0, or 3 mg/m^3) has been shown to induce lung tumors in two species (B6C3F1 mice and F344 rats) and adrenal tumors in female rats (Bucher *et al.,* 1999). The incidence of alveolar and bronchiolar neoplasms was significantly increased in rats exposed to 1.0 and 3 mg/m^3 and mice exposed to 3 mg/m^3. Cobalt sulfate has been recommended to be listed as "reasonably anticipated by the National toxicology Program to be a human carcinogen on the basis of sufficient evidence in animals," is classified as a category 2 carcinogen (labeled R49; "can cause cancer by inhalation") by the European Union, and IARC has concluded that there was *sufficient evidence* in experimental animals for the carcinogenicity of cobalt sulfate (IARC, 2003). Inhalation studies with cobalt metal or hard metal particles are not available.

7.5.2 Human Data

Occupational cohorts have been followed up for cancer mortality in cobalt production plants, in the hard metal industry, and in a porcelain factory.

The follow-up of a cohort of workers employed in an electrochemical plant producing cobalt and sodium, and previously studied from 1950 to 1980 by Mur *et al*. (1987), was extended for 7 additional years (Moulin *et al*., 1993) and did not confirm the hypothesis of a relationship between lung cancer and cobalt exposure (SMR 0.85 [95% CI, 0.18–2.50, 3 cases observed]). IARC concluded that there was "*inadequate evidence* in humans for the carcinogenicity of cobalt metal without tungsten carbide" (IARC 2003).

Hogstedt and Alexandersson (1990) examined retrospectively a cohort of more than 3000 workers from one of three plants in Sweden. There were 292 deaths among persons younger than 80 years of age during the study period (SMR 0.96; 95% CI, 0.85–1.08) and 17 cases of lung cancer versus 12.7 expected (SMR 1.34; 95% CI, 0.77–2.13). Among those workers with more than 10 years employment and deceased more than 20 years since first exposure, a significant excess of mortality by lung cancer was found (7 cases observed vs 2.5 expected; SMR 2.78 95% CI, 1.11–5.72).

Several reports addressing cancer risks among workers in hard metal production facilities in France provide evidence of an increased lung cancer risk related with exposure to hard metal dust. The risk seems to be highest among those exposed to unsintered rather than sintered hard metal dust. There is evidence for an increasing lung cancer risk with increasing duration of exposure in analyses that took into account potential confounding by smoking and other occupational carcinogens. A mortality study (Lasfargues *et al*., 1994) in workers from the hard metal industry in France showed an increased mortality by lung cancer (SMR 2.13, 95% CI, 1.02–3.93, 10 cases observed) that was related to the intensity of exposure (SMR 5.03 in the highest exposure group, 95% CI, 1.85–10.95, 6 cases observed). The excess of lung cancers could not be explained by a confounding effect of smoking. This study has been extended to a cohort of 7459 workers from all hard metal plants in France. The mortality by lung cancer in this cohort was significantly increased (SMR 1.30, 95% CI, 1.00–1.66, 63 cases observed), and a relationship with the intensity and duration of simultaneous exposure to cobalt metal and tungsten carbide particles was found (Moulin *et al.,* 1998). An additional study conducted in one plant already included in the multicentric cohort demonstrated that the risk was the highest among workers exposed in hard metal production before sintering and increased with duration of exposure, exposure scores, and estimated cumulative dose (Wild *et al.,* 2000). On the basis of these data, IARC concluded that "there is *limited evidence* in humans for the carcinogenicity of cobalt metal with tungsten carbide" (IARC 2003).

A mortality study was conducted among Danish women exposed to poorly soluble cobalt-aluminate spinel in a porcelain factory (Tüchsen *et al*., 1996). The airborne cobalt levels in this plant were high (often >1000 µg/m^3). The exposed group had a nonstatistically significant elevated relative risk ratio of 1.2 for lung cancer (95% CI, 0.4–3.8, 8 cases observed) compared with controls, but no relation with duration or intensity of exposure could be found. The influence of smoking could not be taken into account in this study. Among the eight cases of lung cancer identified in the exposed cohort, three had been exposed to cobalt spinel for less than 3 months only. Overall, this study does not provide solid evidence of an increased risk of lung cancer associated with exposure to cobalt spinel.

No study examining the cancer mortality or incidence in populations exposed to other cobalt compounds (soluble salts, oxides) could be located.

7.6 Reprotoxicity

7.6.1 Effects on Reproductive Organs and Fertility

Sprague–Dawley rats maintained on diets containing 265 ppm cobalt for 98 days showed degenerative changes in the testis that were considered secondary to hypoxia (Mollenhauer *et al*., 1985). Decreases in sperm motility and/or increased abnormal sperm were noted in mice, but not in rats, exposed to 3 mg/m^3 or more in 13-week inhalation studies with cobalt sulfate (National Toxicology Program, 1991). After 13 weeks of chronic exposure to 100–400 ppm cobalt chloride in drinking water, male CD-1 mice showed marked dose-related decreases in fertility, testicular weight, and sperm concentration and motility, and increases in circulating levels of testosterone (Pedigo *et al*., 1988). Pedigo and Vernon (1993) also reported that treatment of male mice with cobalt chloride (400 ppm in the diet during 10 weeks) increased preimplantation losses in the dominant lethal assay.

7.6.2 Developmental Effects

Only a few studies have been conducted with soluble cobalt compounds to explore their potential effects on development, and the results are mostly inconclusive because of the high doses used and the frequent presence of maternal toxicity.

Wide (1984) reported that a single intravenous injection of cobalt chloride in pregnant NMRI mice (approximately 1.2 mg/kg bw) on day 8 of gestation significantly affected fetal development; in animals injected at day 3 of gestation, no interference with implantation was noted. Oral administration of cobalt(II) chloride to pregnant Wistar rats (12, 24, or 48 mg/kg per day from day 14 of gestation through day 21 of lactation) significantly affected the late period of gestation, as well as postnatal survival and development of the pups (from 12 mg/kg per day). Signs of maternal toxicity were apparently also noted (Domingo *et al*., 1985). A study conducted in pregnant Sprague–Dawley rats (Paternain *et al*., 1988) concluded that the administration of cobalt chloride (up to a dose of 100 mg/kg by gavage, from day 6–15 of gestation) was not embryotoxic or teratogenic, despite signs of maternal toxicity. The fetal and postnatal developmental effects of cobalt(II) sulfate have been investigated in C57BL mice, Sprague–Dawley rats and/or New Zealand rabbits (Szakmary *et al*., 2001). Several developmental alterations (elevated frequency of fetuses with body weight or skeletal retardation, embryolethality, increased anomalies in several organs) were observed in mice and rats treated with cobalt (0–50 and 0–100 mg/kg/day, respectively). No teratogenic effect was noted in rabbits treated with up to 200 mg/kg/day. Postnatal developmental parameters were also transiently altered in the pups of rats treated daily with 50 mg/kg cobalt sulfate.

In vitro incubation of postblastocyst mouse embryos with cobalt(II) ions (100 µmol/L) adversely affected the development stages and decreased the trophoblast area (10 µmol/L) (Paksy *et al*., 1999).

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CHAPTER

26

Copper

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ABSTRACT

Copper is an essential trace element. Copper is a vital part of several enzymes (e.g., ferroxidases, cytochrome *c* oxidase, superoxide dismutase, tyrosinase, lysyl oxidase, and dopamine beta hydroxylase). The absorption depends on the amount ingested, its chemical form, and the composition of other dietary components such as zinc. Drinking water may contribute significantly to the daily copper intake because of the widespread use of copper pipes. Absorption is regulated by homeostatic mechanisms in the liver, and biliary excretion increases when copper is in excess. No quantitative data on pulmonary absorption are available.

Copper is found in all organs. The highest concentrations, both in adults and in the newborn, are found in the liver. Also the brain is rich in copper. Excretion is mainly through the bile, and only a few percent of absorbed copper is found in urine. Crucial for appropriate biliary excretion of copper is the presence of the copper exporter ATP7B. Mutations in the ATP7B gene are responsible for the genetic disorder Wilson's disease. The biological total body half-time in groups of healthy humans after oral administration has been calculated in the range of 2–5 weeks. Several markers of exposure have been assessed for the purpose of biological monitoring, but none of them have shown clear associations with excessive exposure.

Because copper is a highly reactive metal and thus harmful to the cells if present as free ions, the intracellular copper levels are strictly controlled by a number of integral transmembrane transporters, metallobuffers, and metallochaperones.

Ingestion of a large amount of copper salts causes gastrointestinal disturbances. The first symptom to occur is nausea, with increased reporting starting at approximately 4 mg/L of copper in drinking water. In severe cases, systemic effects, especially hemolysis, liver, and kidney damage, can occur. In contrast to data obtained after ingestion, comparatively little is known about health effects related to the inhalation of copper and copper fumes in the industrial setting. Copper may cause irritation in the respiratory tract and metal fume fever. Lung changes have been attributed to copper sulfate in vineyard workers, but the role of copper has not been fully explained.

For reviews on copper, see Linder and Hazegh-Azam (1996), WHO (1998), Barceloux (1999), Gaetke and Chow (2003), Harris (2003), and Tapiero *et al*. (2003).

1 PHYSICAL AND CHEMICAL PROPERTIES

Copper (Cu) in pure form is a reddish brown metal with high ductility and malleability. The atomic weight is 63.54, atomic number is 29, and the density is 8.94 g/cm^3. The melting point is 1083°C with a boiling point of 2595°C. Copper has two stable isotopes, ^{63}Cu and ^{65}Cu, with natural abundances of 69.2 and 30.8%, respectively (Georgopoulos *et al*., 2001). The water solubility of copper (II) sulfate is 143 g/L at 0°C,

whereas cuprous(I) oxide and copper are practically insoluble (WHO, 1998).

Copper can assume the oxidation states 0, +1, +2, and +3. Cu (0) is very stable, but can dissolve in acids like sulfuric and nitric acids. The cuprous Cu(I) ion is unstable in oxidizing environment. Cu(II) is the most abundant oxidation state in hydrophilic and oxidizing environments, and the ion is stable in most environments. Cu(III) is very unstable and of negligible biological significance (Landner and Lindeström, 1999).

2 METHODS AND PROBLEMS OF ANALYSIS

The introduction of flame atomic absorption spectrometry (FAAS) during the 1960s and later of electrothermal atomic absorption spectrometry (ETAAS), inductively coupled plasma optical emission spectrometry (ICP-OES), and inductively coupled mass spectrometry (ICP-MS) has simplified and improved the copper measurements in biological samples. Copper may be measured in serum without dilution using the microcup FAAS technique (Odland *et al*., 1999). With either a simple aqueous dilution or acid mineralization of serum and urine, copper can be determined by ETAAS, ICP-OES, and ICP-MS simultaneously with other essential and/or toxic elements (Chappius *et al*., 1992; Correia *et al*., 2002; Szpunar *et al*., 1997). Total reflection X-ray fluorescence spectrometry has also recently been shown to be capable of simultaneous measurements of trace elements, including copper, in serum (Hernandez-Caraballo and Marco-Parra, 2003).

3 PRODUCTION AND USES

3.1 Production

The largest copper reserves are found in Chile, followed by the United States, Peru, Zambia, and the Democratic Republic of Congo. The worldwide exploitable copper ores are approximately 90% of sulfidic nature, 9% oxidic, and <1% metallic copper ores. Principal sulfidic ores are chalcocite (Cu_2S) and chalcopyrite ($CuFeS_2$, yellow copper ore), whereas cuprite is the major oxidic ore (Cu_2O, red copper ore). Malachite ($Cu_2(OH)_2CO_3$, green copper ore) and azurite ($Cu_3(OH)_2(CO_3)_2$, blue carbonite copper) are other ores of importance. Also crude phosphates used in the production of phosphate fertilizers may contain significant amounts of copper.

The primary copper production differs somewhat, depending on the type of ore. Both sulfidic and oxidic ores pass extensive, concentration processes. The ore is subject to roasting, converting, and electrolytic refining. Instead of converting, a leaching step is used for the oxidic ores. The secondary copper production, based on recycled copper, has been steadily increasing, and approximately 38% (in 1993) of the copper consumption in the Western world was based on recycled scrap (Landner and Lindeström, 1999). Another source of copper is based on the production of other metals (e.g., electrolytic grade copper from the nickel industry) (Thomassen *et al*., 2004).

3.2 Uses

The most important uses for copper are in wires and cables for transmission of electricity. Various applications within the electronic industry, water pipes and tubes, vessels and containers, and roofing and facing materials for buildings are other important applications. Copper compounds are used in wood preservatives, fungicides, as pigments and antifouling agents in paints, as nutritional additives to livestock, and additive to fertilizers. Copper is an important alloying element, the best known being brass (copper-zinc) and bronze (copper-tin). Cupro-nickels are used as coinage material (Landner and Lindeström, 1999). Copper-beryllium is of particular importance from a health perspective because of the hazards associated with beryllium exposure.

There are also some medical uses of copper, like copper-based alloys used in dental bridges and crowns. Intrauterine contraceptive devices are also widespread (Barceloux, 1999).

4 ENVIRONMENTAL LEVELS AND EXPOSURES

4.1 Food and Daily Intake

The daily intake of copper in adults from food generally varies from approximately 1–2.5 mg, corresponding to 15–45 μg/kg body weight in adults (WHO, 1996).

Organ meats like liver and kidney contain high copper levels. Fish, fruits, cereals, nuts, and green vegetables are good sources of copper, whereas meat contains lower copper levels, and several dairy products including milk contain low copper levels (Georgopoulos *et al*., 2001; Lönnerdal, 1996; WHO, 1998). WHO (1996) suggested daily requirements of 1.2 mg Cu/day for adult women and 1.3 mg Cu/day for adult men. Lower dietary reference intake of 0.9 mg Cu/day has also been proposed for adults, with slightly higher intakes during

pregnancy (1.0 mg/day) and lactation (1.3 mg/day) (Institute of Medicine, 2001; Trumbo *et al*., 2001). The substantial differences in the proposed recommended intakes could reflect the difficulties in determining the optimal daily intake. The daily requirement in children and infants depends on age.

There are large differences between countries in the use of copper pipes in the water supply, and copper can be released from such piping. In drinking water, large variations in copper concentrations may occur depending on type of water (e.g., hardness and pH). Acidic water is associated with higher copper levels. There may also be substantial differences in the copper content of drinking water that has remained in the distribution system overnight as compared with running water (Georgopoulos *et al*., 2001). Copper concentrations from a few micrograms to few milligrams per liter have been reported (WHO, 1998), suggesting that drinking water may add a considerable amount of copper to the daily intake.

4.2 Water, Soil, and Ambient Air

In seawater, most reports have indicated concentrations <1 μg/L (Bruland, 1980; Haraldsson and Westerlund, 1988; Moore, 1978). Much higher levels may be measured in coastal regions and estuaries (Bryan and Langston, 1992; Cabrera *et al*., 1987; Fabiano *et al*., 1988). Generally, higher mean levels (1–10 μg/L) have been reported for rivers and lakes (Bubb and Lester, 1994; Hall *et al*., 1988; 1992; Hurley *et al*., 1996; Ouseph, 1992; Parrish and Uchrin, 1990). The acidity of the water may be related to the measured levels (Filipek *et al*., 1987; WHO, 1998).

Air levels of copper depend on the proximity to major sources of copper release into the ambient air. Average concentrations are usually well below 1 μg/m^3, but higher levels may be found in urban or otherwise polluted areas (Davies and Bennet, 1985; Georgopoulos *et al*., 2001; Romo-Kroger and Llona, 1993; Schroeder *et al*., 1987; Sweet *et al*., 1993). At the South Pole the average copper concentration in air was 0.036 ng/m^3 (Zoller *et al*., 1974). Mean copper concentrations in uncontaminated surface soil vary from 6–80 mg/kg dry weight (Kabata-Pendias and Pendias, 1984). A main release of copper into the soil is from copper mine tailings. Also wastewater sludge may contain significant amounts of copper (Georgopoulos *et al*., 2001).

4.3 Working Environment

Surprisingly, few studies of exposure levels in the work environments of copper-producing plants or plants using copper in their production are found in the scientific literature. Even fewer reported studies have used sampling techniques taking into account health-related aerosol fractions by sampling particles according to size. This latter aspect is of particular importance with respect to copper exposure, because gastrointestinal absorption is well regulated. The respirable and the thoracic aerosol fractions may, therefore, contribute relatively more to the uptake of copper in occupational settings.

Copper smelter workers were exposed to 164 μg Cu/m^3 (range, not detectable–3500 μg Cu/m^3) on average in the total aerosol fraction (Cant and Legendre, 1982). Mean copper fume levels of 390 μg/m^3 (range, 120–988 μg /m^3) in the furnace area and mean copper dust concentrations in the remaining areas ranging from 256–17584 μg /m^3 were reported from a secondary copper refinery (NIOSH, 1981a). The exposure levels to workers producing components for turbine generators ranged from 10 μg–683 μg Cu/m^3 in dust (NIOSH, 1981b). Copper production in a nickel refinery resulted in a geometric mean exposure of 960 μg Cu/m^3 in the inhalable aerosol fraction (Thomassen *et al*., 2004). Casting of a zinc-copper alloy resulted in 113 μg/m^3 of nonrespirable copper compared with only 6 μg/m^3 in the respirable aerosol fraction (Cohen and Powers, 1994). Mining and refining of copper may lead to concomitant exposure to other hazardous metals and metallic compounds, including arsenic and lead (Cant and Legendre, 1982; Enterline *et al*., 1995).

5 METABOLISM

Copper is an essential, yet toxic, trace element and a need, therefore, exists to control transport of the metal in the body. Efficient homeostatic mechanisms have evolved to regulate uptake, distribution, storage, and excretion of copper.

In blood, complexing molecules have long been recognized, whereas cellular mechanisms have largely been unknown. In the past decade numerous new cellular copper transporters have been identified (Solioz and Vulpe, 1996), the first being the copper export pump ATP7A, which is deficient in Menkes disease (Chelly *et al*., 1993; Mercer *et al*., 1993; Vulpe *et al*., 1993).

Immediately after, the homologous transporter ATP7B, which is deficient in Wilson's disease, was identified (Bull *et al*., 1993). A similar mechanism was shown to exist in yeast, and this finding triggered an entire new concept of metal transfer being molecularly well preserved from the most primitive unicellular bacteria to complex eukaryotes. This eventually led to the identification of several new copper specific transporters securing delivery of the

metal to compartments and copper requiring enzymes by specific membrane transporters and cytosolic molecules named copper chaperones (Arnesano *et al*., 2002; Elam *et al*., 2002).

5.1 Absorption

5.1.1 Inhalation

There are no quantitative data from animal or human studies on the extent of absorption of copper compounds after inhalation. A pulmonary half-time of 7.5 hours was reported after intratracheal instillation of $CuSO_4$ to male Wistar rats (Hirano *et al*., 1990). Intratracheal instillation of particles in the respirable range containing CuO to male Wistar rats resulted in a pulmonary half-time of 37 hours (Hirano *et al*., 1993). This suggests a faster pulmonary clearance of more soluble copper compounds compared with less soluble copper compounds. In both studies, an induction of the metallothionein synthesis in the lungs was observed.

5.1.2 Ingestion

Dietary copper is absorbed across the mucosal membrane in the small intestines, but also to a limited extent in the stomach in mammals (Pena *et al*., 1999). Gastrointestinal absorption of copper in adults apparently depends on the amount of copper in the diet, from 55.6% at 0.78 mg/day to 36.3% at 1.68 mg/day and 12.4% at 7.53 mg/day (Turnlund *et al*., 1989). Even higher absorption, ranging from 62–79%, has been reported. Women absorbed slightly more copper than men in that study (Johnson *et al*., 1992). There is some evidence that active transport mechanisms are involved at lower dietary levels, whereas passive diffusion may occur at higher levels (Varada *et al*., 1993). The roles of the highly copper-specific protein Cu transporter 1 (CTR1), as well as the divalent metal transporter 1 (DMT1), in the gastrointestinal absorption from the intestinal lumen across the apical membrane into enterocytes is currently not completely explained (Harris, 2001; Pena *et al*., 1999). Both proteins are expressed in abundance in the intestines (Tapiero *et al*., 2003). Copper seems to modify the expression of DMT1 (Sharp, 2003).

Most absorbed copper is retained within the mucosal cells, bound mainly to metallothionein or glutathione (Tapiero *et al*., 2003). After uptake into the enterocyte, copper is shuttled to the trans-Golgi network (TGN) by the chaperone ATOX1 (antioxidant protein 1) (Bertinato and L'Abbé, 2004; Tapiero *et al*., 2003). The copper efflux into the portal circulation is probably mediated by ATP7A (Menkes protein) (Pena *et al*., 1999; Tapiero *et al*., 2003).

5.2 Distribution

5.2.1 Interorgan and Intracellular Distribution

Copper is bound to albumin, transcuprein, and low-molecular-weight components in the portal circulation (Hyun and Filippich, 2004; Linder *et al*., 1998; Linder and Hazegh-Azam, 1996). The uptake of copper into the hepatocytes is likely to be mediated by the copper transporter CTR1 (Langner and Denk, 2004). ATOX1 is involved in the shuttle of copper to ATP7B (Bertinato and L'Abbé, 2004; Langner and Denk, 2004). ATP7B is localized at the TGN of the hepatocyte.

Copper is incorporated into ceruloplasmin for excretion into the blood at physiological copper levels (Langner and Denk, 2004). The precise mechanism for copper insertion into ceruloplasmin is unknown (Bielli and Calabrese, 2002).

At high copper levels, the intracellular trafficking is targeted to a post-Golgi vesicular compartment for biliary excretion (Langner and Denk, 2004). Excess copper in the hepatocyte is bound to metallothioneins (Wijmenga and Klomp, 2004). Humans with the genetic disorder aceruloplasminemia do not have severe disturbances in the copper homeostasis, suggesting that ceruloplasmin is not of fundamental importance for appropriate targeting of copper to the peripheral tissues (Gitlin, 1998).

Also extrahepatically, the intracellular uptake of copper may be mediated by the high-affinity CTR1 and the low-affinity CTR2, which are differently expressed in human cells (Bertinato and L'Abbé, 2004). High levels of CTR1 are expressed in the liver, heart, and pancreas; intermediate levels in the intestine; and low levels in brain and muscle (Bertinato and L'Abbé, 2004; Pena *et al*., 1999). The highest expression of CTR2 is in the placenta, with very low abundance in liver and intestine (Bertinato and L'Abbé, 2004; Pena *et al*., 1999). Once inside a cell, copper is coupled to chaperones for appropriate intracellular targeting. For instance, copper is shuttled by CCS (Cu chaperone for superoxide dismutase 1) for copper incorporation into the Cu-Zn–dependent superoxide dismutase. Many copper chaperones are known (Prohaska and Gybina, 2004), and some are described later. The total body copper of a healthy 70-kg man has been estimated at 110 mg. The skeleton, muscles, liver, and brain contain the highest total amounts of copper (Linder *et al*., 1998).

5.2.2 Molecular Genetics of Intracellular Transport

Because of its highly reactive nature, copper would be harmful if present as free ion in cells. Both Cu(I)

and Cu(II) can participate in oxidation and reduction reactions, and hydroxyl radicals may arise (Gaetke and Chow, 2003). The production of oxyradicals by the Fenton reaction with subsequent cellular injury could occur (Goldstein and Czapsky, 1986). The intracellular copper levels are thus controlled by means of integral transmembrane transporters, metallobuffers, and metallochaperones.

Copper chaperones are highly specific for their targets and cannot functionally substitute for each other. Each chaperone participates in the delivery of the metal ion to the recipient molecule through a direct interchange between the donor and the recipient (Elam *et al*., 2002; Huffman and O'Halloran, 2001; O'Halloran and Culotta; 2000). Once the metal has been inserted into the target protein, the chaperone is available again for metal delivery.

Cellular uptake across the plasma membrane occurs through an energy-independent copper transporter, CTR1 (Sharp, 2003; Thiele, 2003). The concentration of the transporter seems to be regulated by copper. This mechanism prevents accumulation of surplus metal (Petris *et al*., 2003), although this has been disputed (Eisses *et al*., 2005). The transport molecule contains a specific extracellular site sensing copper (Guo *et al*., 2004), and high levels may result in rapid internalization and degradation of the transporter (Petris *et al*., 2003). On entering the cell, copper is complexed by metal scavenging systems like glutathione, metallothionein, and metallochaperones, and cytosolic free copper is maintained at an extremely low level (Huffman and O'Halloran, 2001; Rae *et al*., 1999).

ATOX1 secures the delivery to the TGN by transferring copper directly to ATP7B in the liver or ATP7A in other cells. The two energy-utilizing transporters pump copper across the TGN membrane and make the metal available for secretion or vesicular enzymes. ATP7B is expressed in the liver, but also in other tissues like the kidney and brain, and plays a critical role in whole-body copper homeostasis. ATP7B directs copper to ceruloplasmin or to biliary excretion interacting with the chaperone COMMD1 (Copper Metabolism MURR1 Domain 1) (Sluis *et al*., 2002).

COMMD1 is presumed to act downstream of ATP7B, suggesting that these two proteins cooperate to regulate hepatic copper excretion (Tao *et al*., 2003).

Besides a role in copper homeostasis, COMMD1 probably exhibits pleiotropic effects that may or may not be linked to copper homeostasis (Biasio *et al*., 2004). Recently XIAP (X-linked inhibitor of apoptosis), a potent suppressor of apoptosis, has also been implicated in copper metabolism by its ability to regulate cellular levels of COMMD1 (Burstein *et al*., 2004).

ATP7A is present in all extrahepatic tissues where it directs copper to enzymes like dopamine beta-monooxygenase, lysyl oxidase, and tyrosinase. ATP7A also pumps the metal out of the cell. Thus, ATP7A also plays a vital role in whole-body copper homeostasis.

When intracellular copper levels increase, ATP7A and ATP7B are directed to the plasma membrane and a vesicular compartment, respectively, thereby promoting export (Hung *et al*., 1997; Yamaguchi *et al*., 1996).

When copper is released, the transporter is rapidly recycled back to the TGN. Copper also affects structure and activity of the export ATPases (Huster *et al*., 2003; Petris *et al*., 1996; Vanderwerf *et al*., 2001; Voskoboinik *et al*., 2001; Walker *et al*., 2002). Thus, copper regulates both the uptake and the export directly (Bertinato and L'Abbé, 2004). If the uptake of copper exceeds the export capacity of the cell, the synthesis of metallothioneins will be induced.

Mitochondria have their own sophisticated copper transport system. The copper containing respiratory chain enzyme cytochrome *c* oxidase (COX) is localized within the inner mitochondrial membrane and acquires copper from a series of free and membrane-bound molecules. The delivery and insertion of copper ions into COX require at least six transporters, COX11, COX17, COX19, COX23, SCO1, and SCO2, but their specific roles are not completely understood. Insertion of copper within the two mitochondrially encoded subunits COX1 and COX2 that make up the two copper centers of COX apparently involve the membrane-bound transporters SCO1/SCO2 (S. Cerevisiae homologue of SCO1/2) for Cu_A (Glerum *et al*., 1996) and COX11 for Cu_B (Hiser *et al*., 2000). COX17 is a soluble copper binding protein that donates the metal in the intermembrane space to SCO1/SCO2 and COX11 (Banci *et al*., 2004; Horng *et al*., 2004).

COX17, also present in the cytosol, may serve in shuttling copper to the mitochondria, although this role has been questioned (Horng *et al*., 2004).

COX23 is a protein equivalent to COX17 that has been identified in yeast, but a human homolog exists. The protein is present in the intermembrane space and in the cytosol, and apparently COX17 is acting downstream of COX23 (Barros *et al*., 2004). COX19 functions within the intermembrane space (Nobrega *et al*., 2002).

APP (beta amyloid precursor protein) is a ubiquitously expressed protein that contains a copper-binding domain structurally related to that of copper chaperones**.** Tissue copper levels are influenced by the activity of APP, and recent studies indicate APP and related proteins to be involved in copper homeostasis as copper efflux proteins (Bellingham *et al*., 2004; Treiber *et al*., 2004).

PrP (prion protein) binds copper, which is necessary for normal conformation (Brown *et al*., 1997). The protein possesses antioxidant activity and may play a role in antioxidant defense. Possibly the protein also regulates copper availability to cells (Kovacs *et al*., 2004).

5.2.3 Uptake into the Brain

The brain uptake of copper implies a controlled copper transfer across the cerebral endothelium and across the barrier at the choroid plexus. CTR1, ATOX1, and ATP7A are highly expressed in the choroid plexus, indicating the participation of this tissue in uptake and/or efflux of copper to and from the cerebrospinal fluid (Nishihara *et al*., 1998). Lack of CTR1 or ATP7A results in low levels of copper in the brain. Menkes babies (see later) have low brain copper levels (Horn *et al*., 1992), and in a mouse model for Menkes disease, copper accumulates in the astrocytes and the vascular endothelial cells (Kodama, 1993), directly demonstrating the low transfer across the BBB. ATP7B also plays a significant role in normal brain function that is nonoverlapping with ATP7A. ATP7A may substitute for ATP7B under certain conditions (Barnes *et al*., 2005). A variant of ATP7B, PINA (pineal night-specific ATPase) is expressed in the pineal gland and retina. This protein is apparently needed for a copper-dependent component involved in circadian rhythm regulation (Borjigin *et al*., 1999).

5.3 Genetic Disorders with a Disturbed Copper Metabolism

Menkes disease and Wilson's disease are the best known diseases of copper metabolism. They are results of mutations in ATP7A and ATP7B, respectively. The study of these diseases has greatly enhanced the understanding of molecular mechanisms of copper metabolism.

Because of the widespread distribution of ATP7A, Menkes disease affects all tissues and organs, except for the liver, where ATP7A is not expressed. The copper transport across the blood–brain barrier is impaired, resulting in severe brain copper deficiency (Horn *et al*., 1992). Copper cannot be delivered to important enzymes, resulting in a copper deficiency phenotype, and Menkes babies usually die before 3 years of age. The disease is characterized by progressive neurological impairment, connective tissue disturbances, and peculiar "steely" hair. Paradoxically, the deficiency symptoms are accompanied by widespread intracellular accumulation of copper in tissues except for the liver and the brain. The accumulation induces large amounts of metallothionein. Cells cultured *in vitro* also accumulate copper, and this is used diagnostically (Horn and Tümer, 2002).

The symptoms can be attributed to lack of function of copper-dependent enzymes secondary to a defect in ATP7A. Thus, hypopigmentation results from deficiency of tyrosinase, low lysyl oxidase activity causes numerous connective tissue disturbances, deficient dopamine beta hydroxylase results in impaired catecholamine production, and poor cross-linking of keratin with resulting steely hair is secondary to inadequate sulfhydryl oxidase activity. In the brain, lack of cytochrome *c* oxidase, superoxide dismutase, peptidyl alpha amidating activity, in combination with aneurysms caused by deficient lysyl oxidase will lead to severe dysfunction with progressive mental retardation.

Wilson's disease (hepatolenticular degeneration) is characterized by a defective biliary copper excretion, resulting in hepatic copper accumulation and subsequent severe liver pathology and destruction of the cells (Llanos and Mercer, 2002). Released copper is deposited elsewhere, primarily in the brain, where neurological abnormalities may occur. ATP7B is also responsible for the formation of the protein PINA that is involved in the control of a normal circadian rhythm, and patients with Wilson's disease may have a disturbed sleeping pattern (Matarazzo, 2002; Portala *et al*., 2002). Copper often deposits in the cornea and forms the diagnostically useful Kayser–Fleischer ring. High amounts of copper are filtered through the kidneys and are, in combination with the high liver copper concentration and low blood ceruloplasmin, useful diagnostic markers.

Excess intake of copper can lead to childhood copper toxicosis like Indian childhood cirrhosis (Tanner, 1998) and endemic Tyrolean infantile cirrhosis (Müller *et al*., 1996). Both are characterized by extremely high copper concentrations in the liver in early childhood. Genetic components have been suggested, but a molecular basis has not been identified.

Human diseases related to the other copper transporters have not been described except for SCO1 and SCO2, where infants suffer from COX deficiency. Patients with mutations of these two genes show distinct phenotypes, SCO1 mutations primarily resulting in early onset hepatic failure and encephalopathy, whereas SCO2 mutations usually give rise to hypertrophic cardiomyopathy and encephalopathy (Horn and Tümer, 2002).

5.4 Excretion

Under normal physiological conditions, approximately 98% of the copper excretion is through the bile and the remaining 2% is through the urine (Wijmenga and Klomp, 2004).

During the first 8 days after intravenous administration of ^{67}Cu, 24.7% of the dose was excreted in the feces compared with 0.5% in the urine (Dekaban *et al*., 1975). Urinary copper output approximately four orders of magnitude lower than fecal output has also been suggested (Scott and Turnlund, 1994). Less than 1% of an intravenous injection of a tracer dose of ^{64}Cu as the acetate was excreted in the urine within 72 hours by normal subjects compared with approximately 9% in the feces (Tauxe *et al*., 1966). The available data show that only minor amounts of copper are excreted in the urine in normal subjects, and that excretion by the bile is dominating.

The liver plays a critical role in copper metabolism, serving as a storage site, as well as regulating the biliary excretion. Renal filtration plays an important role in copper excretion only under rare circumstances, when the tubular reabsorption is exceeded such as in Wilson's disease. ATP7B is localized at the TGN of the hepatocyte, and at normal copper levels, copper shuttled by ATOX1 is incorporated into ceruloplasmin for excretion into the blood (Huster *et al*., 2003; Langner and Denk, 2004). However, when copper is in excess ATP7B is rapidly relocalized to the post-Golgi vesicular compartment of the hepatocyte (Hung *et al*., 1997; Langner and Denk 2004). This fast copper level dependent relocalization of ATP7B does not require *de novo* synthesis of the protein, and a subsequent depletion of copper leads to a rapid return of ATP7B to the TGN (Wijmenga and Klomp, 2004). The post-Golgi vesicular compartment of the hepatocyte is localized in close vicinity to the biliary canalicular membrane and is thereby involved in the biliary excretion (Langner and Denk, 2004). Hepatocytes can sequester excess copper in lysosomes (e.g., in Wilson's disease), and exocytosis of lysosomal contents into biliary canaliculi may be important for biliary copper excretion (Gross *et al*., 1989; Myers *et al*., 1993). The biliary copper excretion in copper-loaded rats can increase at least 10-fold, suggesting this mechanism to be important as protection against copper toxicity (Gross *et al*., 1989). In humans, only a minor part of copper in bile is reabsorbed (Linder and Hazegh-Azam, 1996). In the rat bile, a small fraction of copper is bound to metallothionein (Bremner, 1987; Sato and Bremner, 1984). In human bile, uncharacterized high-molecular-weight components and low-molecular-weight components have been identified (Gollan and Deller, 1973).

The low urinary excretion of copper in animals and in man is consistent with the evidence that little copper in plasma is complexed with lower weight proteins and that a significant part of the ultrafiltered amount is reabsorbed in the renal tubuli (Lindner and Hazegh-Azam, 1996). The mechanisms of the copper transport in the kidney have not been completely described.

Copper filtered through the glomeruli into the crude urine is reabsorbed downstream in the nephron, but the specific site has not been defined. The copper transporters ATP7A and ATP7B are expressed in the kidney at distinct regions (Grimes *et al*., 1997; Moore and Cox, 2001). They are also expressed in the glomeruli through the former in minor amounts and may thus participate in the filtration of copper. In addition, ATP7A is localized in the proximal and distal tubules, whereas ATP7B is expressed in the loop of Henle.

Under normal conditions, the reabsorption is almost complete, whereas disruption of either ATP7A and ATP7B results in disturbed handling of copper. In Mottled mice, copper accumulates downstream in the proximal tubules (Kodama *et al*., 1993) but not in the distal tubules or glomeruli.

The copper chaperone ATOX1 colocalizes with ATP7B in the kidney (Moore *et al*., 2002). CTR1 is expressed at high levels in kidney (Lee *et al*., 2001). It colocalizes with ATP7A at the tubular sites and is likely responsible for the apical copper uptake downstream of the glomeruli.

DMT1 is expressed in the loop of Henle and especially in the distal tubules, and this transporter could, therefore, also play a role in renal copper reabsorption (Ferguson *et al*., 2001).

Patients with primary biliary cirrhosis and other liver diseases have been reported to have lower capacity for gastrointestinal excretion of copper, and increased urinary excretion of copper may be found in such patients (Ritland *et al*., 1977). The urinary excretion of copper increases in some diseases, especially in the nephrotic syndrome (Kleinbaum, 1965). In patients with Wilson's disease, the disturbed renal copper metabolism results in massive excretion of copper in the urine, whereas Menkes babies have near normal urinary copper excretion.

Minimal amounts of copper are lost in human sweat (Turnlund *et al*., 1990). Small amounts of copper are secreted daily by salivary, gastric, pancreatic, and duodenal excretions (Linder *et al*., 1998).

5.5 Biological Half-Time

The total body biological half-time of ^{67}Cu administered orally to normal subjects ranged from 13–33 days for the different age groups (Johnson *et al*., 1992). Females had shorter half-times than males. A similar half-time was found after intravenous injection of ^{67}Cu (Dekaban *et al*., 1975). These data are in accordance with the estimation of several weeks calculated by Tauxe *et al*. (1966) after intravenous injection to normal humans. Three patients with Menkes disease had substantially longer biological half-times, being between 73 and 90 days (Dekaban *et al*., 1975).

6 LEVELS IN TISSUES AND BIOLOGICAL FLUIDS

Average tissue concentrations from approximately 3–10 μg Cu/g wet weight in the liver, between 4 and 6 μg Cu/g wet weight in the brain, between 3 and 4 μg Cu/g wet weight in the heart, and 1.5–4.7 μg Cu/g wet weight in the kidney are common in adult humans. These tissues are regarded to have the highest copper levels (Garcia *et al*., 2001; Sumino *et al*., 1975). The copper content in the liver of the newborn is at the peak at or shortly after birth, being 5–10 times higher than in adults (Davis and Mertz, 1987; Epstein, 1983). Average human hepatic copper levels of approximately 51 μg/g wet weight between 22 weeks of gestational age until 13 weeks after birth, and then declining to the mean level of approximately 5.7 μg/g wet weight have been reported (Klein *et al*., 1991). Higher levels ranging from 13–1218 μg/g dry weight have also been reported (Faa *et al*., 1987). The mean concentration of copper in placenta is approximately 5 μg/g dry weight or 15 μmol/kg (range, 10–20) fresh weight (Osman *et al*., 2000; Thieme *et al*., 1986).

Patients with Wilson's disease may have much higher tissue copper concentrations than healthy adults, in particular in the liver, kidney, and skeletal muscles (Georgopoulos *et al*., 2001). Mean liver copper levels may be 983 μg/g in early Wilson's disease, 493 μg/g in late Wilson's disease (Epstein, 1983), or 1370 μg Cu/g dry weight in the liver of an untreated patient with Wilson's disease (Faa *et al*., 1995). An uneven distribution of the copper brain levels ranging from 88–158 μg/g dry tissue, depending on the anatomical localization, was reported in a patient with Wilson's disease (Faa *et al*., 2001).

Indian childhood cirrhosis is a rare liver cirrhosis of unclear etiology affecting children from 1–6 years of age. Depending on the stage of the disease, liver copper concentrations of 219 μg Cu/g in the early stage and 1461 μg Cu/g in the late stage have been reported (Epstein, 1983; Tanner, 1998).

High liver copper concentrations can be measured in many liver diseases (Beshgetoor and Hambidge, 1998; Danks, 1991). Increased copper levels in the liver are found in patients with chronic extrahepatic or intrahepatic cholestasis (Hunt *et al*., 1963; Smallwood *et al*., 1968; Wonwood *et al*., 1968), usually a twofold to threefold elevation. Marked elevations, in the range seen in patients with Wilson's disease, are reported in children with biliary atresia (Sternlieb *et al*., 1966).

Median copper concentrations of copper smelters were 4.1 mg Cu/kg wet weight (liver), 3.1 mg Cu/kg wet weight (brain), and 1.9 mg Cu/kg wet weight (kidney), which is comparable to normal adult levels (Gerhardsson *et al*., 2002). Humans who had lived in a copper mining and electrolytic copper-producing area had on average 4.6 μg Cu/g wet weight in the liver and 2.3 μg Cu/g wet weight in the renal cortex (Orlowski *et al*., 1996). In one case of human fatal copper sulfate poisoning, 35.1 μg Cu/g wet weight in the liver and 41.4 μg Cu/g wet weight in the kidney were measured (Kurisaki *et al*., 1988). Bile copper concentrations between 0.5 and 8.5 mg/kg were measured in patients with gallstones (Szentmihályi *et al*., 2002).

6.1 Biological Monitoring

In the blood, copper is distributed into a plasma pool associated with larger proteins, an exchangeable fraction of low-molecular-weight copper complexes, and a red cell pool that is partly nonexchangeable. Most of the copper in blood plasma is bound to ceruloplasmin, approximately 15% to albumin, 10% to transcuprein, and small amounts are bound to small peptides and amino acids (Lindner and Hazegh-Azam, 1996; Tapiero *et al*., 2003).

Transcuprein and albumin represent the bulk of the exchangeable copper pool in plasma (Tapiero *et al*., 2003). The percentage of plasma copper bound to ceruloplasmin has been reported in the range from approximately 60–95% (Linder, 1991; Olivares *et al*., 2000; Scott and Turnlund, 1994). The copper concentration in the red blood cells is about the same as in plasma.

In the erythrocytes, approximately 60% of the copper is bound to Cu-Zn-SOD. Significant amounts of copper are also found in the leukocytes (Vitoux *et al*., 1999).

The normal concentration of copper in plasma is approximately 1 mg/L ranging up to approximately 1.5 mg/L (Bergomi *et al*., 1997; Ford, 2000; Romero *et al*., 2002).

Several factors not directly related to copper status, but often related to changes in ceruloplasmin levels, make plasma copper an unreliable marker of copper status, except in more severe deficiency states (Milne, 1998).

Increased serum copper levels have been measured in clinically severe cases of copper sulfate poisoning (Chuttani *et al*., 1965; Oldenquist and Salem, 1999).

The increased concentrations of copper in serum measured in various liver diseases, carcinomas, acute and chronic infections, rheumatoid arthritis, and other conditions may be related to the increased synthesis of ceruloplasmin as an acute-phase reactant (Davis and Mertz, 1987; Linder and Hazegh-Azam, 1996), and thus not related to copper status as such. When rats were administered IL-1a, the serum concentration of ceruloplasmin increased, but not the ceruloplasmin

activity. This finding suggests that the concentration of noncopper-containing apo-ceruloplasmin increases during inflammatory acute-phase reactions (Gitlin *et al*., 1992). The half-time of apo-ceruloplasmin (5 hours) is much shorter than the half-time of holoceruloplasmin (5.5 days) (Gitlin, 1998). Diurnal fluctuations of plasma copper have been reported (Lifshitz and Henkin, 1971).

Women have generally higher plasma copper levels than men (Milne, 1998). Higher levels have also been measured in women taking oral contraceptives and postmenopausal women receiving estrogen (Johnson *et al*., 1992; Milne and Johnson, 1993). Serum copper levels increase during pregnancy (Aaseth *et al*., 2001). The serum ceruloplasmin concentration also increases during pregnancy, reaching values at parturition that are approximately twice those found in nonpregnant women (Flynn, 1982).

In healthy adults, the urinary excretion of copper is generally between 30 and 60 µg Cu/day (Harris, 1991). Dietary intakes of 0.785 mg Cu/day for 42 days, 1.68 mg Cu/day for 24 days, or 7.53 mg Cu/day for 24 days did not result in altered urinary copper excretion (Turnlund *et al*., 1990). This suggests that even an oral intake several-fold higher than the daily recommended intake does not increase the urinary copper excretion. Thus, urinary copper is unsuitable for biological monitoring at moderately elevated oral intakes. However, if this is true when exposure occurs by inhalation, has hardly been studied. An increased urinary excretion >100 µg Cu/24 hours is often observed in patients with Wilson's disease (Langner and Denk, 2004). Urinary copper concentrations as high as 1787 µg Cu/24 hours have been reported in these patients (Wang *et al*., 2004).

The daily excretion of copper into the bile in humans is approximately 2500 µg (Linder and Hazegh-Azam, 1996). The copper content of human milk decreases during lactation, and mean levels of 750 µg/L or 570 µg/L have been measured soon after birth (Davis and Mertz, 1987). Also, the milk content of ceruloplasmin decreases during lactation (Linder *et al*., 1998).

Copper in human milk is more easily absorbed by the infant than copper in cow's milk (Olivares *et al*., 2000).

6.2 Biomarkers of Exposure

Several potential biomarkers of exposure other than copper in serum or in urine have been assessed. Healthy adults with presumably normal copper status had no increase in the levels of CuZn-SOD in erythrocytes, serum ceruloplasmin or copper in serum after supplementation of up to 150 µg Cu/kg/day for two months or 7.53 mg Cu/day for 24 days or 5 mg Cu/l added to drinking water, or 3 mg Cu/day as copper gluconate tablets for 6 weeks (Turnlund *et al*., 1990; Medeiros *et al*., 1991; Pizarro *et al*., 1999; Araya *et al*., 2003). The activities of ceruloplasmin in serum and CuZn-SOD in erythrocytes, but not the concentration of copper in serum, increased when the study was extended to 5 months and the supplementation increased to 7 mg Cu/day (Turnlund *et al*., 2004). These results suggest that gastrointestinal copper intakes several fold higher than the dietary reference intake is necessary to change the levels of these biomarkers, thus not making them very useful for the purpose of exposure surveillance. Whether these biomarkers may be related to exposure at even higher oral intakes remains to be elucidated. Because the described biomarkers of copper status are not sensitive enough to detect moderately increased dietary intakes of copper, other biomarkers like benzylamine oxidase are currently investigated (Turnlund *et al*., 2004). An increase in platelet cytochrome c oxidase and serum diamine oxidase activity suggested that these markers may be more sensitive to copper supplementation than the more traditional biomarkers of exposure (Kehoe *et al*., 2000). No data are available on these biomarkers in the assessment of pulmonary uptake of copper, as for instance in the occupational setting.

In deficiency states it has been shown that infants suffering from malnutrition normalized their levels of SOD in erythrocytes and copper in serum after supplementation with 80 µg Cu/kg/day for 120 days (Uauy *et al*., 1985). The increase of erythrocyte SOD and serum copper in women supplemented with 3 and 6 mg copper daily during 4 weeks was attributed to marginal copper status prior to the study (Cashman *et al*., 2001). It has been suggested that in copper depletion only lower liver copper levels may be found (Olivares et al., 2000). At mild copper deficiency erythrocyte SOD or cytochrome-c-oxidase in leukocytes or platelets may be useful biomarkers (Milne, 1998). At moderate deficiency the serum copper concentration and the serum ceruloplasmin level may be reduced, and eventually also the hemoglobin content of the blood (Olivares *et al*., 2000).

7 EFFECTS AND DOSE-RESPONSE RELATIONSHIPS

Copper is an essential trace element. The redox chemistry makes copper highly suitable as a catalytic cofactor in oxidative enzymes. Copper is involved in numerous biological processes primarily as an integral part of enzymes, and thus in body functions like cellular respiration (cytochrom c oxidase), antioxidant defense (superoxide dismutase), connective tissue formation (lysyl oxidase and related proteins), neurotransmitter

biosynthesis (dopamine beta hydroxylase), peptide hormone maturation (peptidyl alpha amidating enzyme), pigmentation (tyrosinase), keratinisation (sulfhydryl oxidase), and iron homeostasis (ceruloplasmin and hephaestin) (WHO, 1998). Copper may further be implicated in myelination (Horn *et al.*, 1992, Kumar *et al.*, 2003), in regulation of the circadian rhythm (PINA) (Borjigin *et al.*, 1999), and may also be necessary for coagulation (factor V and factor VIII), angiogenesis (Pena *et al.*, 1999; Horn and Tümer, 2002), and sphingolipid metabolism (Tümer *et al.*, 2002). The severe picture of Menkes disease previously described, demonstrates well the essential functions of copper in humans.

Clinically evident copper deficiency occurs relatively infrequent, but many reports of deficiency originate in children and infants (WHO, 1998). Anemia refractory to iron supplementation is often the main manifestation of copper deficiency in normal populations (Olivares *et al.*, 2000). The mechanism is probably related to the ferroxidase activity of ceruloplasmin necessary for oxidizing Fe(II) to Fe(III) in the macrophages of the reticuloendothelial system to mobilize iron for its re-utilization (Hellman and Gitlin, 2002). Anemia caused by copper deficiency is also occasionally reported due to prolonged treatment with zinc containing medications (Willis *et al.*, 2005). At more severe copper deficiency, reduced serum copper and ceruloplasmin levels and anemia, and finally reduction in neutrophils and bone changes may occur (Olivares *et al.*, 2000). Bone abnormalities such as osteoporosis, fractures of long bone or epiphyseal separation, are most often observed in low birth-weight infants and young children (WHO, 1996; WHO, 1998). Increased incidence of infections, increased concentrations of total cholesterol and low-density lipoproteins, altered cardiac rhytm, impaired growth in children and hypopigmentation of the hair are other potential manifestations of copper deficiency (WHO, 1998). Whether marginally reduced copper status has a long-term health impact in humans is not yet known.

Excessive intake of copper has been shown to cause a variety of toxic effects in animal experiments. In humans, toxic effects after industrial exposure to fumes, and after ingestion of large amounts of copper compounds are known. Risk groups may be patients with Wilson disease or with various liver diseases because of their abnormal copper metabolism.

7.1 Local Effects and Dose-Response Relationships

7.1.1 Animals

Inflammatory markers in the broncoalveolar lavage fluid were increased in relation to dose in male Wistar rats administered CuO intratracheally (Hirano *et al.*, 1993). Significantly increased number of macrophages was found after administration of 50 µg/rat and increased protein levels at 20 µg/rat in that study. A similar increase in macrophages was found after intratracheal instillation of $CuSO_4$ at 20 µg/rat (Hirano *et al.*, 1990). Inhalation of 1.6 mg CuO/m^3 resulted in reduced lung ventilatory parameters in Guinea pigs (Chen *et al.*, 1991). Mice inhaling 0.12 mg Cu/m^3, probably in the respirable aerosol fraction, experienced thickening of the pulmonary alveolar walls (Drummond *et al.*, 1986). Exposure to copper sulfate (5 % aqueous solution without neutralizing additives) daily for 4 months resulted in focal accumulation of macrophages in alveoli and interstitial infiltration of cells in all of the 15 mice examined (Eckert and Jerochin, 1982). Accumulation of macrophages, significantly increased in size and containing polymorphic nuclei, crystalline-like inclusions and degenerative membrane structures, was found in the alveolar space one week after a single intratracheal injection of 5 mg CuO (Murthy *et al.*, 1982). Four of six guinea pigs exposed to Bordeaux mixture (1–2 % solution of copper sulfate neutralized with hydrated lime) for six months had micronodular lesions upon X-ray examination and upon microscopical examination of the lungs (Pimentel and Marques, 1969). The exact dose could not be established for the copper component or for the neutralizing calcium additive.

Reduced activity of glucosamine synthetase, the rate-limiting enzyme in the hexosamine synthesis, was shown in rabbits exposed to copper. This could eventually result in impaired synthesis of the gastric mucosal layer (Fujita *et al.*, 1997).

7.1.2 Humans

Acute gastrointestinal disturbances with vomiting, epigastric burns, and diarrhea may occur from accidental ingestion of food or beverages contaminated by copper released from copper vessels, from hot water geysers (Semple *et al.*, 1960), or from drinking water (Wyllie, 1957; Spitalny *et al.*, 1984; Knobeloch *et al.*, 1994). The experience from suicidal copper sulfate poisoning shows that gastrointestinal disturbances occur in all cases admitted to the hospital, suggesting that these symptoms are the first to occur together with the feeling of metallic taste (Chuttani *et al.*, 1965). It was suggested that diarrhea in children below 3 years of age was caused by drinking tapwater containing around 1 mg Cu/L (Stenhammar, 1999). Other authors have considered it unlikely that such low doses of copper should be the causative agent for gastrointestinal disturbances (Elinder and Stråat, 1983; Pettersson

et al., 2003). Experiments in adults have shown mild nausea to be the most frequently reported gastrointestinal symptom, increased reporting starting at around 4 mg Cu/L (as $CuSO_4$) in drinking water (Pizarro *et al.*, 1999; Araya *et al.*, 2001; Olivares *et al.*, 2001). The incidence of vomiting may increase at 6 mg Cu/L drinking water (Olivares *et al.*, 2001). Adding the same amount of copper to an orange-flavored powder drink resulted in less often reporting of nausea and no vomiting (Olivares *et al.*, 2001). Women reported gastrointestinal symptoms more frequently than men when exposed to copper in drinking water (Araya *et al.*, 2004).

Drinking 200 ml of water containing 10 mg Cu/L (as $CuSO_4$) as a single bolus resulted in increased urinary sucrose excretion suggestive of increased permeability of the gastric mucosa (Gotteland *et al.*, 2001). The biological mechanisms for the gastric symptoms have not been completely elucidated, but altered permeability of the tight junctions of human intestinal Caco-2 cells after in vitro exposure to $CuCl_2$ has been shown (Ferruzza *et al.*, 1999).

Although industrial exposure to copper is widespread, well-designed epidemiological studies are lacking from copper exposed populations. Also many occupational exposures consist of a mixture of copper and other compounds, making it difficult to attribute a particular effect to copper alone (WHO, 1998). Some small studies suggest that copper exposure may cause respiratory irritation, which also is compatible with results from animal studies. Long-term occupational exposure to a mixture of copper salts resulted in mucosal and suspected atrophic changes in the mucous membranes of the nose (Askergren and Mellgren, 1979). The study of Suciu *et al.* (1981) reported dyspnoea, thoracic pain, emphysema, and pulmonary fibrosis in copper exposed workers, but methodological weaknesses severly limit the usefulness of this information.

Reports on chronic lung damage after exposure to copper dust or fumes in industry are negative (Gleason, 1968; Cohen, 1974). Pimentel and Marques (1969), and Villar (1974) reported 2 and 15 cases, respectively, of the so-called 'vineyard sprayers lung' that was attributed to the inhalation of copper sulfate during spraying of vineyards with Bordeaux mixture. Macroscopical, greenish blue patches were seen on the lung surfaces. The histological nodular changes resembled those seen in silicosis, but only copper could be demonstrated.

Among the 15 patients described by Villar (1974), 3 cases of lung cancer and 5 cases of tuberculosis were diagnosed.

A form of contact dermatitis associated with copper has been reported (Saltzer and Wilson, 1968), but generally few published cases of dermatitis caused by copper metal or compounds are available from industrial settings.

Copper allergies have been reported, either related to skin contact with copper dust or salts, to copper-containing intrauterine devices, or dental restorations (Barkoff, 1976; Barranco, 1972; Hackel *et al.*, 1991; Nordlind and Lidén, 1992). Only 13 of 1190 eczema patients showed a positive patch test on copper sulfate, although copper has a widespread use in the general population as, for instance, in coinage materials. This suggests that copper may be a quite rare sensitizer (Karlberg *et al.*, 1983). Green skin coloration and itching were reported in workers producing components for turbine generators (NIOSH, 1981b).

7.2 Systemic Effects and Dose-Response Relationships

7.2.1 Laboratory and Domestic Animals

LD_{50} values of copper after a single exposure depend on animal species, copper species, and the route of administration. Soluble copper compounds are generally more acutely toxic than less soluble compounds (WHO, 1998). LD_{50} values between 32 and 1600 mg/kg body weight have been reported after a single oral exposure (WHO, 1998).

Rats were orally administered between 8 and 138 mg Cu/kg body weight as copper sulfate pentahydrate (Hébert *et al.*, 1993). Hyperplasia and hyperkeratosis of the forestomach was observed at 34 mg Cu/kg body weight, and inflammation of the liver in most rats at 67 mg Cu/kg body weight. The kidney lesions were described as minimal nuclear enlargement and degeneration of the tubule epithelium. Also, urinalysis indicated damage to the tubular cells. Pigs accidentally receiving feed containing 700 mg/kg of copper for several months developed an iron-deficiency type of anemia and gastric ulcers (Hatch *et al.*, 1979). The copper concentration in the liver was 100–170 mg/kg wet weight, and hepatic centric lobular necrosis was found.

Hébert *et al.* (1993) also studied the hepatotoxic effects of copper. A chronic-active inflammation of the liver and degenerative changes consisting of increases in the number of secondary lysosomes in the periportal hepatocytes were observed. A concomitant increase in serum enzymes related to the liver function was also found.

Sheep are more vulnerable to copper toxicity than many other species. During copper loading, the number of copper-containing lysosomes increase. Necrosis of hepatocytes may occur by further increase in the intracellular copper levels. This may result in a sharp

increase of plasma copper levels followed by an uptake by the erythrocytes. An increase in the plasma concentrations of β-glutamyltransferase and aspartate aminotransferase is evident before a hemolytic crisis (Lippi *et al*., 2003). However, in rats and mice, nephrotoxic and hepatotoxic effects are observed without preceding hemolysis, suggesting that this phenomenon may be species-related (Hébert *et al*., 1993).

Reactive oxygen species may be produced in the liver of experimental animals administered copper (Gaetke and Chow, 2003). There is some evidence that the liver toxicity may be associated with the induction of oxidative damage with resulting lipid peroxidation (Gaetke and Chow, 2003). Lipid peroxidation of mitochondrial membranes of liver cells (Britton, 1996), membranes of hepatic lysosomes (Bremner, 1998), rat hepatic mitochondria (Sokol *et al*., 1990), and malondialdehyde in the livers of copper-loaded rats (Ohhira *et al*., 1995; Zhang *et al*., 2000) have been observed in different studies.

The Long–Evans Cinnamon (LEC) rat has a mutation homolog to the human Wilson's disease gene and is used as a model for this disease (Harris, 2003; Klein *et al*., 1998; Powell, 1994). Copper toxicosis has been reported in several dog breeds, and the prevalence of the condition is particularly high in Bedlington terriers (Hyun and Filippich, 2004). This may be because albumin has a low affinity to copper in many dogs because its N-terminal histidine is missing (Hyun and Filippich, 2004). The clinical manifestations of copper toxicosis in Bedlington terriers resemble those of Wilson's disease. Therefore, copper toxicosis in these animals was originally considered to be a model for Wilson's disease (Coronado *et al*., 2003). Recent research has shown that the copper toxicosis in Bedlington terriers is not related to the human Wilson's disease gene ATP7B, but perhaps to the gene Murr1 (Coronado *et al*., 2003; Harris, 2003; Hyun and Filippich, 2004).

7.2.2 *Humans*

7.2.2.1 Metal Fume Fever

Metal fume fever is an influenza-like syndrome with fever, myalgias, profuse sweating, and other symptoms that usually occur 3–10 hours after heavy exposure to a variety of metal oxides. The symptoms usually disappear after 24–48 hours (Mueller and Seger, 1985). Metal fume fever has also been reported after exposure to copper-containing fumes and fine dusts; the first reports date back at least to the start of the last century (Friberg and Thrysin, 1947; Hansen, 1911). Sixteen workers diagnosed as having metal fume fever after cutting pipes containing 90% copper, 10% nickel, and traces of zinc were suggested to have increased urinary copper concentrations (Armstrong *et al*., 1983). No dose-response relationship has ever been reported.

7.2.2.2 Hemolysis

Case reports of acute copper sulfate poisoning have indicated the occurrence of acute hemolysis (Bhowmik *et al*., 2001; Liu *et al*., 2001; Oldenquist and Salem, 1999). In two cases, 50 g/L and 65 g/L hemoglobin were measured on admission to the hospital, and 1.8 mg Cu/L in serum and 5.3 mg Cu/L in whole blood, respectively, were reported. The liver copper concentration of 19.0 μg/g fresh weight reported in one case of hemolysis is somewhat higher than normal, but substantially lower than what is found, for instance, in Wilson's disease. In 5 of 123 cases of acute copper sulfate poisonings, acute intravascular hemolysis was reported as a complication of the poisoning, suggesting this condition to be rare in humans (Ahasan *et al*, 1994).

Hemolysis has been reported after application of solutions of copper salts to large areas of burned skin (Holtzman *et al*., 1966) or after the introduction of copper from copper-containing semipermeable membranes and copper tubing into the circulation during hemodialysis (Blomfield *et al*., 1969; Klein *et al*., 1972; Manzler and Schreiner, 1970). No dose-response relationship between serum copper levels and the tendency to intravascular hemolysis has been reported. *In vitro*, hemolysis of human erythrocytes was observed at concentrations of copper > 4–5 mg/L (Sivertsen, 1980).

7.2.2.3 Effects on Liver and Kidneys

During a period of 6 years, 123 cases of copper poisoning with suicidal intention were treated in southern Bangladesh. The mortality was 24.9%, and the severe medical complications were mainly related to hepatotoxicity ($n=46$), gastrointestinal tract bleeding ($n=8$), acute renal failure ($n=5$), and acute intravascular hemolysis ($n=5$) (Ahasan *et al*., 1994). However, in fatal poisonings, multiple organ pathologies are often reported (Bhowmik *et al*., 2001; Kurisaki *et al*., 1988). No substantial difference in the mean serum copper concentrations was found between mild cases of poisoning with gastrointestinal symptoms only and severe cases with kidney or liver manifestations. The serum reference level was approximately 1.5 mg/L compared with approximately 3.0 mg/L in the two groups of patients with mild or severe symptoms (Chuttani *et al*., 1965).

Jaundice is often observed in cases of copper sulfate poisoning (Chuttani *et al*., 1965). It has been proposed that the jaundice may either be related to hepatotoxicity or hemolysis (Chuttani *et al*., 1965). Hepatomegalia and centrilobular necrosis of the liver may be found

(Chuttani *et al*., 1965; Kurisaki *et al*., 1988). In severe cases, biliary stasis may be observed as well (Chuttani *et al*., 1965).

In 48 patients admitted to a hospital because of acute copper sulfate poisoning, 13 patients developed renal clinical symptoms like anuria or oliguria (Chuttani *et al*., 1965). In two cases kidney biopsy revealed swelling and necrosis of the tubular cells, and in four fatalities tubular cell necrosis, congestion of glomeruli, and in some instances hemoglobin casts were observed. Intravenous injection of copper sulfate resulted clinically in acute renal failure and oliguria, without any gastrointestinal disturbances (Bhowmik *et al*., 2001). Patchy tubular atrophy with interstitial fibrosis and focal chronic interstitial inflammation was found, but the glomeruli were largely unaffected. Renal insufficiency with necrosis of tubular epithelium and edema of the medulla was found in another case (Kurisaki *et al*., 1988).

Hemolysis is not a necessary event for the induction of nephrotoxicity. Data on administered dose in the cases of poisoning are unprecise and also sparse, and it is, therefore, difficult to establish dose-response relationships for the induction of the acute toxic effects on the basis of these data.

7.3 Mutagenic, Carcinogenic, and Teratogenic Effects

Errors in the DNA synthesis (Sirover and Loeb, 1976), reduced DNA synthesis (Garrett and Lewtas, 1983; Sirover and Loeb, 1976), and increased occurrence of DNA strand breaks (Sideris *et al*., 1988; Sina *et al*., 1983) have been observed *in vitro* after administration of different copper salts. This is in contrast to the mostly negative results from reverse mutation testing in *Salmonella* or *Saccharomyces* (Marzin and Phi, 1985; Singh, 1983; Tso and Fung, 1981; Wong, 1988). Several *in vivo* animal studies have reported increased occurrence of micronuclei and chromosomal aberrations (Agarwal *et al*., 1990; Bhunya and Jena, 1996; Bhunya and Pati, 1987). An increased load of p53 mutations was observed in the livers of patients with Wilson's disease (Perwez Hussain *et al*., 2000).

There is evidence to suggest that the mutagenic effects may be related to the induction of oxidative damage (Gaetke and Chow, 2003).

The association between cancer and serum copper or serum ceruloplasmin levels has been investigated in several epidemiological studies (WHO, 1998). In some studies, these biological markers have been collected at the time of diagnosis and may thus reflect the status of ceruloplasmin as an acute-phase reactant. Other studies have not been able to show convincing dose-response relations. A general problem in occupational cancer studies of copper-exposed populations is that copper handling often is associated with other cancer risk factors like mining or arsenic exposure in copper smelting. For instance, the increased incidence of lung cancer reported among workers in copper refineries and copper-ore mines (Kuratsune *et al*., 1974; Milham and Strong, 1974) has been attributed to arsenic exposure.

Overall, no convincing evidence of copper being a human carcinogen has been presented so far (WHO, 1998). Also, animal studies have not convincingly established copper as a carcinogen, but few such studies have been carried out so far, and several of them have been too small or have had too short a follow-up time for addressing this question. It has even been suggested that copper can inhibit some tumors under certain circumstances (Burki and Okita, 1969; Carlton and Price, 1973; Oberley *et al*., 1982). However, the LEC rats with the genetic defect in the ATP7B gene, homologous to that of Wilson's disease, are highly susceptible to the development of hepatitis and hepatocellular carcinomas (Eagon *et al*., 1999).

Rats inhaling $CuCl_2$ had increased incidence of abnormal sperms, reduced sperm motility and testis weight, and also a reduction in testosterone levels (Gabuchyan, 1987). Similar results were found after intraperitoneal exposure to rats (Chattopadhyay *et al*., 1999). Incubation of human spermatozoa with metallic Cu resulted in a significant fall in the percentage of motile sperms (Battersby *et al*., 1982). A large study on male and female mice and rats did not report any changes in testes weight, epididymis, spermatid counts, or sperm motility in males and vaginal cytology or estrous cycle length in the females (Hébert *et al*., 1993).

O'Shea and Kaufman (1979) reported that 4 mg/kg of copper administered intramuscularly early in pregnancy affected the development of the CNS of the fetuses. Developmental malformations in mice were observed after oral administration of 3 g copper sulfate/kg of feed (Lecyk, 1980). Reduced ossification in rats administered up to 0.185% copper acetate in drinking water was observed (Haddad *et al*., 1991).

7.4 Biological Interactions

Ceruloplasmin (as holoprotein) has a significant impact on iron metabolism. In particular, the mobilization of iron from the macrophages of the reticuloendothelial system by the capacity of ceruloplasmin to oxidize Fe(II) to Fe(III) is important (Hellman and Gitlin, 2002). A result of reduced mobilization is less iron available for new synthesis of hemoglobin and consequently anemia. A similar role has been ascribed to the copper-containing protein hephaestin, which

is integrated in the membrane of the enterocyte (Anderson *et al*., 2002). It has recently been shown that rats on a copper-deficient diet had lower hephaestin levels in the enterocytes, indicative of lower iron mobilization from the enterocytes into the body (Reeves *et al*., 2005).

There are also indications that ceruloplasmin is synthesized and secreted in astrocytes of the brain and is believed to have a role in iron efflux from these cells (Hellman and Gitlin, 2002; Qian and Ke, 2001). This function could explain the clinical picture of humans having the rare genetic disease aceruloplasminemia. These patients usually present their clinical picture in the fourth or fifth decade of life, with dysarthria, dystonia, dementia, insulin-dependent diabetes mellitus, and retinal degeneration (Hellman and Gitlin, 2002). The patients also have mild anemia. In contrast to the demonstrated iron accumulation in these patients, the levels of copper in the body seem to be normal (Gitlin, 1998).

It has been shown in animals that high oral zinc intake may induce synthesis of intestinal metallothionein. Copper has a higher affinity than zinc to metallothionein and will thus bind in the enterocytes. The mucosal cells are subsequently shed and excreted in the feces (Danks, 1991; Lönnerdal, 1996). It seems that quite high zinc intakes are needed to have a negative impact on the intestinal copper absorption, and this mechanism of interaction has not been shown to be relevant at normal zinc intake. However, at higher zinc intake, copper deficiency may be induced. For details, consult Chapter 47.

Several other dietary factors like animal proteins, amino acids, phytate, fibers, ascorbic acid, and carbohydrates have also been reported to modify the gastrointestinal absorption of copper (WHO, 1998).

High intake of molybdenum and sulfur in the food of ruminants causes a decreased bioavailability of copper. Tetrathiomolybdate was identified to complex copper gastrointestinally and in the tissues. A redistribution of copper from the liver to other tissues occurs. The redistribution to the kidney results in increased urinary copper excretion (Danks, 1991). In humans, the urinary excretion of copper is influenced by the molybdenum intake; a low molybdenum concentration in the diet causes a low excretion of copper, and a high intake provokes a considerable increase in excretion of copper (Deosthale and Gopalan, 1974; Kovalskii *et al*., 1961). A decrease in blood copper levels has been reported from an area where people had a high intake of molybdenum (Kovalskii *et al*., 1961). Tetrathiomolybdate has been used in the treatment of Wilson's disease (Brewer, 1995).

Drugs that can affect the copper metabolism include phenobarbital, phenytoin, morphine, and dithiocarbamates. The dithiocarbamates have been identified as metabolic products after exposure to carbon disulfide and after ingestion of disulfiram and are reported to inhibit the biliary excretion of copper in rats and affect the interorgan distribution (Aaseth *et al*., 1981; Alexander and Aaseth, 1980). Phenytoin and phenobarbital increase the serum ceruloplasmin level and change the copper metabolism as a result of hepatic enzyme induction (Tutor *et al*., 1982).

8 PREVENTIVE MEASURES AND TREATMENT

Oral intake of toxic doses of copper salts should be treated as early as possible with gastric lavage with a 1% solution of potassium ferrocyanide (Moeschlin, 1965). This treatment leads to the formation of insoluble cupric ferrocyanide. If the oral antidotic resin ferrocyanide is unavailable, medical charcoal should be instilled immediately after gastric lavage to minimize the absorption of copper.

In cases of systemic signs of poisoning, adequate replacement of fluid, electrolytes, or blood is important. Corticosteroids may be efficacious particularly in the severe cases with jaundice, hemoglobinuria, and hematuria.

$CaNa_2EDTA$ or BAL should be used in severe poisoning (Barceloux, 1999). Other chelating agents that afford protection against experimentally poisoned animals are triethylenetetramine (TETA), dimercaptopropane sulfonic acid (DMPS), and dimercaptosuccinic acid (DMSA). Clinical cases have also been treated successfully with these agents (Andersen, 1999). In cases of acute poisoning with acute renal failure, hemodialysis has been used (Oldenquist and Salem, 1999).

The major cause for chronic copper intoxication is Wilson's disease. Patients with this disease have been treated successfully with D-penicillamine or TETA (Andersen, 1999; El-Yousesef, 2003; Roberts and Schilsky, 2003). It should be emphasized that continuous lifelong treatment with one of the copper-chelating agents is necessary to avoid progression of this disease. Such long-term treatment requires periodic clinical and laboratory control to detect possible side effects of the drugs, and, in particular, the use of D-penicillamine is associated with numerous side effects (Roberts and Schilsky, 2003). Recent reviews conclude that TETA is better tolerated than penicillamine (Andersen, 1999; Roberts and Schilsky, 2003). In China, hundreds of patients with Wilson's disease have been treated successfully with DMSA during the past 30 years (Andersen, 1999). Patients with Wilson's disease should avoid foods with high copper contents (Barceloux, 1999).

Oral zinc induces the metallothionein synthesis in the enterocytes. This mechanism is used therapeutically to reduce copper uptake in Wilson's disease. Metallothionein will complex copper in these cells that subsequently will be shed off into the feces (Barceloux, 1999; Danks, 1991; Lönnerdal, 1996; Roberts and Schilsky, 2003).

Liver transplantation must be considered in decompensated liver disease (Roberts and Schilsky, 2003).

Allergic contact dermatitis caused by copper has been treated successfully with hydrocortisone ointment and with the removal of the offending agent (Saltzer and Wilson, 1968).

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CHAPTER

27

Gallium and Semiconductor Compounds

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ABSTRACT

Gallium is a member of Group III of the Periodic Table of Elements, and new uses have been found for different chemical forms of this metal over the past two decades in the fields of semiconductor materials, lasers, cancer and malaria chemotherapy, and dental materials. The production of gallium for these uses has increased greatly during this period. Analytical methods for gallium include atomic absorption/emission spectrometry, X-ray fluorescence, and, more recently, inductively coupled plasma spectrometry. The main health concerns for gallium have been centered on workers in the semiconductor industry engaged in production of gallium arsenide–based devices. Gallium is transported in the circulation bound to transferrin, and competition with iron for transferrin binding seems to be one mechanism by which it may act as an anticancer agent. Toxicity studies in experimental animal systems have shown gallium nitrate and gallium arsenide to produce toxicity to the lungs, immune system, kidneys, and hematopoietic systems. The International Agency for Research on Cancer (IARC) has classified gallium arsenide as a human carcinogen. Studies of semiconductor workers are limited, but clinical trials in humans treated with gallium nitrate for various cancers have demonstrated renal toxicity as the primary side effect.

1 PHYSICAL AND CHEMICAL PROPERTIES

Gallium (Ga), atomic number, 31; atomic weight, 69.735; valence states, +2, +3; melting point, 29.78°C; specific gravity, 5.904 at 29.6°C.

2 METHODS AND PROBLEMS OF ANALYSIS

Atomic emission spectroscopy, X-ray fluorescence, ICP (inductively coupled plasma) with an LOD (limit of detection) of .03 μg/mL electrothermal atomic absorption spectrometry has been reported to provide a detection limit of 0.08 μg/cm^3 after preconcentration by precipitation with synthetic zeolites (Minamisawa *et al.*, 2004). Cathodic adsorptive voltammetry with a gallium-alizarin red S complex has been reported to provide a detection limit of 0.01 μg/L (Li *et al.*, 2004). Spectrophotometric determination of gallium as thiocarbohydrazone derivatives has been reported (Lucena *et al.*, 1994) to provide limits of detection in the 5–8 ng/mL range.

3 PRODUCTION AND USES

World production of gallium has risen steadily in the past two decades to reach a peak in 1998 of 93.3 metric tons with a recent decline to 61 metric tons in 2002 (USGS, 2004). The recent decline may be a reflection of the increasing use of indium compounds in the III–V semiconductor industry (see Figure 1).

3.1 Uses

Gallium has a number of uses, most notably in the semiconductor industry as a dopant material for silicon computer chips and in the formation of III–V semiconductors such as gallium arsenide (GaAs) (Fowler and Sexton, 2002; Jenkins *et al.*, 1994; Pan *et al.*, 2003).

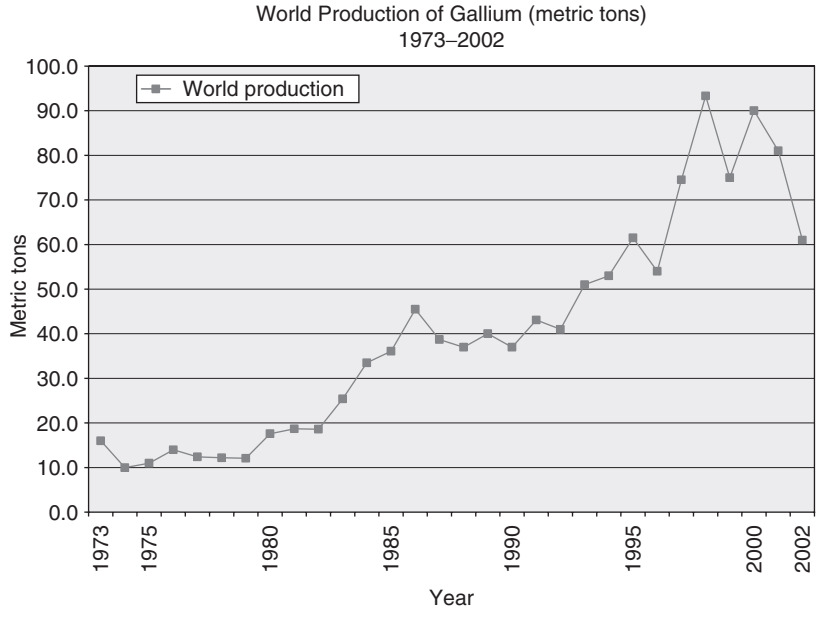

FIGURE 1 World Production of Gallium (metric tons) from 1973–2002.

Gallium isotopes such as ^{67}Ga are widely used for organ screening (e.g., Hodgkin's disease and lymphomas) (Foo *et al*., 2004; Friedberg *et al*., 2004; Galanski *et al*., 2003, Kellenberger *et al*., 2004; Ng *et al*., 2005; Shah Sayed *et al*., 2004; Tuli *et al*., 2004) and receptor-based nononcological diseases (Sosabowsky *et al*., 2003). These include infections (Love and Palestro, 2004), and sarcoidosis (Abad *et al*., 2004; Nakazawa *et al*., 2004). The uptake of ^{67}Ga into tumor cells has been reported to be stimulated by hypoxia related to tumor cell transferrin receptors, because Ga binding to transferrin was not altered under hypoxic conditions (Kinuya *et al*., 2004). Gallium nitrate has received expanded use as an antitumor agent for tumors of the bladder and lymphatic system (Chitambar, 2004a). Gallium exerts part of its effect by means of competition with iron on transferrin (Chitambar, 2004a,b; Jakupec and Keppler, 2004a,b) and inhibits ribonucleotide reductase (Chitambar, 2004a). Gallium has also been found to initiate apoptosis by activation of the caspase system (Chitambar, 2004c). Gallium has also been found to exert an attenuating effect on hypercalcemia related to a variety of cancers (Leyland-Jones, 2004). This may occur in part by a mechanism involving inhibition of tyrosine phosphatase (Berggren *et al*., 1993). In addition, synthesis of gallium metalloporphyrins (Begum *et al*., 2003) and complexes with amin phenol ligands (Harpstrite, *et al*., 2003) have been tested as antimalarial agents against *Plasmodium falciparum*.

In addition, GaAs lasers operated at 904 nm have been used in myofacial pain syndrome (Gur *et al*., 2004) and bone formation (Khadra *et al*., 2004a,b). Gallium alloys have also been used for dental implants (Claire and Williams, 2001; Garhammer *et al*., 2004; Hero *et al*., 1997).

4 ENVIRONMENTAL LEVELS AND EXPOSURES

There are limited published data on environmental exposure levels to gallium.

4.1 Food and Daily Intake

Although arsenic intake from food has been reported (see Chapter 19), no data on food intake specifically for Ga or GaAs could be found despite investigations showing the dissociation of the gallium and arsenic moieties (see Section 5.1.1). Relevant to issues regarding gallium from food are the reports of children's Ga intake from breastfeeding because of the presence of Ga in human milk (Wappelhorst *et al*., 2002).

4.2 Water, Sediments, Soil, and Ambient Air

Orians (1993) reported open seawater concentrations of gallium in the range of 2–60 picomolar after preconcentration and ICP–MS analysis. Anand *et al*. (2003) reported Ga concentrations over a range of

9.74 – 16.02 mg/kg along a smelter-damaged landscape gradient in Sudbury, Ontario. The USGS (2004) reported sediment concentrations measured in 1998 in the Bear River basin, Utah, over a range of 1.08–19.4 μg/g.

5 METABOLISM

5.1 Absorption

5.1.1 Inhalation

Absorption of gallium from the lungs after inhalation exposure partially depends on whether it is in a particulate form such as GaAs or Ga_2O_3 (National Toxicology Program, 2000). Acute intratracheal instillation studies from a number of investigators (Rosner and Carter, 1987; Webb *et al*., 1984; 1986; 1987) or intraperitoneal injection (Yamauchi *et al*., 1986) has shown dissociation of the gallium and arsenic moieties to release both elements. Dose absorption and dissolution rates also seem to vary with dose level and duration of exposure. The metabolism of gallium arsenide has been recently reviewed by Carter and coworkers (2003).

5.1.2 Ingestion

The absorption of Ga after ingestion of GaAs particles in experimental animals has been shown to be very low (Yamauchi *et al*., 1986).

5.2 Distribution

Absorbed gallium is distributed to a number of organs with highest concentrations in the liver, kidneys, and spleen as a result of its binding to and transport in the circulation by transferrin. Absorbed gallium is bound to transferrin in the circulation and distributed to a number of organ systems with major concentrations in the liver (Sun *et al*., 1998), bone, bone marrow, spleen, and kidneys (Belozerov, 1966; Collery *et al*., 1996), in humans and in animals (Straw *et al*., 1975).

5.3 Excretion

The main route of excretion for gallium from the body is through the urine, with lesser amounts excreted in the feces (Rosner and Carter, 1987; Webb *et al*., 1984; 1986; 1987; Yamauchi *et al*., 1986). In humans, ^{67}Ga has been reported in the breast milk of nursing mothers (Paterson *et al*., 1976; Rubow *et al*., 1991; Tobin and Schneider, 1976; Weiner and Spencer, 1994) treated with this agent. Studies of the nursing infant showed ^{67}Ga in only in the gastrointestinal tract (Rubow *et al*., 1991). More recent studies (Wappelhorst *et al*., 2002) have shown that gallium has a high transfer coefficient from ingested food into milk of lactating mothers.

5.4 Biological Half-Time

The biological half-time of gallium nitrate in humans with normal renal function has been found to be biphasic with mean half-times of 1 hour for the fast, first-phase component and 25 hours for the second phase after intravenous injections with colorimetric measurement of gallium (Hall *et al*., 1979). Kelson *et al*. (1980) performed similar studies in humans and also obtained a biphasic pattern with mean half-times of 8.3–26 minutes for the fast phase and 6.3–196 hours for the second phase component. The intersubject variations may reflect differences in renal function among the test patients.

6 LEVELS IN BIOLOGICAL FLUIDS

Liao and his coworkers (2004) reported gallium concentrations in blood and urine from workers in Taiwan engaged in the production of III–V semiconductors. The geometric mean values of the gallium blood levels were 0.57, 0.53, 0.44, 0.48 μg/L for workers in fabrication equipment maintenance, workers in dopants and thin film, fabrication supervisors and engineers, and office workers (controls), respectively. Gallium concentrations in urine were 0.28, 0.23, 0.23, and 0.15 μg/L for the same groups of workers. Although blood levels were not consistently higher in "exposed" workers than office workers, urinary gallium was significantly higher ($P < .05$) in all other workers compared with the officer workers.

7 EFFECTS AND DOSE-RESPONSE RELATIONSHIPS

7.1 Animal Studies

Numerous studies regarding the target organ toxicity of GaAs compounds in rodents (Flora *et al*., 1998; 2002; Goering and Rehm, 1990; Goering *et al*., 1988; Webb *et al*., 1984; 1986) have reported pulmonary, renal, and effects on the heme biosynthetic pathway. The pulmonary effects were most marked in rodents given single intratracheal instillations of the GaAs. More chronic exposures to GaAs particles by intratracheal instillation to hamsters (Tanaka *et al*., 2000) or by inhalation to rats and mice (National Toxicology Program, 2000) produced marked toxicity and inflammation in the

lungs, including the development of alveolar and bronchioalveolar tumors. Marked toxicity to alveolar macrophages was observed. *In vitro* studies on hamster alveolar macrophages (Okada *et al*., 1999) demonstrated direct cellular and death primarily by necrosis versus apoptosis. Tanaka (2004) has recently reviewed the relative toxicity of GaAs, InAs, and AlGaAs and concluded that GaAs is of lesser toxicity than InAs but of greater toxicity than AlGaAs. Morgan *et al*. (1995) studied the comparative acute pulmonary toxicity of copper gallium diselenide relative to indium diselenide and cadmium telluride and found that it produced pulmonary inflammation and hyperplasia of the type II cells but that copper gallium diselenide was the least toxic of the three compounds tested.

In addition to the pulmonary effects of GaAs, this compound has been shown to produce a number of marked immunosuppressive effects. Studies in mice by Sikorski *et al*., (1991) that examined splenic cell function from a single intratracheal instillation of GaAs particles were found to have suppressed IgM production capability. Splenic cells were found to have a decreased ability to process and present particulate but not soluble antigens to T-cell populations. Subsequent studies (Burns and Munson, 1993a,b) showed that GaAs exposure produced suppression of the antibody-forming cell (AFC) response, but that protein components in the vehicle (VH) cultures were able to reverse this suppression over time. They studied productions of the interleukins (IL) and found that *in vivo* GaAs treatment produced altered expression of interleukins IL-2, IL-4, IL-5, and IL-6. The addition of IL-2 to cultures was shown to reverse GaAs suppression. In contrast, IL-4 suppressed AFC responsiveness and did not reverse the suppressive effects of GaAs treatment. These data indicate some very specific IL regulation of the immunosuppressive effects of GaAs. Lewis *et al*. (1996) studied the effects of GaAs treatment on macrophage processing of various antigens and induction of IL-2 production in $CD4^+$ helper T cells. Overall, the results of these studies indicated that GaAs-exposed macrophages showed decreased capacity to elicit T-cell responses to some soluble protein antigens but not others. The investigators concluded that GaAs treatment produced a time- and antigen-dependent alteration in antigen processing that is required for T-cell stimulation by $CD4^+$. Lewis *et al*. (1998) examined proteolytic cathepsin activities and antigen processing by macrophages in mice injected with 200 mg/kg (intraperitoneal) of GaAs particles and found differential effects on splenic and peritoneal macrophages. The splenic macrophages showed inhibition of cathepsins and antigen processing, whereas the peritoneal macrophages showed generally elevated cathepsin activities and antigen-processing capabilities. These data suggested differential effects of GaAs particles on these two macrophage populations. Subsequent studies from this laboratory (Becker and McCoy, 2003) reported that treatment of mice at 200 mg/kg intraperitoneally selectively up-regulated inflammatory cytokine expression at the injection site. More recent studies by Harrison *et al*. (2003) showed that GaAs treatment *in vivo* increased the activities of cathepsins L and B in macrophages apparently by an up-regulation of genes coding for these proteases. In contrast, other studies (Gondre-Lewis *et al*., 2003) on splenic B cells showed marked inhibition of cathepsin L and B activities, which are thiol dependent, suggesting that GaAs may inhibit antigen processing in splenic B cells by means of this mechanism. Hartmann and McCoy (2004) conducted *in vitro* studies that showed impaired processing of sheep red blood cell (SRBC) particulate antigen by splenic macrophages derived from mice treated *in vivo* with GaAs that resulted in attenuated SRBC activation of lymph node T cells.

The testes are another target organ for GaAs toxicity as demonstrated by repetitive intratracheal instillation studies by Omura *et al*. (1996 a,b) and more recently by the chronic inhalation studies conducted by the National Toxicology Program (2000). Omura *et al*. (1996a) demonstrated that intratracheal instillation of GaAs particles twice per week for 8 weeks to hamsters produced a marked decrease in sperm count and an increase in abnormal sperm morphology and the incidence of degenerating spermatids. Omura *et al*. (1996b) repeated the preceding studies but decreased the duration of dosing to 7 weeks and found that GaAs treatment produced spermatid retention in the testes and decreased epididymal sperm counts. Chemical analyses for Ga and As showed much greater concentrations of Ga relative to As in the treated animals, suggesting that Ga may have been the primary causative agent for testicular toxicity. Similar toxic effects were observed in the testes of rats and mice exposed to GaAs particles through inhalation by the NTP (2000).

The developmental toxicity of gallium nitrate was studied in mice by Gomez *et al*. (1992). The investigators found that intraperitoneal injection of gallium nitrate to pregnant Swiss mice on days 4, 6, 8, 10, 12, and 14 of gestation with sacrifice on day 18 produced embryo/fetal toxicity as judged by a decreased number of implants. No increase in the incidence of malformations was noted among the offspring.

In vitro (Burns *et al*., 1993) and *in vivo* studies (Flora *et al*., 2004) have demonstrated the usefulness of dimercaptosuccinic acid (DMSA) and DMSA or dimercaptopropane-sulfonate (DMPS) (Flora and Kumar, 1996) in attenuating the toxicological effects resulting from GaAs exposure in experimental systems.

7.2 Human Studies

The literature over the past two decades is replete with expressions of concern regarding exposures of workers in the semiconductor industry (Flora, 2000; Fowler and Sexton, 2002; Fowler and Silbergeld, 1989; Fowler and Woods, 1979; Nordberg and Nordberg, 1996; Tanaka, 2004; Webb, *et al*., 1984;Yamauchi, *et al*., 1989). Although frequently based on substantial animal data, such as cited in this chapter, which establish toxicity levels in identified organ systems for specific metals, such as gallium arsenide, these concerns have resulted in a fairly modest and uneven database regarding human health. The problems, such as access to workers and records, changing work environments and sites, the identification and documentation of the specific exposure chemicals and levels, have limited the accumulation of evidence and understanding for this ever-growing number of workers (Fowler and Silbergeld, 1989). Some editorials have attributed at least some of the lag in assembling a body of evidence to the industry's desire to disregard any findings suggestive of a health problem (Wadman, 2004; Watterson and LaDou, 2003). Certainly, factors such as the movement of production sites from developed to developing countries (Tenenbaum, 2003), law suits (Wadman, 2004), technological changes in manufacturing (Chepesiuk, 1999; Flora, 2000), and industry-controlled studies (Brumfiel, 2004; Watterson and LaDou, 2003) have made the assembly of a solid base of data regarding the human health effects resulting from exposure to chemicals used in the semiconductor industry difficult. Overall, the scientific evidence is far less than needed. Earlier studies focused on reproductive effects (Correa *et al*., 1996; Pastides *et al*., 1988) and more recent studies on other conditions (Wang *et al*., 2002). The results can be examined for consistency of findings with those from animal and cellular studies. In addition, gallium, and other semiconductor materials, such as indium, have been used at lower doses as adjuvant therapeutic agencies. Some properties of these chemicals (e.g., production of apoptosis) at high doses have been found to be useful in medical treatment of quite distinct health problems, such as treatment of cancer or wound healing. Nevertheless, as noted in the following, questions of safety for workers exposed to these agents during the manufacture of III–V semiconductors or light-emitting diodes remain.

7.2.1 Toxicity

The International Agency for Research on Cancer (IARC) has classified gallium arsenide as a Group 1 carcinogen to humans. In reaching this conclusion, they stated: "Gallium arsenide was evaluated as *carcinogenic to humans* (Group 1). For gallium arsenide itself, no data on human cancer were available, and the evidence of carcinogenicity in experimental animals was considered *limited* on the basis of increased incidence of bronchioloalveolar neoplasms observed in female rats in one study. However, once in the body, gallium arsenide releases a small amount of its arsenic moiety, which behaves as inorganic arsenic, already evaluated as *carcinogenic to humans* in Volume 84 (in press) and Supplement 7 (1987) of the *IARC Monographs*. The gallium moiety may be responsible for the pulmonary neoplasms observed in rats in view of the apparent resistance of this species to the carcinogenic potential of arsenic" (International Agency for Research on Cancer, in press). Despite the classification by organizations such as IARC and the U.S. National Toxicology Program of gallium arsenide along with other semiconductor compounds as extremely hazardous, there is very little in the literature on the biomonitoring of workers. Liao and colleagues (2004) in Taiwan found blood indium and urine gallium to be elevated in the 103 workers tested relative to the 67 controls. The higher level was also found to be related to job title, gender, educational level, and use of preventive equipment.

Although no longer available, the United States Bureau of Labor statistics earlier provided a database from which an assessment of incidences of reported illnesses in semiconductor workers could be made. Data by the Standard Industrial Classification (SIC) codes were reported separately for workers in the manufacture of semiconductors and related devices (SIC 3674) for more than a decade. Fowler and Sexton (2002) analyzed the available data and concluded that relative to workers in other manufacturing jobs, semiconductor workers seemed to have higher rates of illness and that the rates were higher in the 1980s than in the 1990s. Chee and Rampal (2003) conducted a survey of female production workers in 18 semiconductor factories in Malaysia. They found that those in wafer polishing jobs had higher illness rates compared with assembly workers.

In animals, pulmonary (Goering *et al*., 1988), hematopoietic (Goering *et al*., 1988), renal (Goering *et al*., 1988), immunological (Sikorski *et al*., 1991), and reproductive (Tanaka, 2004) effects from gallium have been reported. However, there is little documentation relating these conditions to workers exposed to gallium arsenide. Nevertheless, the limited correlative human data are consistent with those from animals.

7.2.1.1 Pulmonary

As a part of a clinical survey, 249 semiconductor workers in one plant in Taiwan were examined for

pulmonary functioning. The investigators (Luo *et al.*, 1998) found that men who were ion-implantation workers had almost four times the risk of having an abnormal level of functioning than controls after an adjustment for smoking and age. As a part of the Semiconductor Health Study, more than 3000 individuals from eight manufacturing sites in the United States completed questionnaires. After adjusting for potential confounders, McCurdy and coworkers (1995) reported a small, but statistically significant (relative risk = 1.08), increase in respiratory symptoms among fabrication workers relative to other workers. Although these data are not strong enough to draw a conclusion, they do suggest the need for additional studies on pulmonary effects.

7.2.1.2 Immunologic

Blood samples from almost 1000 semiconductor workers in Taiwan were assayed.

Significantly lower white blood cell counts were found in men in photolithography and in implantation jobs than male control workers. Photolithography workers were also found to have a significantly higher prevalence of leukopenia (30%) than control workers (6%) (Luo *et al.*, 2002).

7.2.1.3 Reproductive

In 1988, Pastides and his coworkers (Pastides *et al.*, 1988) reported an increase in spontaneous abortions (SAB) among semiconductor workers in the United States. An additional three cohorts with larger numbers of subjects later reported an increased risk of SABs among semiconductor workers (Beaumont, *et al.*, 1995; Correa, *et al.*, 1996; Pinney and Lemasters, 1996). Two small case-control studies (Elliott *et al.*, 1999; Shusterman, *et al.*, 1993) failed to find an elevated risk of SABs. This body of work has been previously synthesized (Fowler and Sexton, 2002). Because a cohort study design is stronger than a case-control study design in establishing a true association between exposure and outcome and because the results from the cohort studies are consistent, Fowler and Sexton concluded that overall the results suggest an elevated risk of SABs in female semiconductor workers. There have been no studies of other types of reproductive outcomes, such as shorter gestation, lower birth weight, congenital malformations, and perinatal deaths. A few investigators have reported on fertility status related to men working in semiconductor jobs (Correa *et al.*, 1996; Pastides *et al.*, 1988; Samuels *et al.*, 1995; Schenker *et al.*, 1995). Although some findings have reported lower indices of fertility (e.g., longer time before conception, lower fertility rates) among the female partners of male semiconductor workers, these studies have been plagued with small numbers and inconsistent findings. Thus, not enough information is available on male fertility for a reasonable assessment of risk to be made.

7.2.2 Therapeutic

7.2.2.1 Low-level laser therapy (LLLT)

LLLT is used investigationally in the United States but is used clinically in other areas, such as Canada, Europe, and Asia, for treatment of a wide variety of disorders, ranging from chronic pain in different parts of the body to almost every kind of refractory wound (Bulow *et al.*, 1994, Gur *et al.*, 2002; 2003; 2004; Ilbuldu *et al.*, 2004; Pipitone and Scott, 2001; Posten *et al.* 2005; Simunovic *et al.*, 2000; Tascioglu *et al.*, 2004; Trock *et al.*, 1994). Generally, the lasers have a gallium, arsenide, and aluminum base and are predominantly used as adjuvant therapies to deliver light deep into the body tissues to promote wound healing and cellular growth (Whelan *et al.*, 2001). Thus, the patient does not come in direct contact with the gallium itself, and toxicity has not been a concern. Many, but not all, investigators have reported positive results (Kubota, 2004), leaving the efficacy of the laser treatments uncertain for specific disorders and for defined therapeutic parameters. In an attempt to overcome the small number of study subjects and inconsistencies in findings, as well as differences in the quality of the studies, pooled analyses of the randomized clinical studies have been conducted. For the treatment of both osteoarthritis (Akai and Hayashi, 2002; Brosseau *et al.*, 2004) and wound healing (Lucas *et al.*, 2003), a meta-analysis was conducted. The conclusion from the meta-analysis for each of these conditions was that the data suggested an independent positive contribution, but efficacy could not be definitively established (Cullum *et al.*, 2001; Flemming and Cullum, 2001). For other conditions the data need to be strengthened before making even a tentative conclusion regarding efficacy.

7.2.2.2 Hypercalcemia

Results of studies that used gallium nitrate for a variety of reasons indicated that its properties might be useful for the treatment of hypercalcemia, a life-threatening metabolic disorder of cancer patients (Hughes and Hughes, 1992; Warrell, 1997). In a randomized, double-blind trial, for example, that compared etidronate and gallium nitrate (administered as a 200 mg/m^2/day continuous intravenous infusion for 5 days), it was found that 82% of those receiving gallium nitrate achieved normocalcemia compared with 43% receiving etidronate (Warrell *et al.*, 1991). Frequency of renal toxicity has been reported as 10% and nausea and vomiting as 14% (Zojer *et al.*, 1999). Although the mechanism is not fully

understood, gallium nitrate seems to stimulate bone formation and to inhibit osteoclast-mediated bone resorption (Chitamabar, 2003; Leyland-Jones, 2004).

7.2.2.3 Antineoplastic

Gallium nitrate binds to the iron transport protein transferrin. The inhibition of tumor growth by gallium seems to relate, at least in part, to its close resemblance to ferric iron and its ability to interfere with the cells' handling of iron both in acquiring it from blood and its intracellular availability (Jakupec and Keppler, 2004a,b). Studies of intravenous infusion of gallium nitrate, often combined with other drugs, to treat many different kinds of tumors have reported mixed results. Studies of non-small cell lung cancer using a short infusion of gallium nitrate have resulted in little efficacy (Chang *et al*., 1995; Webster *et al*., 2000). Reported adverse reactions were renal impairment and optic neuritis. Senderowicz *et al*. (1999) reported the early termination of a trial to test the efficacy of gallium nitrate (200 mg/m^2/day for a week administered every 21 days) with individuals who had prostate cancer, because the regimen was poorly tolerated. Adverse drug reactions included anemia, blindness, with partial reversal, pulmonary infiltrates, hypoxemia, and fever. Treatment with a mixture of gallium nitrate, vinblastine, and ifosfamide (VIG) for cancer of the cervix and ovary, which had been unresponsive to other regimens, has shown either minimal or no promising effect (Dreicer *et al*., 1998; Malfetano *et al*., 1995). VIG was used in a Phase II trial of 45 patients with locally advanced or metastatic carcinoma of the urothelium. Dreicer *et al*. (1997) reported therapeutic response in 44% but also reported two deaths that were possibly related to treatment, leukopenia in 62%, anemia in 69%, and cardiac events in 13%. Gallium nitrate has been found to benefit patients with lymphoma, possibly because the expression of transferrin receptors is particularly high on lymphoma cells (Chitambar, 2004a; Warrell, Jr. *et al*., 1983). Significant response rates have been observed in patients with diffuse large cell lymphoma, small lymphocytic lymphoma, and follicular lymphoma (Straus, 2003). Gallium nitrate is used in refractory bladder cancer that has been previously treated with multiple drugs (e.g., methotrexate, vinblastine, doxorubicin, cisplatin). Gallium nitrate either used as a single agent or in combination regimens has significant therapeutic activity (Chitambar, 2004b; Einhorn, 2003; Fagbemi and Stadler, 1998). It has been suggested that some of the limitations experienced with gallium nitrate as an antineoplastic drug may result from the use of a long exposure to steady-state levels in blood and may be overcome by the use of oral gallium compounds (Jakupec and Keppler, 2004a).

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CHAPTER

28

Germanium

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ABSTRACT

Animal experimental data show that germanium compounds, both inorganic and organic, are rapidly and almost completely absorbed from the lungs and the gastrointestinal tract. The distribution among the organs and tissues is fairly uniform, and there is no evidence of preferential uptake or accumulation. Absorbed germanium is rapidly excreted, mainly in urine. Data on biological half-times are inadequate, but for the rat the whole-body retention half-time has been estimated at approximately 1.5 days.

Germanium tetrachloride is a strong irritant of the respiratory system, skin, and the eye, possibly because it is easily hydrolyzed producing hydrogen chloride; in mice, high-level inhalation exposures caused necrosis of the tracheal mucosa, bronchitis, and interstitial pneumonia. Systemic toxicity of germanium compounds is comparatively low. The specific target or critical organs cannot be identified, but nephropathy, neuropathy, and hepatotoxicity are usually observed. Trialkylgermanium compounds are less toxic than the corresponding lead or tin alkyls. Germanium compounds do not seem to be carcinogenic. Dimethylgermanium oxide is teratogenic in chickens, but sodium germinate has not produced malformations in hamsters.

There is little information on the toxicity of inorganic germanium compounds to man, except that germanium tetrachloride may produce skin irritation. In clinical trials, spirogermanium, an organogermanium antitumor agent, has been shown to be neurotoxic. Recently, germanium was reported as having anticancer effects. A patient with 175 ppm of germanium hair level showed a variation of the fiber sizes of the skeletal muscles with dense cytoplasmic bodies, reduction of cytochrome *c* oxidase activity, mitochondrial myopathies, and vacuolar degeneration; similar findings were observed in rats treated with germanium oxide.

High doses of germanium compounds (taken as supplements) induced remarkable lactic acidosis, hydropic degeneration of the proximal convoluted tubules with presence of inclusion bodies, and some cellular necrosis and subsequent renal failure; however, the renal glomeruli and the renal interstitial tissue seemed normal. Neurological effects involved negative deep tendon reflexes in the lower extremities and persistent tingling sensation of the palms and soles. In addition to severe cardiac dilation, vacuolar degeneration of myocardial cells and interstitial edema were observed.

Inhalation is the main route of exposure under occupational conditions; the main source of germanium for the general population is food.

Short reviews of the toxicology of germanium and its compounds have been published by Mogilevskaja (1973), Underwood (1977), and Aldridge (1978). Recent reviews on the adverse health effects were conducted by Ohri *et al*. (1993) and Tao and Bolger (1997).

1 PHYSICAL AND CHEMICAL PROPERTIES

Germanium (Ge): atomic weight, 72.59; atomic number, 32; density, 5.32 (25°C); melting point, 937.4 °C; boiling point, 2830°C; in elemental form it is a grey-white, brittle solid; oxidation states +2 and +4. It belongs to

group IVa of the periodic table together with carbon, silicon, tin, and lead. Germanium is a metalloid that exhibits both metallic and nonmetallic properties. Important properties that may be important in germanium chemistry and toxicology are the first ionization energy (kJ mol^{-1}), electronegativity, and covalent radius (pm) that are, respectively, for carbon 1086, 2.5, and 77 and germanium 760, 1.8, and 122. Bond energies of carbon and germanium for the halogen series do not vary greatly. Like other group IVA elements exhibit some p-bonding, form tetrahedral compounds, and form some chiral compounds (Cotton and Wilkinson, 1988). Ge is an n- or p-type semiconductor, depending on the impurities present.

2 METHODS AND PROBLEMS OF ANALYSIS

Inductively coupled mass spectrometry (ICP-MS), atomic absorption spectroscopy (AAS), emission spectrography, and spectrophotometry with phenylfluorone are widely used for the analysis of germanium in environmental and biological samples. The limit of detection of AAS is of the order of 1.5 mg/L and can be reduced approximately 100 times by using a graphite tube atomizer (Amos and Willis, 1966; Johnson *et al*., 1973). To reduce losses because of volatility, biological samples should be ashed at low temperature. Interference of other elements can be eliminated by carbon tetrachloride extraction. Samples are treated in the same way for spectrophotometry with phenylfluorone, for which the limit of detection is approximately 0.1–0.5 mg/L (Luke and Campbell, 1956). The achievable precision (relative standard deviation) of the spectrophotometric method is 5–10% (Schroeder and Balassa, 1967a). There is not enough information presented in these early reports to evaluate the accuracy of the analytical methods used. Atomic emission spectrometry of germanium in environmental samples was described by Braman and Tompkins (1978); germanium compounds are converted in aqueous solution to volatile hydrides by reduction with sodium borohydride. By use of atomic emission spectrometry, the detection limit was estimated at approximately 0.004–0.014 mg/L and the precision at approximately 3%. The detection limit for spark source mass spectrometry (SSMS) (human tissue samples) is 0.007 mg/kg wet weight (Hamilton *et al*., 1972; 1973). Proton activation can also be used for the analysis of germanium in biological samples and has a detection limit of approximately 0.7 mg/kg (Bonardi *et al*., 1982). Diffusion phoresis has been proposed for the analysis of gas mixtures containing germanium compounds (Minjajlo *et al*., 1978). Swennen *et al*. (2000) have described an air-sampling method that uses a CIP-10 dust sampler that maintains 10 mL of air/minute flow through high-speed rotary polyurethane foam filters on which small Ge particles (inhalable or respirable) collected separately. Collectable dust on the filter is then measured using AAS with a flame (N_2O; C_2H_2) or modified by procedures using AA-10 or FIAS-400. The measurement reliability for this technique was reported as being down to 10 ng Ge/m.

Inductively coupled plasma mass spectrometry (ICP-MS) is the preferred technique for measurement of multiple elements in the same sample, including Ge. Different applications have been developed for controlling various parameters, including speciation, molecular structure, and total concentration (Krystek and Ritsema, 2004). Urinary germanium concentrations can be used as a biomarker of inhalation exposure to airborne dust from metallic germanium or germanium dioxide in an occupational setting. A novel approach for analyzing such samples involving a hydride generation-based method coupled with flow-injection graphite furnace atomic absorption spectrometry (HG/FI-GFAAS) has been described by (Roels and Buchet, 2001). The limit of detection (LOD) for this method was 0.25 µg/L germanium. The determination of germanium in human samples with graphite furnace atomic absorption spectrometry (GFAAS) and microwave-induced plasma mass spectrometry (MIP-MS) was described by Shinohara *et al*. (1999). The limits of detection were 3 ng/mL with GFAAS and 0.05 ng/mL with MIP-MS. The sensitivity of GFAAS was found to be lower than that of MIP-MS (Shinohara *et al*., 1999). A similar analytical method was described by Swennen *et al*. (2000) to measure Ge urine concentrations in workers after occupational exposure. Ge levels in urine were measured by flow injection graphite furnace atomic adsorption spectroscopy (FI-GFAAS). This method consisted of five consecutive steps, mineralization of urine, pH conditioning, generation of gaseous GeH_4 by flow injection, GeH_4 trapping in an iridium-coated graphite furnace, and ultimate electrothermal atomization of Ge. The reported limit of detection was 0.25 µg Ge/L of urine.

Krystek and Ritsema (2004) reported a combined HPLC-ICPMS analytical system for speciating both inorganic and organic Ge compounds in commercially available medical compounds used in clinical treatments for cancer and AIDS. These analytical methods were confirmed by NMR. The total germanium concentration was found to be 4.6 mg/g of sample by HR-ICP-MS and 5.7 mg/mL by NMR.

3 PRODUCTION AND USES

3.1 Production

Some zinc and lead-zinc-copper sulfide ores are readily available sources of germanium, but there is no widely occurring germanium ore. The concentration of germanium in the earth's crust ranges between 1.5 and 7 ppm (Scansetti, 1992). The total estimated world production of germanium was approximately 114,000 kg in 1982, but this does not include production in Zaire, which has one of the largest resources but for which no production data are available. In recent years, the world production was estimated to be 44,000 kg in 2003 and 50,000 kg in 2005 (USGS, 2005). Approximately 60% of germanium metal used each year is recovered from the recycling of electronic and optical devices (USGS, 2005). Germanium could also be recovered from waste products of coal and coke technology such as ash and flue dusts (U .S. Bureau of Mines, 1983).

3.2 Uses

The largest end use of germanium is now in the production of infrared sensing and identification systems. The use in fiber optical systems has also increased, whereas the consumption for semiconductors has continued to decline because of advances in silicon semiconductor technology. The estimates of the end uses for germanium worldwide are as follows: polymerization catalysts, 35%; infrared optics, 25%; fiberoptic systems, 20%; electronics/solar electrical applications, 12%; and other (phosphors, metallurgy, and chemotherapy), 8%. For the United States, the estimates are somewhat different: fiberoptic systems, 40%; infrared optics, 30%; electronics/solar electrical applications, 20%; and other (phosphors, metallurgy, and chemotherapy), 10%. The primary difference is the absence of germanium as a polymerization catalyst in the United States (USGS, 2005). Other applications include phosphors, correction inhibition in alloys, glass of high refractive index, and spirogermanium for cancer therapy (Scansetti, 1992). Germanium tetrachloride is an intermediate in the preparation of germanium dioxide and organogermanium compounds. Several germanium compounds have potential uses in pharmacology, pharmacy, and cosmetics (Kenney, 1980; Ohara, 1977; Tomizawa *et al*., 1978), and cancer chemotherapy (Budman *et al*., 1981; Kakimoto and Miyao, 1979; Kumano *et al*., 1978; Schein *et al*., 1980). Of industrial and toxicological interest are elemental germanium, germanium dioxide, sodium germanate, germanium tetrahydride (germane), germanium tetrachloride, and some organogermanium compounds, such as alkylgermanes and hexaalkyldigermanium oxides. An example of an organogermanium compound of therapeutic interest is spirogermanium, an azaspiran with germanium substituted for one-carbon moiety in the ring structure (Jao *et al*., 1990; Schein *et al*., 1980). Germanium compounds have the potential to be used as antidotes for reducing the acute toxicity and increasing urinary excretions of selenium. For example, 40 mg Ge/kg subcutaneously has been reported to reduce the selenate-induced body weight loss, and pretreatment with germanium sesquioxide enhanced urinary excretion of ^{75}Se in a dose-related manner in rats (Paul *et al*., 1989).

Germanium detectors are used in various industrial and clinical settings. Lithium-drifted germanium semiconductor (GeLi) detectors for measuring radioactivity require cooling with liquid nitrogen and were introduced in the 1970s. These have been largely replaced with high-purity intrinsic germanium detectors that allow temperature cycling. These detectors are used to measure the radioactivity of biological and environmental media, as well as the quantity of radioactive material in humans. Although the detection efficiency is less than that for thallium-activated sodium iodide detectors of the same volume, the energy resolution is greatly superior, allowing a peak resolution of a few MeV. Germanium detectors are also used to image X-rays and measure the bone mineral content, particularly of vertebrae, after adjusting for lean and adipose tissue thickness (Jonson *et al*., 1986). A high-purity germanium detector has been used as a component monitoring system for routine assessments of ^{125}I uptake in the thyroid gland of radiation workers. By use of a whole-body counter with a protective shielding chamber, an activity of 2.2 Bq (MDA) was detectable. The system could measure an activity of 128 Bq with an uncertainty of ±5% (Kopp *et al*., 1994).

4 ENVIRONMENTAL LEVELS AND EXPOSURES

4.1 Food and Daily Intake

A 1994 total diet study in the United Kingdom found the germanium concentration to range from <2–4 μg Ge/kg fresh food weight, with the highest levels found in cereals (4 μg/kg) and bread (3 μg/kg) (FSA, 1997). A spectrophotometric method that used phenylfluorone showed the presence of germanium in all food items analyzed (Schroeder and Balassa, 1967a). However, only five items had more than 2 mg Ge/kg (raw clams, canned tuna, dried pan fish, canned baked beans, and tomato juice), and 15 items contained more than 1 mg Ge/kg. Selected hospital diets contained on

average 0.88 mg Ge/kg wet weight. The daily intake of germanium may vary from 0.4–3.5 mg (Schroeder and Balassa, 1967a). Hamilton and Minski (1972, 1973) estimated that the mean daily intake in the United Kingdom to be 0.367 ± 0.159 mg by SSMS. The estimated dietary intake for a "reference man" is approximately 1.5 mg Ge/day of which 96% or more is absorbed (Snyder *et al*., 1975). Estimation of the mean of human daily intake of Ge from food is 1.5 mg/day (with a range of 0.40–3.40) (Okada *et al*., 1991; Shauss, 1991).

4.1.1 Water, Soil, and Ambient Air

Braman and Tompkins (1978) used emission spectrometry to measure Ge concentrations in U.S. freshwater and saline samples. They reported a concentration range from less than 4– 600 ng/L (Oregon well waters) and in some saline waters, the concentration range was from less than 4 ng/L to approximately 200 ng/L. Reimann *et al*. (1978) analyzed 145 hard rock ground water and surface water samples from Norway and Finland and found the median content of germanium to range between 0.015 and 0.44 µg/L.

Samples of soils in the United States contained 0.6–1.3 mg Ge/kg (Schroeder and Balassa, 1967a), which is of the same order of magnitude as the abundance of germanium found in the earth's crust (1.5 mg/kg) (Fyfe, 1974).

Considerable amounts of Ge are discharged into the atmosphere by coal combustion. Paone (1970) estimated 2000 tons per year to be discharged in stack gases, flue dusts, and ashes from coal-burning plants in the United Kingdom. Coal ash may contain 20–280 mg Ge/kg (Coal Research Section, 1972). Concentrations of germanium in air particulates may range from less than 0.01 ng/m^3 to approximately 1700 ng/m^3 (Braman and Tompkins, 1978). Lifetime consumption of 5 ppm Ge in drinking water resulted in liver and kidney dysfunctions of the experimental animals.

4.1.2 Plants, Fishery Products, and Microbial Organisms

Germanium is a constituent of various medicinal plants and microorganisms (Park and Han, 1979; Slawson *et al*., 1992; Staufer, 1980). Ge has been reported in sargassum and its epiphytes at concentrations of up to 4.2 µg/kg (Johnson and Braman, 1975). Also, germanium has been found to accumulate in a variety of microorganisms. The accumulation rate of germanium depends on environmental pH, temperature, and silicic acid concentration within the cell. The growth inhibition by GeO_2 was more pronounced in yeast than bacteria (Lee *et al*., 1990). Traces of Ge usually exist in soil, plants, and animal tissues; however, high concentrations (2–9 ppm) of Ge may be found in beans, tomato juice, oysters, tuna, garlic, aloe vera, ginseng, shelf fungus, and green tea. Tissue levels higher than 5 ppm are toxic to most plants.

4.2 Working Environment

Exposure to Ge tetrachloride and its hydrolysis products (GeO_2 and HCI) may occur in the production process of germanium and its compounds. Dust concentrations ranging from 5–70 mg/m^3 (corresponding to germanium concentrations up to approximately 7 mg/m^3) have been reported to occur in the production of germanium monocrystals. In some cases, the samples contained up to 30% of free silica (Goldman, 1960; Mogilevskaja, 1973). Swennen and coworkers observed, for exposed and control workers, no significant differences in the germanium urine levels taken at the beginning and the end of the workweek. These authors also reported no overt clinical health effects for the two groups of workers; however, slightly higher than control levels of albumin and transferrin and some low-molecular-weight proteins were reported in exposed workers as discussed later. The geometric mean of the cumulative frequency of germanium levels in the air during 5 working days ranged from 1.07–5.11 µg/m^3 (Swennen *et al*., 2000).

5 TOXICOKINETICS

5.1 Absorption

5.1.1 Inhalation

The rate of clearance of deposited elemental germanium particles (mean size, 1.4 µm) from the lungs of rats was found to be exponential (52% in 24 hours, 18% remaining 7 days after exposure). Radiochemical examination of the tissues showed that part of the material entered the circulation and reappeared in the kidney and liver 1 hour after exposure. The clearance of germanium dioxide particles (mean size, 0.4 µm) was more rapid than that of elemental Ge particles (79% within 24 hours) (Dudley, 1953).

5.1.2 Ingestion

Neutralized Ge dioxide was rapidly absorbed in rats after gavage (73.4% in 4 hours; 964% in 8 hours) (Rosenfeld, 1954). Equally rapid absorption of tetraethylgermanium from the gastrointestinal tract of mice was found by Caujolle *et al*. (1963). Schroeder and Balassa (1967a) reported that the concentrations of germanium in human urine were approximately the same

as the concentrations in the diet and concluded that dietary germanium was well absorbed. Ge was almost absorbed completely after oral exposure and excreted from human body within 3 days (Ohri *et al.*, 1993).

5.2 Distribution

In rats, absorbed Ge was found to be fairly uniformly distributed between erythrocytes and plasma (2:3) and not bound to plasma proteins (Rosenfeld, 1954). In exposed rabbits and dogs (intravenous injection of GeO_2), the highest concentrations were identified in the kidney, liver, spleen, gastrointestinal tract, and bone (Dudley and Wallace, 1952). Schroeder and Balassa (1967b) and Schroeder *et al*. (1968) kept mice and rats for life on normal laboratory diet (0.33 mg Ge/kg) and drinking water containing 5 mg Ge/L as sodium germinate. Germanium was widely distributed in the body, and there was no selective retention or storage. Essentially the same distribution was found in mice after ingestion of tetraethyl germanium (Caujolle *et al*., 1963). In a case study of a patient who took 600 mg/day of a germanium-containing elixir and subsequently died of kidney failure, germanium was found to be present at elevated levels in almost every organ compared with a nonconsumer who died of liver cirrhosis. The respective organ concentrations in µg/gm wet tissue for Ge patient/nonconsumer were spleen, 19.804/(0.108); vertebra, 16.926/(8.820); renal cortex, 3.184/(0.046); brain, 3.108/(0.189); and skeletal muscle, 2.758/(0.030) (Nagata *et al*., 1985). In addition, Morita *et al*. (1986) and Asaka *et al*., (1995) reported high accumulation of germanium in hair (56.4–173.7 µg/g), fingernails (5.4–35 µg/g), and toenails 14–15.8 µg/g) in three individuals who consumed a germanium-containing nutritional supplement for a year to a year and a half.

5.3 Excretion

Absorbed germanium is preferentially excreted in urine both in man (Schroeder and Balassa, 1967a) and in animals. In rats, 64.8% of an intraperitoneal dose of sodium germanate (100 mg/kg) was excreted in urine and 4.7% in feces within 1 day, the total excretion in 5 days amounting to 78.8% and 13%, respectively (Rosenfeld, 1954). Excretion of germanium by rabbits in urine after an intravenous injection of germanium dioxide (13.5 mg in terms of Ge) amounted within 72 hours to 60–77% of the dose, and 6–12% was excreted in feces (Dudley and Wallace, 1952). In dogs, 90% of an injected germanium dioxide dose (3.4 mg/kg in terms of Ge) was excreted in urine within 72 hours (Dudley and Wallace, 1952). Roels and Buchet (2001) collected urine samples at the beginning and the end of the day shift from workers occupationally exposed to Ge or GeO_2 by inhalation. Urinary elimination kinetics was studied in seven workers, and the urinary elimination rates (half-times) ranged from 8.2–18.1 hours. Therefore, germanium urinary excretion may be used for biomonitoring of occupational Ge exposures.

5.4 Biological Half-Time

By use of a simple exponential model and Rosenfeld's data (1954) for rats, the biological half-times can be roughly estimated at 1.5 days for the whole-body retention, 2 days for the liver, and 4.5 days for the kidney. The biological half-lives in mouse tissues were reported as 6.3 hours in brain, 1.2 hours in blood, and approximately 4.5 hours in pancreas (Shinogi *et al*., 1990). Lin *et al*. (1999) reported the kinetics of Ge in male Wistar rats after the administration of a single 100 mg/kg orally and found that maximal mean serum concentration was achieved in 0.7 hour and the mean elimination half-time was 2.3 hours. They also conducted a multiple dose for 4 weeks and found highest concentrations of Ge in the peripheral nerves and kidneys.

6 LEVELS IN TISSUES AND BIOLOGICAL FLUIDS—BIOLOGICAL MONITORING

Hamilton *et al*. (1972; 1973) published the following values of germanium content in some "normal" human tissues in the United Kingdom (mean concentration in mg/kg wet weight using SSMS): lymph node, 0.0009; muscle, 0.003; liver, 0.04; lung, 0.09; brain, 0.1; blood, 0.2; testes, 0.5; and kidney, 9.0. The concentrations of germanium in human lung tissue (SSMS, 247 samples), as reported by Brown and Taylor (1975), ranged from 0.08–12 mg/kg dry weight. Schroeder and Balassa (1967a) found 0.29 mg/L in serum, 0.65 mg/L in erythrocytes, and 1.26 mg/L in urine (spectrophotometry with phenylfluorone). In one patient who ingested a total dose of 30 g of Ge oxide in capsules over a period of 8 months, Ge concentrations in µg/gm of wet tissue at autopsy were 2.51 in renal cortex, 17.70 in renal medulla, 4.73 in myocardium, 20 in spleen, 1.99 in cerebrum, and 8.67 in lymph nodes (Matsusaka *et al*., 1988). Swennen *et al*. (2000) reported that urinary Ge concentrations could be used for biological monitoring of occupational Ge exposure. They reported urinary concentrations of <0.25 µg Ge/g creatinine in control workers. In exposed workers, they reported geometric mean urinary concentration ranges from 1.36 (Monday) to 3.6 (Friday) µg Ge/g creatinine before the working

shift to 4.22 (Monday) to 4.34 (Friday) µg Ge/g creatinine after the working shift. Ranges of individual values before the shift were 0.23–70 µg/g creatinine and after the shift 0.16–195 µg/g creatinine.

7 EFFECTS AND DOSE-RESPONSE RELATIONSHIPS

7.1 Inorganic Compounds

7.1.1 Local Effects and Dose-Response Relationships

7.1.1.1 Animals

Rats showed thickening of alveolar walls and hyperplasia of pulmonary lymphatic vessels 7 months after an intratracheal administration of 30, 50, and 70 mg of germanium dioxide (Mogilevskaja, 1973). Changes in the respiratory system after single (1.4–20 g/m^3, 2 hours) and repeated (0.5–0.7 g/m^3, 2 hours/day, 5 days/week, 8 weeks) inhalation exposure of mice to germanium tetrachloride were dose-related and ranged from irritation of the respiratory system to necrosis of the tracheal epithelium, catarrhal desquamative bronchitis, and interstitial pneumonia (Kal'sada, 1964). No changes in the respiratory system were reported after long-term inhalation exposure of rats (3, 4, 5, and 7 mg/m^3, calculated as HCl, duration 4 hours/day, 5 days/week, for 7 months) to germanium tetrachloride (Kurljandskij *et al*, 1968). No local respiratory effects were reported after inhalation of germanium tetrahydride either in acute or chronic experiments (Gus'kova, 1974). On the other hand, oral treatment with the compound carboxyethylgermanium sesquioxide (Germanium-132 or Ge-132) showed a remarkable inhibition of viral growth in the mouse lung. It provided a significant protective effect against a dose of 10 times the LD_{50} of an influenza virus aerosol challenge to the mouse. It increased survival rate, prolonged survival time, and prevented the development of lung consolidation, and 20 mg/kg/day for 6 days was found to be the most effective dose protocol (Aso *et al*., 1989).

Neutralized Ge dioxide (topical application, twice a day for 2 weeks) induced no irritation of the shaved skin of the rat (Rosenfeld, 1954). However, ulceration and necrosis of the skin were observed in rabbits after 2 hours of exposure to germanium tetrachloride. The effects observed in guinea pigs and rats were much milder (Kal'sada, 1964; Kurljandskij *et al*., 1968). Germanium tetrachloride had a pronounced irritating action on the conjunctivae of the eye in rats, rabbits, and mice (Kal'sada, 1964; Kurljandskij *et al*., 1968). Local effects of germanium tetrachloride are very likely caused by a combined action of the compound and its hydrolysis products, such as GeO_2, HCl, and Ge_2C1_6 (Kurljandskij *et al*., 1968).

7.1.1.2 Humans

Mild irritation of the skin is produced by germanium tetrachloride (Kal'sada, 1964).

7.1.2 Systemic Effects and Dose-Response Relationships

7.1.2.1 Animals

Inorganic germanium compounds seem to have a comparatively low systemic toxicity. The LD_{50} values of germanium dioxide (GeO_2) for mice ranged from 2025 mg/kg (females, intraperitoneally) to 6300 mg/kg (males, orally.). The corresponding values for rats were 1620 mg/kg and 3700 mg/kg. Toxic signs included reduced spontaneous activity, sedation, hypothermia, vasodilation, ptosis, cyanosis, and tremor. Death was caused by respiratory paralysis (Hatano *et al*., 1981a). Eight weekly intraperitoneal doses of neutralized GeO_2 did not inhibit the growth of rats, but the ingestion of the same compound in food (1000 mg/kg) or drinking water (l00 mg/L) inhibited the growth of young rats and caused 50% mortality in 4 weeks After this period, the survivors seemed to recover even though they continued to ingest approximately the same dose for another 8 weeks (Rosenfeld and Wallace, 1953). GeO_2 in doses >150 mg/kg/day, subcutaneously, for >30 days inhibited body weight gain and motor activity and reduced erythrocytes and hemoglobin concentrations in blood and uric acid in urine. There were heart hypertrophy, intestinal hemorrhage, and lung congestion in some rats. Intraperitoneal administration of Ge-132 to rats under urethane anesthesia induced a dose-related dropoff in the means of arterial blood pressure and heart rate. The effects of Ge-132 on blood pressure and heart rate were induced probably through stimulation of the parasympathetic efferent and inhibition of sympathetic efferent central nervous system (Ho *et al*., 1990). No specific pathological changes were observed in a 90-day study with rats (subcutaneously, 3–50 mg/kg/day) (Hatano *et al*., 1981a).

7.1.2.2 Liver

Degenerative changes and necrosis, sometimes associated with functional impairment (for example, reduced detoxifying capacity–hippuric acid test), were observed in acute, subacute, and chronic studies by Kal'sada (1964) (mice, GeC14 inhalation, 1.7–40 g/m^3, single 2-hour exposures; and 0.5–0.7 g/m^3, 2 hours/day, 40 days), by Kurljandskij *et al*. (1968) (rats, $GeC1_4$ inhalation, 0.30–0.7 g/m^3, 4 hours/day, 5 days/week, for 7 months), and by Gus'kova (1974) (rats and guinea

pigs; GeH_4 inhalation, 0.26–1.4 g/m^3, single exposures of 2 hours for mice and 4 hours for rats and other animals). Increased incidence, compared with controls, of fatty degeneration of the liver was found in rats after a lifetime exposure to 5 mg Ge/L as sodium germanate in drinking water (Schroeder *et al*., 1968), but there was no effect in a similar experiment with mice (Schroeder and Balassa, 1967b).

7.1.2.3 Kidney

Degenerative changes in the epithelium of the proximal tubules were noted by Kal'sada (1964), Kurljandskij *et al*. (1968), and Gus'kova (1974) after high-level inhalation exposure to germanium tetrachloride and germanium hydride, respectively (for exposure conditions and animal species used, see Section 7.1.1) The same morphological changes, accompanied by proteinuria, had higher incidence in rats exposed to sodium germanate than in the controls (Schroeder *et al*., 1968). Rats treated with germanium dioxide for 24 weeks showed weight loss, decreased creatinine clearance, increased BUN, and increased serum phosphate. Morphological examination revealed vacuolar degeneration of the kidneys' distal convoluted tubules, electron-dense inclusions in the swollen mitochondria, and fibrous connective tissue 16 weeks after cessation of the treatment. High germanium dioxide deposition was observed in the distal tubular epithelium at the end of the treatment (24 weeks) and 16 weeks later (40 weeks) (Sanai *et al*., 1991).

7.1.2.4 Hematological Effects

Bailey *et al*. (1925) treated rabbits with germanium dioxide through repeated intraperitoneal injections for 7 weeks with total doses of 28–280 mg/kg, and Rochow and Sindler (1950) treated rabbits and hamsters, dimethylgermanium oxide, single and repeated subcutaneously doses up to 1 g Ge/kg and reported no hematological effects. Similarly, multiple intraperitoneal doses of sodium germanate (100 mg/kg) and repeated oral doses of neutralized GeO_2 (0.9–170 mg/kg body weight) also showed no hematological effects in rats (Rosenfeld and Wallace, 1953). Some changes in the blood of uncertain biological significance, such as increased hemoglobin and increased numbers of erythrocytes and leukocytes, were observed by Kurljandskij *et al*. (1968) (rats, $GeC1_4$ inhalation, 3–7 mg/m^3, 4 hours/day, 5 days/week for 7 months) and Gus'kova (1974) (rats and guinea pigs, GeH_4 inhalation, 50–250 mg/m^3, 4 months).

7.1.2.5 Nervous System

Lethal exposures of rats to germanium dioxide (600–1200 mg/kg, single intraperitoneal dose; Rosenfeld and Wallace, 1953) and of mice to germanium tetrachloride (20–40 g/m^3, inhalation 2 hours; Kal'sada, 1964) and germanium hydride (2 g/m^3 inhalation, 2 hours for mice and 4 hours for rats and other animals; Gus'kova, 1974) were accompanied by various neurobehavioral signs (excitation, impairment of locomotor activity, listlessness, hypothermia, and convulsions). Histochemical changes in the brain observed in rats by Kurljandskij *et al*. (1968) after chronic inhalation exposure to germanium tetrachloride (3–7 mg/m^3, 4 hours/day, 5 days/week for 7 months) are difficult to interpret. Wistar rats were maintained on a diet containing 0.15% GeO_2 (average, 100 mg/kg/day) for 8 months, resulting in segmental demyelination/remyelination, neurocytic edema, disintegration of the cytoplasm, increased Schwann cell volume, and deposition of electron-dense material within their mitochondria. These lesions suggest that the Schwann cells are the primary target of Ge toxicity (Matsumuro *et al*., 1993).

Wu *et al*. (1992) and Yim *et al*. (1999) reported ultrastructural/biochemical and histochemical studies on muscles of rats treated with Ge for up to 24 weeks and found a number of alterations in mitochondrial enzyme functions, suggesting Ge mitochondrial toxicity as a model for understanding human mitochondrial myoencephalopathy.

Arts *et al*. (1994) studied the acute and subacute toxicity of germanium oxide in Wistar rats of each sex after inhalation exposure to either amorphous or hexagonal germanium oxide over a dose range from 0–309 mg/m^3 for 6 hours/day/5 days per week for 4 weeks. Additional groups (2) of rats of each sex were kept for 33 days after cessation of exposure to either 0 or the 309 mg/m^3 dose levels to assess recovery. At the end of the study, a number of major organs showed increased weights and histopathological changes. These were accompanied by alterations in serum clinical chemistries. These toxic alterations were found to persist in the treatment groups after the 33-day recovery period.

Lee *et al*. (2004) performed toxicity studies on Geranti Bio Ge® and organic germanium fortified yeasts at single (2000 mg/kg) and repeated (0, 500, 100, 2000 mg/kg) daily dosed for 13 weeks in beagle dogs. They observed some alterations in blood chemistries, hematologies, and heart R-R interval and QT corrections in male, but not female, dogs. Only mild histological alterations were observed in major organ systems.

Chiu *et al*. (2002) reported that *in vitro* exposure of CHO cells to germanium oxide inhibited the G2 to M phase. These changes occurred at Ge concentrations up to 5 mmol/L after 12 hours of exposure but decreased at concentrations >5 mmol/L. Examination of cells labeled with 5-bromodeoxyurindine (BrDU)

showed a dose-related delay in the progression from S phase. The authors examined cyclin content and cyclin-dependent kinase activity and found that cyblin B1 levels were not altered but that Cdk1 activity was decreased that was restored after 9 hours in control media resulting in resumption of the G2-M phase transition.

Ozeki *et al*. (2006) studied the toxicity of germanium apatite in gingival fibroblasts and reported that the cellular growth rate was near that of controls at a concentration of 0.1 mg/mL, but growth rate was attenuated at 1.0 mg/mL. The observed growth rate was increased at 1.0 mg/mL when the pH of the cell growth medium was adjusted to 7.60.

7.1.3 Humans

Humans have been shown to experience adverse health effects associated with high exposures to germanium compounds.

7.1.3.1 Occupational Exposures

Swennen *et al*. (2000) reported some clinical renal differences between occupationally exposed germanium workers and control workers. These included slightly higher than control levels of low molecular proteins (microglobulin, retinol binding protein, and kallikrein) in urine samples of the Ge-exposed workers relative to controls. In addition, the means of concentrations of albumin and transferrin were also significantly increased in exposed workers compared with controls. In the exposed group, the prevalence of increased albumin (>17.6 mg/g creatinine) and transferrin (>832 µg/g creatinine) were also significantly higher than in the control group (12.0 vs 1.3%; $P = 0.006$ and 20 vs 8.9%; $P = 0.04$, respectively). The authors concluded that the increase in urinary levels of albumin and transferrin probably reflected glomerular changes in the kidneys.

7.1.3.2 Germanium Food Supplements

7.1.3.2.1 Kidney A case report indicated that a 53-year-old man who took a total of 400 g of germanium oxide as a health supplement over 14 months resulting in weight loss (16 kg in 3 months), azotemia (BUN 60 mg/dL, creatinine 2.6 mg/dL), and asthenia (primarily of lower extremities); 2 months later his condition degraded further. He was admitted to the hospital with chronic renal failure. Histological examination of the kidneys revealed no antibody deposits, lipofuscin accumulation, vacuolar degeneration, but some cell death (Kim *et al*., 1998). In Europe, germanium has been marketed as an ingredient in nonprescription drugs and recommended by distributors for AIDS and cancer treatment. However, the use of Ge for these purposes is not approved by regulatory agencies in most European countries. In Japan, it is used as an elixir to maintain or restore health. Asaka *et al*. (1995) reported a case of sensory ataxia in a patient who took an inorganic Ge compound at a dose of 36 mg/day for approximately 6 years. Gait disturbances, irreversible peripheral neuropathy, and renal damage were observed. At autopsy, the patient presented with degenerative changes of the dorsal root ganglia and dorsal column of the spinal cord. The neurodegenerative changes seemed to be more directly related to Ge toxicity rather than secondary to uremia, because the renal changes were more modest in nature.

A patient with 175 ppm of germanium hair level showed a variation of the fiber sizes of the skeletal muscles with dense cytoplasmic bodies, reduction of cytochrome *c* oxidase activity, mitochondrial myopathies, and vacuolar degeneration. Similar findings were observed in rats treated with germanium oxide (Higuchi *et al*., 1989).

Takeuchi *et al*. (1992) reported a case of a 55-year-old woman who ingested a total dose of 49g of Ge over a prior 19-month period. She presented with increased BUN and serum creatinine values and evidence of distal tubular damage without glomerular and vascular changes at autopsy. These data suggest that the distal tubular epithelium is also a target cell population for Ge toxicity.

After 2 months of ingestion of a total dose of 25 g of elemental germanium by a 43-year-old white woman, severe lactic acidosis, renal failure, and hepatotoxicity developed. The autopsy findings were hydropic degeneration and the presence of inclusion bodies in the proximal convoluted tubules; however, the renal glomeruli and the renal interstitial tissue seemed normal and unaffected. Extensive steatosis of the liver was observed in addition to a high level of germanium in urine, blood, kidneys, liver, muscles, and pancreas (Krapf *et al*., 1992). One case report addressed a 58-year-old man who consumed 426 g of germanium-lactate-citrate (Ge content 18 weight %) over 6 months as a natural remedy. He lost 10 kg of weight ending at 64 kg and suffered from nausea, yellowish skin, and uremia. Blood chemistry indicated increased sedimentation rate, normal leukocyte count, reduced erythrocyte and thrombocyte counts, and electrolyte levels that were close to the lowest part of the normal range (Luck *et al*., 1999). This patient survived after long medical intervention. Similar kidney lesions and renal failure were described on autopsy of a patient who died after intake of 600 mg of GeO_2 daily for 18 months as a remedy (Nagata *et al*., 1985; Obara *et al*., 1991; Okada *et al*., 1988; Omata *et al*., 1986; Sanai *et al*., 1990a,b; Schauss,

1991a,b). Schauss (1991) and Tao and Bolger (1997) summarized the literature for human Ge toxicity from dietary supplements and reported approximately 23 cases of germanium-related toxicity worldwide; 21 of these cases involved Japanese users. The duration of intakes ranged from 4–24 months, with doses varying between 14 and 324 g of germanium compounds.

7.1.3.2.2 Neurological Neuropathy involving mostly the sensory nerves was demonstrated in a 53-year-old man who took 400 g of germanium oxide as a health supplement over 14 months (Kim *et al*., 1998). Observations included grade IV motor strength, negative deep tendon reflex in the lower extremities, and persistent tingling sensation of the palms and soles. A 63-year-old woman consumed 36 mg inorganic germanium/day for 6 years (total dose equivalent to 80 gm) and subsequently developed difficulty in writing, gait discoordination, and peripheral neuropathy. The neuronal damage was manifest as irreversible, and an autopsy showed degeneration of dorsal root ganglion cells and degeneration of the dorsal column of the spinal cord. The patient died from sepsis and pneumonia (Asaka *et al*., 1995).

7.1.3.2.3 Heart Effects Ingestion of 30 g of GeO_2 as a remedy in capsule form over 8 months resulted in remarkable cardiac dilation, vacuolar degeneration of myocardial cells, and interstitial edema (Matsusaka *et al*., 1988).

7.2 Organometallic Compounds

The toxicity of several trialkylgermanium compounds was studied by Cremer and Aldridge (1964). Triethylgermanium acetate was toxic to rats in intravenous doses of 50 mg/kg body weight, and orally at 250 mg/kg. Tri-*N*-butylgermanium acetate was tolerated orally in single doses up to 375 mg/kg. The toxicity of trialkylgermanium compounds was less than one tenth of the toxicity of triethyltin or triethyllead and did not seem to have a predominant effect on the CNS. Among the tetraalkylgermanium compounds evaluated by Caujolle *et al*. (1966), the least toxic were those with saturated normal symmetrical radicals. The halogenation of organic radicals increased the biological activity. Higher hexaalkyldigermanium oxides (butyl-, amyl-, hexyl-) were less toxic than the ethyl homologs (Bouissou *et al*., 1964). Spirogermanium was neurotoxic to human cancer patients after intravenous administration in doses from 32–160 mg/m^2. The reversible effects included lethargy, vertigo, ataxia, and grand mal seizures (at high doses). Neurotoxic effects were also observed in dogs (Schein *et al*., 1980). Another organogermanium antitumor agent, carboxyethyl Ge sesquioxide, was considered to restore the impaired immunoresponse in aged C3A/HeJ mice (Mizushima *et al*., 1980). This compound was found to induce hypotension and bradycardia in the rat by promoting the activation of the parasympathetic efferent and inhibition of the sympathetic efferent inputs (Ho *et al.*, 1990). In 1988, the FDA accepted the use of germanium sesquioxide as a food additive, but in 1995, they issued an alert to remove the food additive on the basis of its renal toxicity (FDA, 1995). In October 2003, the FDA refused an entry of 20 kg of bulk Ge sesquioxide in human dietary supplements, because germanium has caused nephrotoxicity (kidney injury) and death when used chronically by humans, even at recommended levels of use (FDA, 2004).

7.3 Carcinogenicity, Mutagenicity, and Teratogenicity

Studies by Kanisawa and Schroeder (1967) on mice and by Schroeder *et al*. (1968) on rats (lifetime exposure to 5 mg Ge/L in drinking water as sodium germanate) did not produce any evidence of carcinogenicity of Ge compounds.

There is no definite evidence of mutagenicity of Ge compounds, although Ge tetrachloride was positive in the rec-assay (Kanematsu and Kada, 1978). Spirogermanium inhibits DNA and RNA synthesis in HeLa cells and is cytotoxic *in vitro* (Schein *et al*., 1980).

Dimethylgermanium oxide, an analog of acetone, was shown to produce a variety of malformations in chick embryos at 72 and 96 hours of incubation, the ED_{50} being 1.8 and 2.5 mg/embryo, respectively, compared with 17.9 and 25.1 mg/embryo for acetone (Caujolle *et al*, 1965). Ferm and Carpenter (1970) did not find any teratogenic or fetotoxic effect in hamsters treated with sodium germanate (40–100 mg/kg, intravenous injection, 8-day gestation). Hatano *et al*. (1981b) did not observe any adverse effect of Ge dioxide (19, 38, and 75 mg/kg, subcutaneously, pregnant rats) on mothers, embryos, or newborns. Ge oxide in 0.05 or 0.1 mg/kg inhibits the dose-related increase in frequency of sperm with abnormal head morphology after treatment with cadmium chloride in mice. However, germanium oxide alone did not have any effect on the frequency of sperm-head abnormalities (Han *et al*., 1992).

8 TREATMENT TRIALS

Spirogermanium was very effective in suppression of experimental autoimmune encephalomyelitis induced by myelin preparation from guinea pig

spinal cord (GPSC). The rats suffered acute episodes of paralysis and weight loss 10 days after intraperitoneal injection of GPSC. However, treatment with three different doses (2.5, 3.5, 5 mg spirogermanium) twice a day intraperitoneally on day 7 through day 14 resulted in suppression of both clinical and histological signs in a dose-dependent manner (Sacks *et al*., 1987). Spirogermanium also exhibited antiarthritic and immunoregulatory activity in rats (DiMartino *et al*., 1986). Because of the induction of suppressor cells in the splenic cell population and the exhibited antiarthritic and immunoregulatory effects of spirogermanium, spirogermanium may have a therapeutic potential for autoimmune diseases (Badger *et al*., 1985). Spirogermanium was administered at a dose of 80 mg/m^2 three times a week for 28 days by intravenous infusion as a treatment for advanced renal cell cancer, and the dose was adjusted to 120 mg/m^2 when there were no symptoms of neurotoxicity. Although 32 patients were entered in the trial, only 26 patients were evaluated for response to therapy. Spirogermanium administrations in those doses had nonsignificant effects on the regression of advanced renal cell cancer (Saiers *et al*., 1987) and on advanced non-small cell lung cancer (Dhingra *et al*., 1986). Schulman *et al*. (1984) and Volgelzang (1985) used similar treatment therapies with spirogermanium and reported similar findings. The administration of sera containing interferon (IFN) obtained from Ge-132–treated mice to mice with ascites tumors resulted in the inhibition of tumor growth (Suzuki *et al*., 1986). McMaster *et al*. (1990) conducted treatment trials on 17 patients with advanced colorectal carcinoma for 13 months. Patients started on 80 mg of spirogermanium/m^2 /day, IV over 1 hour three times per week in combination with a bolus dose of 600 mg of fluorouracil/m^2 once/week. The spirogermanium dose increased to a maximum dose of 120 mg/m^2/day when the patients did not show severe reactions to the initial doses. None of the patients had complete recovery. All patients had severe toxicity from this treatment, and two toxicity-related deaths occurred. An additional five patients were removed from the treatment because of intolerable side effects. The toxicities of spirogermanium include paresthesias, dizziness, and neutropenia that required hospitalization. The treatment was discontinued because of high toxicity and low response rate.

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CHAPTER

29

Indium

BRUCE A. FOWLER

ABSTRACT

Indium (In) compounds are poorly absorbed when ingested and moderately so when inhaled. Muscle, skin, and bone constitute the main storage sites, but the excretion routes vary depending on the form. Ionic indium is excreted primarily in urine; fecal elimination is the predominant route for removal of colloidal indium. A biphasic pattern of excretion and a whole-body biological half-time on the order of 2 weeks have been reported for both forms of indium.

Ionic indium is concentrated in the kidneys, producing renal failure; colloidal indium is taken up by the reticuloendothelial system, causing damage to the liver and spleen. Ionic indium has been shown to produce marked ultrastructural damage in the endoplasmic reticulum of both hepatocytes and renal proximal tubule cells with associated disruption of heme metabolism and hemoprotein function.

Intravenous administration of ionic indium to pregnant hamsters has been reported to produce malformations of the fetal digits.

The most common routes of exposure for the general population are inhalation and ingestion; for occupationally exposed persons it is inhalation. The use of In for nanotechnology may increase the possibility of dermal absorption in work environments.

The toxicology of indium has been reviewed by Izrael'son (1973), Smith *et al*. (1978), Fowler (1979; 1986), and Fowler and Sexton (2002).

1 PHYSICAL AND CHEMICAL PROPERTIES

Indium (In): atomic weight, 114.8; atomic number, 49; density, 7.31; melting point, 156.6°C; boiling point, 2080°C; in elemental form, it is a soft silver-white metal, tetragonal; oxidation states +1, +2, and +3. Only In (III) compounds are stable in aqueous systems. Indium compounds of toxicological interest include indium trichloride, indium oxide, indium sulfate, indium sulfide, indium sesquioxide, and colloidal indium hydroxide. Indium also forms organometallic compounds such as trimethylindium, a polymeric solid. Indium phosphide and indium arsenide are used in the manufacture of III–V semiconductors, and indium phosphide is used in the production of nanoparticles, nano wires, and dots (Akaogi *et al*., 2004; Bakkers *et al*., 2004; Fujita *et al*., 2004; Larsson, *et al*., 2004; Mikkelsen *et al*., 2004; Yu *et al*., 2003).

2 METHODS AND PROBLEMS OF ANALYSIS

A spectrochemical method for determining indium in biological materials has been reported by Kinser *et al*. (1966). The detection limit of this method is 0.002 μg indium with a coefficient of variation of approximately 10–15%. Neutron activation analysis (NAA) has been used to analyze for indium in rocks (Kuznecov, 1979; Rey *et al*., 1970) and seawater (Matthews and Riley, 1970). The detection limit for indium in rocks has been reported

as 0.01 mg/kg, with a standard deviation of 0.2 mg/kg (Kuznecov, 1979). The detection limit for indium in seawater is 0.006 mg/L, and the coefficient of variation is approximately 5% (Matthews and Riley, 1970).

Polarography has been reported to permit indium analysis in water with a detection limit of 1 µg/L and a precision of approximately 1% (Maienthal and Taylor, 1968). In one report, Armannsson and Ovenden (1980) described a demountable hollow cathode lamp for atomic absorption spectroscopy by use of a graphite furnace with a reported detection limit of approximately 1 µg In/L. More recent advances in atomic absorption spectroscopy have been reported to provide a detection limit of 0.8 µg/g (Shiue *et al.*, 2001), whereas atomic emission spectroscopy has been found to provide a limit of detection of 0.20 µg/g (Taddia *et al.*, 2002). An interesting fluorimetric method that uses indium chelation by 5-bromine-salicylaldehyde salicyloylhygrazone with a detection limit of 2.4 ng/mL was reported by Wang *et al.* (2001).

More recently, inductively coupled mass spectrometric techniques have been used for indium with detection limits on the order of 0.17 µg/g (Lewen *et al.*, 2004).

3 PRODUCTION AND USES

3.1 Production

The world production of indium was <50 metric tons in 1982 and greatly increased to approximately 335 metric tons in 2002 (US Geological Survey, 2004) in part because of increased use in the production of semiconductor materials, optic electronics, and plasma televisions. Detectors on the nanoscale may prove useful for a variety of analytical purposes (Wang *et al.*, 2001; Werle *et al.*, 2002; Zhou *et al.*, 2004). Please see Figure 1 for world production data and Table 1 for a summary of uses for indium.

Indium is recovered as a by-product of zinc smelting. Acid leaching of indium from crude zinc liquors with subsequent precipitation as a phosphate or acid leachate followed by a precipitation on metallic zinc rods are two widely used methods (Smith *et al.*, 1978).

3.2 Uses

The more important uses of indium include the incorporation into solders, alloys, semiconductors (Kato *et al.*, 2004), optoelectronics, plasma television screens, and, more recently, nanoparticles, nano whiskers, and dots (Liu, 2001; Pan *et al.*, 2003; Su *et al.*, 2002; Yu *et al.*, 2003). It is also used in automobile bearings as a hardening agent that improves resistance to corrosion. Indium oxide is used as a coloring glaze, and indium sulfide is used in electroplating. Radioisotopes of indium in compounds such as indium trichloride and colloidal indium hydroxide are used in the treatment of tumors and in organ scanning (Hart and Adamson, 1971; Haslett *et al.*, 1989; Limouris *et al.*, 2005; Oberg and Eriksson, 2005; Okpala *et al.*, 2005; Olkkonen *et al.*, 1977; Pasieka *et al.*, 2004; Smith *et al.*, 1978; Stern *et al.*, 1966; 1967; Stokkel *et al.*, 2003). More recently, gallium and indium alloys have been

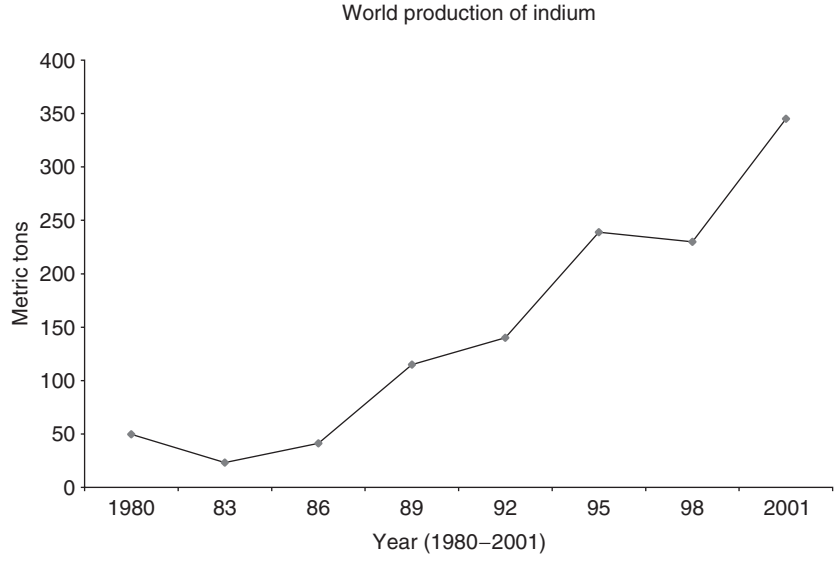

FIGURE 1 World production of indium from 1980 to 2002, showing marked increases over this time period. Data source: USG (2004).

TABLE 1 Uses of Indium

Uses	Chemical Form/Isotope
Automobile bearings	Alloys with steel
Glazes	^{111}In isotope /^{114}In isotope
Organ scanning	
Dental amalgams	InHg
Semiconductor dopants for silicon chips	In with Ga, Al, As, Sb
III-V semiconductors	InAs, InP, InAlGa
Plasma television screens	InP
Fiberoptics/light-emitting diodes	InAs, InP
Nano-particles, nano-whiskers, nano-fibers, nano-dots	InP, InSb, InSnO

incorporated into dental alloys as surface hardening agents for dental restorations (Claire and Williams, 2001; Uo *et al*., 2003).

4 ENVIRONMENTAL LEVELS AND EXPOSURES

4.1 Food and Daily Intake

The indium content of plants and animal tissues consumed as food ranges from below detection to 0.01 mg/kg for beef muscle and ham; however, algae and fish and shellfish collected near smelter outfalls have been reported to contain concentrations ranging from 0.4–7 mg/kg (for algae) up to 10–15 mg/kg (for fish and shellfish). The average daily human intake of indium has been estimated at about 8–10 µg In/day (Smith *et al*., 1978).

4.2 Water, Soil, and Ambient Air

Indium concentrations in seawater have been found to average <20 µg/L (Goldberg, 1965). Neutron activation studies (Matthews and Riley, 1970) have reported the concentration of indium in Atlantic Ocean water to be about 0.1 ng/L, whereas other investigations in a Norwegian fjord (Johansen and Steines, 1976) using the same technique have reported a value of approximately 0.7 ng/L. This element is also rare in soil as it comprises only about 1×10^{-5}% of the earth's crust (Sunderman and Townley, 1960). Haury and Schikarski (1977) estimated that the average indium concentration in soil was approximately 11 µg/kg on the basis of ^{115}In content. Ragaini *et al*. (1977) studied soils near a smelter in Idaho using NAA and reported a mean value of 2.05 mg/kg. Smith and coworkers (2005) reported indium in a large number of soil samples obtained from a U.S. Geological Survey in several U.S. states and Canada. In general, the concentrations of indium were approximately .04 ppm in soil samples taken in Manitoba and somewhat less in those taken in Minnesota.

Indium concentrations in England in air and rain have been reported to be approximately 0.3 ng/m^3 and <0.59 µg/L, respectively (Peirson *et al*., 1973). Studies by Ragaini *et al*. (1977) near a smelter in Idaho showed a mean indium concentration of 5.79 ng/m^3. Air in a residential area of Liege, Belgium, was found to contain 1.91 ng/m^3 by NAA (Dams, 1973). A more comprehensive review of indium in air, water, and soil was given by Smith *et al*. (1978).

5 METABOLISM

5.1 Absorption

5.1.1 Inhalation

Smith *et al*. (1957) evaluated the absorption, distribution, and excretion of $^{114}In(OH)_3$ or ^{114}In citrate after intratracheal instillation in rats and found that most of the administered dose was taken up by the tracheobronchial lymph nodes. Similar results were observed by Leach *et al*. (1961), who exposed rats through inhalation to In_2O_3 at an average indium concentration in dust of 64 mg In_2O_3/m^3 with little apparent movement of indium from the lung to tracheobronchial lymph nodes. The absorption of inhaled ^{114}In sesquioxide particles (2.5 mg In/m^3) by rats has been estimated to be between 3 and 6% of the total dose after a single 1-hour treatment, and approximately 18% for sequential 1-hour exposures on four successive days (Morrow *et al*., 1958). Isitman *et al*. (1974) administered $InCl_3$ or ^{111}In-diethylenetriaminepentaacetic acid (DPTA) to adult humans by ultrasonic nebulization and found deposition in the major airways with little (1.3–4.4%) alveolar deposition. More recent studies on indium arsenide particles (Yamauchi *et al*., 1992) have demonstrated biological dissolution *in vivo* with release of both indium and arsenic components.

5.1.2 Ingestion

The intestinal absorption of indium in rats has been reported to be approximately 0.5% of the administered dose (0.38 or 0.8 g In/kg) given as either an indium hydroxide or indium citrate complex (Smith *et al*., 1960). The intestinal absorption of indium in humans has been studied by Heading *et al*. (1971), who found that adult volunteers absorbed less than 2% of a 200 µCi dose of ^{113}In as a DPTA complex. Coates *et al*. (1973) reported no detectable absorption of a 500-µCi dose of ^{113}In administered as $InCl_3$ to human adults. Similar low-absorption

percentages were recently reported by Van Hulle *et al*. (2005) in rats after oral administration of ^{114}In as InAs.

5.2 Distribution

Ionic indium is transported in the blood bound to transferrin (Castronovo and Wagner, 1973; Hosain *et al*., 1969; Zhang *et al*., 2004) and has been found to be cleared within 3 days from the blood of mice given an intravenous injection (Castronovo, 1970; Castronovo and Wagner, 1971). Uptake of ^{111}In and ^{59}Fe have been found to be different from that of^{67} Ga in partially hepatectomized rats, suggesting that differences in the binding affinity of these elements for transferrin may explain differences in uptake (Sato *et al*., 2004). In was found to be largely in the plasma with only small amounts in the red cells, but Fe had a larger percentage bound to the red cell fraction.

Distribution of indium in body tissues is largely determined by the metal's chemical form. Ionic indium is extensively accumulated by the kidney, whereas colloidal indium oxide is accumulated by the liver, spleen, and the reticuloendothelial system. Three days after a single intravenous injection, approximately 20% of a tracer dose and 30% of an LD_{100} dose of ionic ^{114}In was found in the kidneys of mice. In contrast, mice injected with colloidal ^{114}In concentrated approximately 64% of the tracer dose and 40% of the LD_{100} dose in their livers after 3 days (Castronovo, 1970; Castronovo and Wagner, 1971). Hamsters treated with indium arsenide showed major In deposition in the liver, kidneys, and spleen with lesser amounts in the lungs (Yamauchi *et al*., 1992). Similar results have been recently reported by Van Hulle *et al*. (2005), who found the major sites of deposition for rats subcutaneously injected with ^{114}In as InAs were the liver, kidneys, and spleen. Intracellular fractionation demonstrated that most of the ^{114}In was bound in the cytoplasmic fraction followed by the mitochondria. Most of the ^{114}In was bound in the high-molecular-weight fractions of the serum and cytoplasmic fractions of the liver, kidneys, and spleen. Similar distribution and renal excretion patterns have been reported for a number of organoindium compounds (Sun *et al*, 1991) after intravenous injections in rats. Similar findings have been reported for ^{111} Inlabeled polymethacrylic nanoparticles in mice after intravenous injection (Rolland *et al*., 1989).

5.3 Excretion

The primary route of indium excretion from the body is determined by the chemical form administered. Ionic indium is mainly excreted in urine, whereas colloidal indium complexes are primarily excreted in feces. Mice have been found to excrete 52% of an administered dose of ionic indium through urine and 53% of a dose of colloidal indium through feces (Castronovo, 1970; Castronovo and Wagner, 1971; 1973). Yamauchi *et al*. (1992) treated mice with a single subcutaneous dose of indium arsenide particles (100 mg/kg), and approximately 0.05% of the indium was excreted per day over a 30-day time period. Overall, the urine was the primary route of elimination from the body, but fecal elimination was also a major excretory pathway.

A general diagram for the known *in vivo* absorption of indium compounds from the lungs and gastrointestinal tract, transport in the blood, target tissue deposition, and elimination are presented in Figure 2. The possible absorption of nanoparticles across the skin is not considered at this point because of a lack of published data.

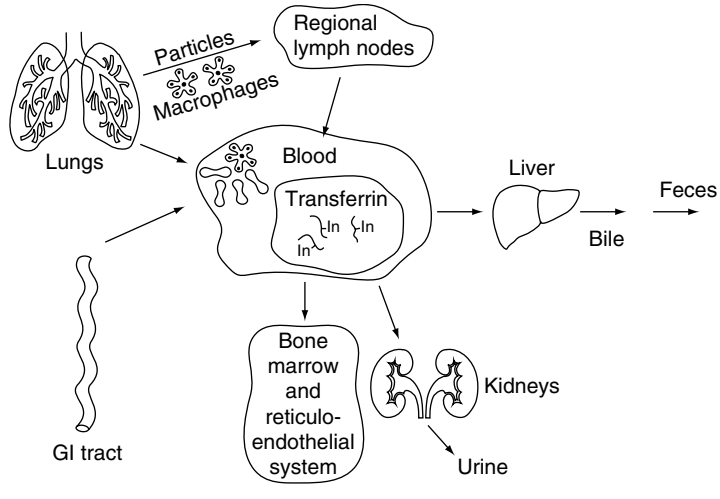

FIGURE 2 Generic diagram of *in vivo* indium uptake, binding to transferrin in the circulation, and tissue distribution.

5.4 Biological Half-Time

The biological half-time for indium depends somewhat on the chemical form administered, and excretion seems to follow a biphasic pattern. Mice given intravenous injections of ^{114}indium chloride showed a biological half-time of 1.9 days for the fast-phase component representing approximately 50% of the body burden, and 69 days for the slow phase. Hydrated ^{114}indium oxide had a biological half-time of 2 days for the fast-phase component representing approximately 25% of the body burden, and 73.8 days for the slow phase after intravenous injection (Castronovo, 1970; Castronovo and Wagner, 1971; 1973). Stern *et al*. (1967) found a biological half-time of 3.5 days for clearance of hydrated indium oxide from the lungs of mice after intravenous administration. A whole-body biological half-time of 14–15 days was reported by these authors.

Smith *et al*. (1960) reported that approximately 60% of an intratracheal injection of radioactive indium left the lungs of rats within 16 days. Excretion of indium from the bodies of these animals was also biphasic. The biological half-time for inhaled indium sesquioxide particles has been found to be approximately 8–10 days in rat lungs (Morrow *et al*., 1958). Isitman *et al*. (1974) reported the biphasic half-times for $^{111}InCl_3$ in lungs of adult human subjects exposed to an aerosol to be 16 and 35 minutes, respectively. Yamauchi *et al*. (1992) reported the results of intratracheal instillation studies of InAs particles in hamsters and observed the greatest rate of excretion (0.07%/day) during the first 5 days after treatment for the In component.

6 LEVELS IN TISSUES AND BIOLOGICAL FLUIDS

Smith *et al*. (1978) have extensively reviewed the literature concerning reported tissue concentrations of indium in animal and human tissues, and most values have been found to be <0.05 ppm. Tissues of fish and shellfish collected near a smelter outfall, however, have been found to range up to 10–15 mg/kg (wet weight).

7 EFFECTS AND DOSE-RESPONSE RELATIONSHIPS

Indium is considered to be a nonessential element. At present, there are no published data on the toxicity of organoindium compounds or on indium-containing nanoparticles.

7.1 Local Effects and Dose-Response Relationships

7.1.1 Animals

Podosinovskij (1965) administered 50 mg of In_2O_3 intratracheally to rats and found pneumonic and initial fibrotic changes in the lungs. The pulmonary toxicity of indium diselenide was reported by Morgan *et al*. (1995), who observed increased pulmonary inflammation in lungs of female rats 72 hours after intratracheal instillation of this compound. Similar inflammatory changes associated with markers of oxidative stress and the development of lung carcinogenesis were observed in rats exposed to an aerosol of InP for 105 weeks (Gottschling *et al*., 2001). Tanaka (2003) reported that InAs was more toxic to the lung of experimental animals than GaAs or AlGaAs after intratracheal instillation.

7.1.2 Humans

Raiciulescu *et al*. (1972) reported that 3 patients of a total of 770 persons injected with colloidal ^{113}indium for liver scanning developed severe vascular shock within 20 minutes after treatment. Shock was found to last from 10 minutes to 1 hour. Increased blood and urinary concentrations of indium have been reported among optoelectronics industry workers in Taiwan (Liao *et al*., 2004), and pulmonary fibrosis has been reported in a person exposed to inhaled indium-tin oxide for 4 years in a flat-panel display facility in Japan (Homma *et al*., 2005).

7.2 Systemic Effects and Dose-Response Relationships

7.2.1 Animals

The primary adverse effects of ionic indium are exerted in the kidneys, whereas colloidal hydrated indium oxide damages the reticuloendothelial system. A single intravenous injection of indium chloride has been reported to cause extensive necrosis of the renal proximal tubules both in rats (3.6 mg/kg) and mice (16.5 mg/kg) (Castronovo, 1970; Castronovo and Wagner, 1971; Downs *et al*., 1959). Administration of colloidal hydrated indium oxide by this route produced necrosis of cells in the liver and spleen (Castronovo, 1970; Castronovo and Wagner, 1971; Downs *et al*., 1959; Stern *et al*., 1967). Lung damage has also been reported in mice given subcutaneous injections of $In(SO4)^3$ (Yoshikawa and Hasegawa, 1971). Lower hemoglobin and neutrophil counts have been observed in rats, mice, and rabbits injected with ionic indium (Downs *et al*., 1959; McCord *et al*., 1942; Steidle,

1933; Yoshikawa and Hasegawa, 1971). Administration of ionic indium at a concentration of 5 mg/L in drinking water has also been reported to cause mild growth-depression in mice (Schroeder and Mitchener, 1971). Other studies (Fowler *et al*., 1983; Woods and Fowler, 1982; Woods *et al*., 1979) concerning the intracellular mechanisms of indium-induced cell injury have demonstrated marked disruption of the endoplasmic reticulum associated with marked alteration of cellular heme metabolism in both hepatocytes and renal proximal tubule cells.

Studies by Conner *et al*. (1993) demonstrated both altered protein expression patterns in kidneys of hamsters treated with indium, arsenic, or indium arsenide particles. These investigators (Conner *et al*., 1995) also demonstrated indium, arsenic, and indium arsenide–specific proteinuria patterns in hamsters treated *in vivo* with these agents 10 and 30 days after treatment.

Intratracheal exposure of rats to indium selenide has been reported to produce acute pulmonary toxicity in rats characterized by a marked inflammatory reaction (Morgan *et al*., 1995). Similar findings of inflammation linked to oxidative stress were observed in male and female rats exposed to an indium phosphide aerosol for 6 hours/day, 5 days/week for up to 105 weeks (Gottschilling *et al*., 2001). *In vitro* studies by Okada *et al*. (2002) demonstrated mild toxicity to alveolar macrophages and no evidence of cell death after *in vivo* exposure of hamsters to particles of ferric oxide (60 µg) and indium arsenide over a dose range of 2–20 µg InAs per animal. *In vitro* exposure studies of rat thymocytes to soluble indium chloride were found to induce apoptosis (Bustamente *et al*., 1997). The pulmonary toxicity of indium phosphide was studied in rats by Oda (1997) after intratracheal instillation of at doses of 0, 1.2, 6.0, and 62 µg/kg. Eight days after treatment, only rats at the 62-µg/kg dose level had pulmonary inflammation. Tanaka and coworkers (1996; 2000) studied the chronic comparative pulmonary toxicity of indium phosphide and indium arsenide particles (Tanaka *et al*., 1996) and indium arsenide, arsenic trioxide, and gallium arsenide particles (Tanaka *et al*., 2000) in hamsters. They found pulmonary inflammation, fibrotic changes, and evidence of pulmonary squamous cell metaplasia and bronchial cell hyperplasia after 8 weeks of treatment (Tanaka *et al*., 2000) and that indium arsenide was the most toxic compound to the lung. The pulmonary toxicity of indium arsenide and other III–V semiconductor compounds has been recently reviewed by Tanaka (2004) in relation to cancer. The National Toxicology Program (Gunnels *et al*. (2001) studied the toxicity of indium phosphide after inhalation of indium phosphide particulates by mice and rats at 14 weeks and 2 years. Pulmonary inflammation was observed at 14 weeks, and alveolar and bronchiolar carcinomas were noted in both male and female mice.

The developmental toxicity of indium chloride has been studied by Nakajima and coworkers in rats (1998; 1999) in rats and mice (Nakajima *et al*., 2000). These investigators concluded that both rats and mice were susceptible to embryo toxicity after parenteral administration but that mice were less susceptible than rats to the teratogenic effects of this element. Ungvary and coworkers (2000; 2001) and Morvai and coworkers (2001) studied the embryotoxic and teratogenic effects of indium chloride administration in rabbits and rats (Ungvary *et al*., 2000), on the hemodynamics of the placenta in rats (Morvai *et al*., 2001a), and on chondrogenic ossification in rats (Ungvary *et al*., 2001). Overall, the results of these studies show altered placental hemodynamics and a number of embryo lethal and teratogenic effects in rats involving normal limb ossification.

The testicular toxicity of indium arsenide was evaluated by Omura *et al*., (1996a,b), who found no evidence of testicular toxicity for this compound at doses of 7.7 mg/kg administered by intratracheal instillation a total of 14 times.

Implantation of indium-treated silver discs into rabbits has been found to produce only foreign body reactions (Harrold *et al*., 1943).

7.2.2 Humans

One case of pulmonary fibrosis has been reported in a person exposed to indium-tin oxide by inhalation for 4 years in a flat panel plasma manufacturing plant in Japan (Homma, *et al*., 2005).

7.3 Carcinogenicity, Mutagenicity, and Teratogenicity

There are a number of published studies on the carcinogenicity or mutagenicity of indium phosphide (Gottschilling *et al*. 2001; Gunnels, 2001). The IARC has determined that indium phosphide is a probable human carcinogen (2A) on the basis of these data coupled with the *in vitro* studies by Bustamente *et al*. (1997) that demonstrated the ability of In to induce apoptosis in rat thymocytes. On the basis of this evaluation and the increasing use of indium compounds in nanotechnology, it may be prudent to revisit current occupational exposure standards such as that of ACGIH that is currently 0.1 mg In/m^3. Intravenous administration of ionic indium to pregnant hamsters has been reported to produce malformations of the fetal digits at doses <1 mg/kg and embryo lethality at higher doses of 2–20 mg/kg after injection on day 8

of gestation (Ferm and Carpenter, 1970). Similar and additional teratogenic and embryo lethal effects have been reported in rats and mice by Nakajima *et al*. (2000) at doses of 0.4 mg/kg in rats and 0.8 and 1.6 mg/kg in mice. These authors concluded that both rats and mice are susceptible to the embryo lethal effects of indium but noted greater sensitivity of rats relative to mice with regard to the teratogenic effects, because no malformations were observed in this species. Similar teratogenic effects were reported in rats by Ungvary *et al*. (2000; 2001).

7.4 Interactions with Ferric Dextran, Thorium Dioxide Sol, and Gelatin

Concomitant administration of ferric dextran to rats and hamsters given intravenous doses of ionic indium has been reported to protect against liver damage and embryopathic effects in these species (Ferm, 1970; Gabbiani *et al*., 1962). Administration of thorium dioxide sol and gelatin prevented damage to the reticuloendothelial system by colloidal indium hydroxide (Evdokimoff and Wagner, 1972).

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CHAPTER

30

Iron

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ABSTRACT

Iron is the fourth most abundant metal in the earth's crust and the most abundant transition metal. Iron can easily change valence and form complexes with oxygen, and iron-mediated reactions support the respiration of nearly all aerobic organisms. However, unless appropriately shielded, iron catalyzes the formation of radicals that can damage biological molecules, cells, tissues, and entire organisms. Exposure to excess iron—typically from multiple blood transfusions over many years—can lead to numerous pathological consequences. By contrast, severe iron deficiency may also have serious health consequences. Because of the inherent danger of iron, specialized molecules for the acquisition, transport, and storage (ferritin) of iron in a soluble nontoxic form have evolved. Delivery of iron to most cells occurs after the binding of transferrin to transferrin receptors on the cell membrane. The transferrin–receptor complexes are then internalized by endocytosis, and iron is released from transferrin by a process involving endosomal acidification and reduction. Iron is then transported through the endosomal membrane by the Fe(II) transporter DMT1/Nramp2. Importantly, this identical transporter is involved in the absorption of inorganic iron in the duodenum, a process facilitated by the ferric reductase Dcytb, which presumably provides Fe(II) for DMT1/Nramp2. Organisms and cells possess limited ability to excrete excess iron, and only some specialized cells have active mechanisms to export iron. Iron release from these "donor cells" (primarily enterocytes and macrophages that recycle hemoglobin iron) is mediated by ferroportin 1. The ferroxidase activity of copper-containing proteins, hephaestin and ceruloplasmin, facilitates the movement of iron across the membranes of enterocytes and macrophages, respectively. Cells are also equipped with a regulatory system that controls iron levels in the labile pool. Levels of iron modulate the capacity of iron regulatory proteins (IRPs) to bind to the iron-responsive elements (IREs) present in the untranslated regions of mRNAs for several proteins involved in iron metabolism (e.g., ferritin, transferrin receptor, DMT1); these associations, or lack of them, in turn control the expression of these proteins. In fact, important information about the regulation of iron metabolism came from studies of proteins (e.g., HFE, ferroportin, and hepcidin) coded by genes, mutations of which cause different types of hereditary hemochromatoses. Despite these homeostatic mechanisms, organisms can face the threat of either iron deficiency or iron overload.

Acute iron overload resulting from unintentional or intentional overdose is potentially life threatening. Chronic iron overload leads to slowly developing (and, *in extremis*, lethal) damage to organs such as the heart and liver. However, the nature of the accumulated damage that eventuates in such organ failure is not yet fully known. In addition, occupational inhalation exposures to iron may give rise to siderosis, a benign condition easily diagnosed by chest X-ray. Our developing knowledge of iron metabolism increasingly emphasizes the simultaneously vital and threatening nature of this most important and abundant metal.

1 PHYSICAL AND CHEMICAL PROPERTIES

Iron (Fe): atomic weight, 55.8; atomic number, 26, density, 7.9; melting point, 1535°C; boiling point, 2750°C;

major valences, +2 and +3 forms unstable ferryl and perferryl intermediates.

Iron is found in numerous inorganic compounds with oxygen (e.g., as magnetite), carbonate, sulfur, chloride, and carbonyls. Ferrous (Fe(II)) salts are soluble but at neutral pH readily oxidize and precipitate as insoluble $Fe(OH)_n$ (rust). Because of its malleability and ductility when hot and its hardness when cold (~5 on the Mohs scale), iron has been particularly important in the technological evolution of *Homo sapiens*.

2 METHODS AND PROBLEMS OF ANALYSIS

Soluble iron is most easily quantified with colorimetric reactions using reagents that form chromophoric complexes with the metal. In the past, these reagents have included thiocyanate, phenanthroline, and ferrozine. At this time, perhaps the most sensitive chromophore is 3-(2-pyridyl)-5,6-bis(2-(5-furylsulfonic acid))-1,2,4-triazine (Ferene S), the Fe(II) complex of which has a molar absorption coefficient of 35,500 cm^{-1} (Artiss *et al*., 1981). Alternative analytical procedures are used on a variety of metals, and these include atomic absorption spectroscopy (flame or electrothermal ionization) and inductively coupled plasma mass spectrometry. The latter has recently been applied to high-throughput analyses of iron balance, because it, in combination with mass spectrometry, permits measurements of various stable iron isotopes (Chen *et al*., 2005).

3 PRODUCTION AND USES

Iron ores (most in the form of taconite, because deposits rich in magnetite and hematite have been largely exploited) are mined, processed to remove noniron components, and smelted into metallic iron. Major sites of iron mining include South Africa, the United States, Australia, China, Russia, Japan, Ukraine, India, and Brazil. The aggregate worldwide production in 2004 was approximately 1 billion metric tons of raw ore. The steel produced from this ore typically contains small percentages of carbon (<2%) and is sometimes combined with other metals, either purposefully or through contamination of the original ore. The products of iron mining are used in a variety of products, ranging from structural elements in buildings to iron-based pigments and pharmaceuticals.

4 ENVIRONMENTAL LEVELS AND EXPOSURES

In the general environment, iron levels in water and soil vary widely. Very iron-rich soils yield edible plants that are inappropriately iron rich. Iron overload from their consumption has not been reported. In the air, other than that encountered in foundries and other industrial environments, iron concentrations are generally negligible. However, Sentz and Rakow (1969) reported air iron levels of 20–30 mg/m^3 in some steel manufacturing plants, levels that would very likely lead to at least pulmonary accumulation of iron. The limit value for occupational exposures recommended in 2006 by the American Conference of Governmental Industrial Hygienists (ACGIH) is 5 mg/m^3 for iron oxide and 1 mg/m^3 for soluble iron salts.

The typical daily western European/American dietary intake of iron is approximately 15 mg/day, only a fraction of which (approximately 10%) is actually absorbed. Iron content of foodstuffs varies greatly from iron-rich foods, such as red meat, to extraordinarily iron-poor foods like milk. As reviewed in the following, the extent of absorption of dietary iron depends on a number of factors. These factors include the form of iron (e.g., heme iron is absorbed far more readily than other forms); other components of the diet (e.g., polyanionic iron-binding compounds like phytic acid can limit absorption of ionic iron); body iron status (iron absorption is homeostatically increased in iron deficiency states); and genetic status (certain genetic defects predispose one to absorption of abnormally large amounts of iron) (see Section 6.2); and, consequently, to iron overload.

Inadvertent contamination of foods with excess iron can occur, but resultant iron overload after long-term consumption of these foods has been reported only in individuals with genetic susceptibility for increased iron absorption. This may occur through contamination of foods (such as beer and grain) with iron from the environment or from cooking and brewing vessels. A classic case of diet-induced iron overload involved the consumption of iron-rich beer by members of the Bantu tribe, leading ultimately to pathological iron overload (Bothwell *et al*., 1964). Iron intakes of >100 mg occurred, and the iron overload led to cirrhosis, diabetes, and, perhaps, predisposition to hepatoma. However, it was subsequently shown that this form of iron overload arises from a conspiracy of iron intake and (presently uncharacterized) predisposing genetic factor(s) (Moyo *et al*., 1998).

5 BIOLOGICAL FUNCTION AND METABOLISM

5.1 Overview of Iron Metabolism

Iron is a precious metal for the organism because of its unsurpassed versatility as a biological catalyst. It is involved in a broad spectrum of essential biological functions such as oxygen transport, electron transfer, and DNA synthesis. Hence, iron is an essential element required for growth and survival. However, the very chemical properties of iron that allow this versatility also create a paradoxical situation, making acquisition by the organism of the fourth most abundant element in the earth's crust exceedingly difficult. At pH 7.4 and physiological oxygen tension, the relatively soluble ferrous ion is readily oxidized to the ferric ion, which on hydrolysis forms virtually insoluble ferric hydroxides. The concentration of aquatic Fe(III) in aqueous solutions (pH 7.4) cannot exceed 10^{-17} mol/L. Moreover, unless appropriately "shielded," iron promotes the formation of harmful oxygen radicals, which ultimately cause peroxidative damage to vital cell structures. Because of this virtual insolubility and potential toxicity, iron must constantly be chaperoned; specialized mechanisms and molecules for the acquisition, transport, and storage of iron in a soluble, nontoxic form have evolved to meet the cell's and the organism's iron requirements. Moreover, organisms are equipped with sophisticated mechanisms that prevent the accumulation of a catalytically active intracellular iron pool, while maintaining sufficient concentrations of the metal for metabolic use (Aisen *et al*., 2002; Andrews, 2005; Eaton and Qian, 2002; Harrison and Arosio, 1996; Hentze *et al*., 2004; Ponka *et al*., 1998; Richardson and Ponka, 1997).

Iron, both in its heme and nonheme forms, is present in every cell in the body, and most cells, except for mature red cells and terminally differentiated lens epithelium, have the capacity to acquire iron. However, cellular iron acquisition and its proper intracellular targeting into functional iron proteins depend on an array of other proteins. "Traditional" proteins involved in iron metabolism include transferrin, transferrin receptor, and ferritin, but a remarkable flurry of activity in the past several years has identified a large number of novel genes whose products emerge as important players in iron metabolism (Table 1).

The bodies of adult men and women contain 55 mg and 45 mg of iron per kilogram of body weight, respectively. Normally, 60–70% of total body iron is present in hemoglobin in circulating erythrocytes. In vertebrates, iron is transported within the body between sites of absorption, storage, and use by the plasma glycoprotein transferrin that binds iron (III) very tightly but reversibly. The turnover of transferrin iron is approximately 30 mg/24 hours and, normally, approximately 80% of this iron is transported to the bone marrow for hemoglobin synthesis in developing erythroid cells. Senescent erythrocytes are phagocytosed by macrophages of the reticuloendothelial system, where the heme moiety is split from hemoglobin and catabolized enzymatically by means of heme oxygenase-1 (HO-1). Iron, which is liberated from its confinement within the tetrapyrrole ring inside macrophages, is returned almost quantitatively to the circulation. The remaining 5 mg of the daily plasma iron turnover is exchanged with nonerythroid tissues, primarily the liver. Approximately 1 mg of dietary iron is absorbed per 24 hours, and the total iron balance is maintained by a daily loss of 1 mg by means of nonspecific mechanisms (mostly cell desquamation) (Andrews, 2005; Ponka, 2003; Ponka *et al*., 1998). This process is an extraordinarily delicate balancing act, given that iron amounts to approximately 1/100,000,000th part (w/w) of the human body.

5.2 Cellular Iron Acquisition from Transferrin

With some notable exceptions (e.g., enterocytes), virtually all the cells in the organism take up iron from transferrin. Delivery of iron to cells occurs after the binding of transferrin to its cognate receptors on the cell membrane (Hentze *et al*., 2004; Ponka and Lok, 1999; Richardson and Ponka, 2003). The transferrin–receptor complexes are then internalized by endocytosis, and iron is released from transferrin by a process involving endosomal acidification (Figure 1). It has been shown that the transporter, DMT1 (also known as Nramp2 or DCT1), is likely responsible for the egress of iron from the endosome (Canonne-Hergaux *et al*., 2001; Fleming *et al*., 1997; Gunshin *et al*., 1997). The protein is encoded by a gene that belongs to the *Nramp* (Natural resistance-associated macrophage protein) family of genes identified by Gros and coworkers (Cellier *et al*., 1995). Mutations of one form of Nramp were found to predispose organisms to diseases (such as leprosy and tuberculosis) caused by intracellular pathogens. Interestingly, *Nramp2* generates two alternatively spliced mRNAs that differ at their 3′ untranslated regions (UTR) by the presence or absence of the iron-responsive element (IRE, discussed later) and that encode two proteins with distinct carboxy termini (Canonne-Hergaux *et al*., 1999; 2001). In collaboration with Dr. Philippe Gros, we have recently found it is isoform II (derived from non-IRE containing mRNA) that is the major DMT1 protein isoform expressed in the developing erythroid cells (Cannone-Hergaux *et al*., 2001). One of us (Ponka) was a member of the team who identified the first human

TABLE 1 Some Proteins Involved in Iron Metabolism

Protein	Function	Result of deficiency	Reference
ABC7	Non-heme Fe export from mito. (?)	XLSA with ataxia	Lill, 2005, Napier, *et al*., 2005
ABC-me	Mitochondrial transport function related to heme synthesis	Unknown	Shirihai, *et al*., 2000
ALA-S2/eALA-S	1st enzyme of heme synthesis; erythroid-specific 5-aminolevulinic acid synthase	X-linked sideroblastic anemia	Cox, *et al* 1991, Ponka, 1997, Fitzsimons, 1996
Ceruloplasmin (Cp)	Regulation of Fe export from cells	Neurodegeneration; hypochromic microcytic anemia	Hellman, 2002
DMT1/Nramp2	Membrane transporter for Fe^{2+}	Hypochromic microcytic anemia	Fleming, *et al*., 1997
Duodenal cytochrome b (Dcytb)	Ferric reductase (suggested to provide Fe^{2+} for DMT1 in duodenum)	Unknown	McKie, *et al*., 2001
Ferritin (H and L)	Cellular Fe storage	H: embryonic lethality	Harrison, 1996 Richardson, 1997 Ferreira *et al*., 2000
Ferrochelatase	The last enzyme of heme synthesis; Fe^{2+} insertion into protoporphyrin IX	Erythropoietic protoporphyria	Ponka, 1997, Dailey, 2002, Cox, 1997
Ferroportin 1 / MTP1 / Ireg1	Fe export from cells	Hemochromatosis type 4; anemia in childhood	Donovan, *et al*., 2000 Pietrangelo, 2004
Frataxin	Involvement in [Fe-S] synthesis	Friedreich ataxia	Lill, 2005, Napier, *et al*., 2005, Gerber, 2003
HFE	Unknown; binds TfR; restricted expression (enterocytes, macrophages)	Hemochromatosis type 1	Feder, *et al*., 1996 Pietrangelo, 2004
Heme oxygenase-1 (HO-1)	Recycling of hemoglobin Fe	Severe anemia and inflammation	Yachie, *et al*., 1999 Maines, 2004
Hemojuvelin, also known as Repulsive Guidance Molecule C	Unknown; expressed in striated muscle and the myocardium	Juvenile hemochromatosis (subtype of type 2)	Papanikolaou, *et al*., 2004
Hepcidin	Plasma peptide which inhibits Fe absorption and heme Fe recycling in macrophages	Juvenile hemochromatosis (subtype of type 2); overexpression of hepcidin causes severe Fe deficiency anemia	Park, *et al*., 2001 Nicolas *et al*., 2002 Ganz, 2005
Hephaestin	Regulation of Fe export from enterocytes (membrane-bound Cp homolog)	Hypochromic microcytic anemia (sex-linked anemia in mice)	Vulpe, *et al*., 1991
IRP (1 and 2)	Fe "sensors"; bind to IREs	IRP2: brain Fe overload; anemia	Richardson, 1997 Mikulits, *et al*., 1999 Pantopoulos, 2004 Cooperman, *et al*., 2005
Lactoferrin	Member of the "Tf family" of proteins; anti-bacterial and antiviral activities	No change in Fe homeostasis	Richardson, 1997 Ward, *et al*., 2003
Melanotransferrin	Unknown	Unknown	Richardson, 1997
Mitochondrial ferritin	Mitochondrial Fe storage (?)	Unknown; high expression in "ring" sideroblasts	Napier, *et al*., 2005 Levi, *et al*., 2001 Nie, *et al*., 2005
Mitoferrin (coded for by *frascati*)	Fe transport to mitochondria	Hypochromic anemia	Shaw, *et al*., 2006
Sec15l1	"Vesicle docking"	"Haemoglobin- mice deficit" (hbd) mice	Lim, *et al*., 2006. White, *et al*., 2005 Zhang, 2006
Sideroflexin 1	Mitochondrial transport function related to Fe metabolism	Siderocytic anemia (mice)	Fleming *et al*., 2001
Steap3	Endosomal ferrireductase	Hypochromic anemia (mice)	Ohgami *et al*., 2005
Transferrin (Tf)	Fe(III)-carrier in plasma	Severe anemia (Fe unavailable for erythropoiesis; generalized Fe overload	Richardson, 1997 Ponka, 2002
Tf receptor (TfR)	Membrane receptor for Fe_2-Tf	Embryonic lethality	Richardson, 1997 Ponka, 1999, Aisen, 2004, Levy *et al*., 1999
TfR2	Unknown	Hemochromatosis type 3	Kawabata *et al*., 1999 Camaschella *et al*., 2000

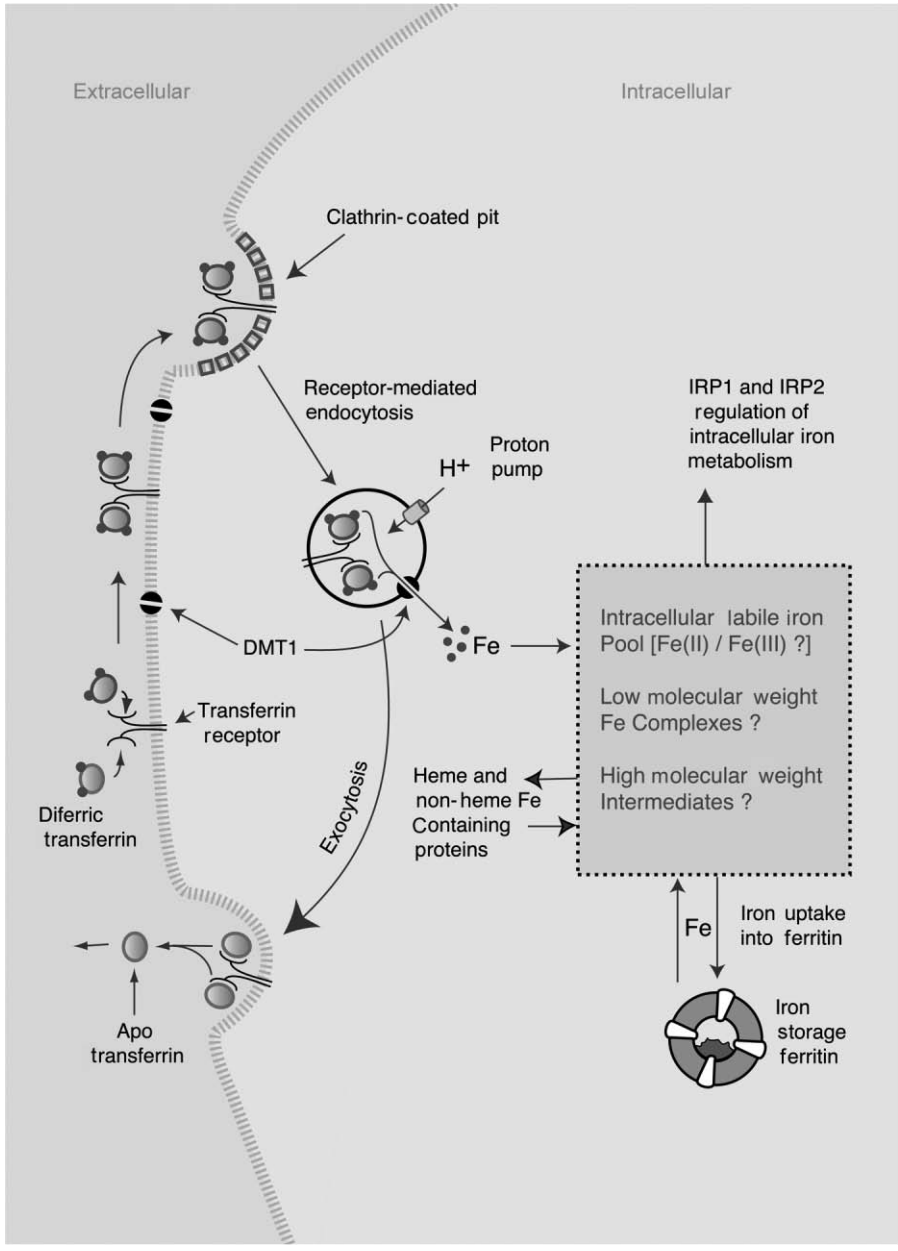

FIGURE 1 Schematic representation of iron uptake from transferrin (Tf) by means of Tf receptor–(TfR) mediated endocytosis in mammalian cells. Extracellular Fe_2^{3+}–Tf is bound by the membrane-bound TfR and internalized by means of receptor-mediated endocytosis into an endosome. Iron is released from Tf by a decrease in pH and is then transported through the endosomal membrane by DMT1/Nramp2. Physiologically, all iron in the circulation is Tf-bound and, therefore, DMT1 expressed at the plasma membrane of erythroid and perhaps other cells has no substrate. Obviously, DMT1 can assume its function of Fe^{2+} transporter only after its recruitment into endosomes where it colocalizes with TfR (Cannone-Hergaux *et al*., 2001). Because reticulocytes acquire Fe^{2+} at rates that are much higher than those of iron uptake from Fe_2Tf (Sheftel A and Ponka P, unpublished results), DMT1 is not a limiting factor in iron uptake through a Tf-dependent pathway in these cells. Once the iron has passed through the membrane, it then enters a very poorly characterized compartment known as the intracellular labile iron pool (LIP); however, developing erythroid cells may deliver the metal directly from Tf-containing vesicles to mitochondria (Zhang *et al*., 2005). Apo-Tf remains bound to the TfR and is then released by exocytosis. Iron that enters the cell can be used for metabolic functioning or can be stored in ferritin. It is thought that iron in the intracellular iron pool modulates the activity of iron regulatory proteins 1 and 2 (IRP1 and IRP2). (Adapted from Richardson and Ponka, 1997.)

mutation of DMT1 causing severe hypochromic microcytic anemia (Mims *et al*., 2005; Priwitzerova *et al*., 2004; 2005). Because the substrate for DMT1 is Fe(II), reduction of Fe(III) must occur in endosomes. Importantly, Ohgami *et al.* (2005) recently identified a gene, *Steap3* (six transmebrane, epitheilal antigen of the prostate 3), whose product is a compelling candidate for endosomal ferric reductase. The protein coded by this gene is highly expressed in hematopoietic tissues, is present in endosomes and colocalizes with transferrin, transferring receptors, and DMT1. Significantly, Steap3 contains an oxido-reductase domain, based on the presence of a flavin-NAD(P)H binding structure and a putative heme binding site.

After its escape from endosomes, iron is transported to intracellular sites of use and/or storage in ferritin, but much about this aspect of iron metabolism—including the nature of the elusive intermediary pool of iron and its cellular trafficking—remains enigmatic. One exception is that in erythroid cells some evidence points to specific targeting of iron toward mitochondria within which the enzyme ferrochelatase inserts Fe(II) into protoporphyrin IX, producing heme. This is documented by our results showing that in hemoglobin-synthesizing cells, iron acquired from transferrin continues to flow into mitochondria, even when the synthesis of protoporphyrin IX is markedly suppressed (Napier *et al*., 2005; Ponka, 1997). More recently we showed that ^{59}Fe flux from endosomal ^{59}Fe-transferrin into heme can be intercepted by membrane-permeable ferric and ferrous iron chelators, but ^{59}Fe-chelates can be formed only in metabolically labeled cells (Zhang *et al*., 2005). Moreover, we have found that inhibition of endosome motility decreases the rate of ^{59}Fe incorporation from ^{59}Fe-labeled endosomes into heme (Zhang *et al*., 2005). These observations suggest that in erythroid cells a transient mitochondrion–endosome interaction may be involved in iron translocation to ferrochelatase. Nothing is known about the postendosomal phase of iron trafficking in nonerythroid cells of mammals.

5.3 Iron Export from Cells to Transferrin

There are specialized mammalian cells that must export iron. Absorption of dietary iron for transfer to transferrin in plasma requires iron efflux across the basolateral surface of the intestinal epithelia. A second major site of iron release is from macrophages where senescent or damaged red cells are degraded to export the metal from hemoglobin and provide it for binding to transferrin. Moreover, iron delivery to brain, placenta, and testis requires transport into and across endothelial cells (Ponka, 2004; Richardson and Ponka, 1997).

Iron release from "donor cells" (primarily enterocytes and macrophages) to plasma transferrin is poorly understood, but a number of recent studies have provided new clues in this important area of iron metabolism. A likely candidate for iron export from cells is ferroportin 1 (Donovan *et al*., 2001), also known as MTP1 (Abboud and Hailey, 2000) or Ireg1 (McKie *et al*., 2000), and the ferroxidase activity of hephaestin (named after the Greek god of fire and iron Hephaestos) (Anderson *et al*., 2002; Vulpe *et al*., 2001) and ceruloplasmin (after the Latin *caeruleus* for blue, the color of the purified protein) (Hellman and Gitlin, 2002) facilitate the movement of iron across the membranes of enterocytes and macrophages, respectively. Ceruloplasmin and hephaestin exhibit a high degree of homology, because both proteins contain several copper atoms that are necessary for their ferroxidase (i.e., oxidation of Fe(II) to Fe(III)) activity.

5.4 Recycling of Hemoglobin Iron

At the end of an erythrocyte's life, it is phagocytosed by macrophages of the reticuloendothelial system (e.g., Kupffer cells of the liver), and iron is liberated from the vice-like grip of the protoporphyrin ring by heme oxygenase-1 (HO-1; Maines, 2004; Maines and Gibbs, 2005). These cells have an enormous capacity to purge themselves of iron, which is likely exported through ferroportin 1 (Figure 2). It has recently been proposed that hepcidin (originally discovered as an antimicrobial compound in urine—hepatic bactericidal protein) is involved in the regulation of iron release from macrophages (see later).

5.5 Iron Absorption

No information is available that would allow assessment of iron acquisition by the organism through inhalation. General knowledge on uptake of airborne particles is summarized in Chapter 3. Normally, the body iron content in humans is maintained within narrow limits by the regulation of intestinal iron absorption (Miret *et al*., 2003). Both heme and elemental iron are absorbed through the brush border of the upper small intestine. Heme iron is more readily available for absorption but usually constitutes only a small fraction of dietary iron. Heme (derived from hemoglobin or myoglobin) is taken up intact, probably by means of specific high-affinity heme binding sites in the mucosal brush border (Grasbeck *et al*., 1982; Shayeghi *et al*., 2005; Worthington *et al*., 2001) (Figure 3). After entering the intestinal epithelial cells, iron is enzymatically released from heme by HO-1.

As mentioned earlier, elemental Fe(III) is virtually insoluble at neutral pH, and, therefore, the availability of dietary iron for intestinal absorption depends on the composition of intestinal secretions, as well as

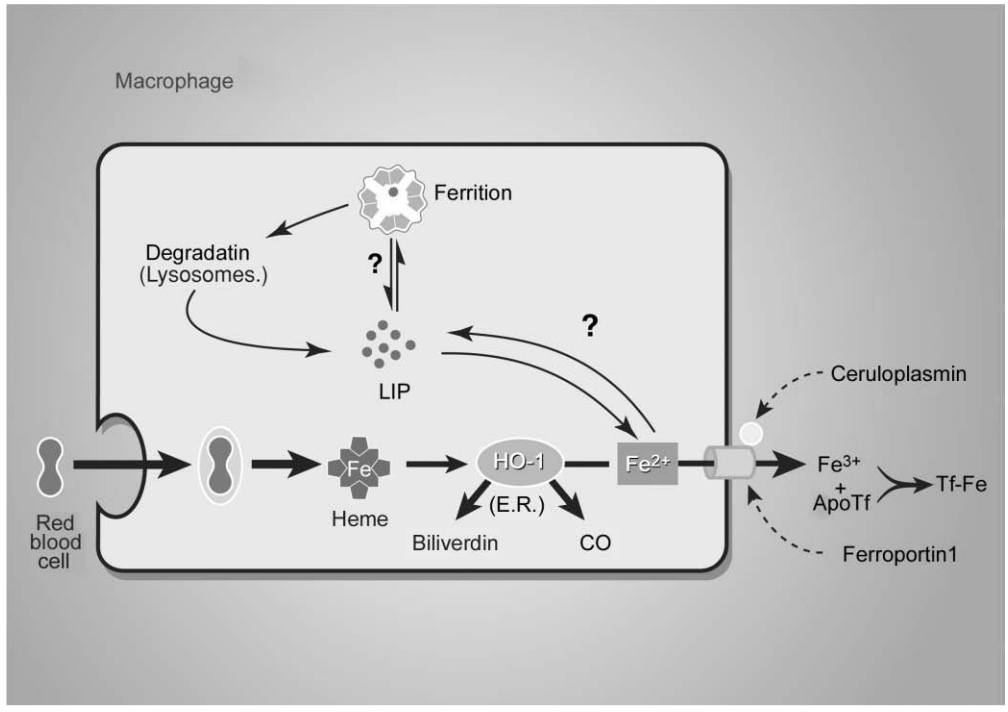

FIGURE 2 Scheme of possible iron pathways in reticuloendothelial macrophages involved in the recycling of hemoglobin iron. After phagocytosis of senescent red blood cells (RBC), the erythrocyte membrane is lysed, and heme is transported to the endoplasmic reticulum (ER) to be degraded by heme oxygenase-1 (HO-1). Most of the iron derived from hemoglobin catabolism is promptly returned to the circulation, likely being transported across the plasma membrane by ferroprotein 1. In Kupffer cells, ferroprotein 1 (MTP1, Ireg1) is present not only at the plasma membrane but also in the cytoplasm (Abboud and Hailey, 2000).

on ligands and reducing agents present in the diet. Ascorbic acid is the most powerful promoter of nonheme iron absorption, which is also enhanced by the organic acids (e.g., citric acid and amino acids). On the other hand, compounds that form insoluble complexes with iron (e.g., phosphates, phytates, and tannin) prevent absorption. Similarly, conditions in which there is a failure of gastric acid secretion (e.g., atrophic gastritis) may significantly reduce the availability of iron for absorption (Miret *et al*., 2003).

The process of inorganic iron absorption is not fully understood, but a compelling candidate for iron transporter has recently been identified. DMT1, which is involved in iron transport across the endosomal membrane (see previously), is also a principal transporter of iron in the intestine (Canonne-Hergaux *et al*., 1999; Gunshin *et al*., 1997). DMT1 transports only the ferrous (reduced) form of iron, and this explains why reducing agents enhance iron absorption. Moreover, the duodenal brush border contains a ferric reductase, Dcytb (McKie *et al.*, 2001), which has been proposed to play a role in the formation of Fe(II) before its transport into the enterocyte. However, recently, Andrews and coworkers inactivated the murine *Dcytb* gene and showed that Dcytb deficiency did not impair intestinal iron absorption when mice were fed normal chow (Gunshin *et al*., 2005). Hence, Dcytb does not seem to be an essential component of the intestinal iron absorption system in mice. The chemical nature of iron in the labile intermediate pool in enterocytes is unknown, but recently a protein necessary for iron egress from enterocytes was identified. This protein, ferroportin 1 (Abboud and Hailey, 2000; Dononvan *et al*., 2000; McKiet *et al*., 2000), is identical to the Fe(II) exporter involved in iron egress from macrophages (see Figure 2). The ferroxidase activity of hephaestin (Anderson *et al*., 2002; Vulpe *et al*., 2001), a membrane-bound ceruloplasmin homolog, also plays an important role in iron export from intestinal epithelial cells to the circulation (Hellman and Gitlin, 2002). Hephaestin is not an iron transporter itself but likely interacts with the ferroportin1 to facilitate the movement of iron across the membrane (Figure 3). Hephaestin is mutated in sex-linked anemia (*sla/sla*) mice that take up iron from the intestinal lumen into the epithelial cells normally, but the subsequent exit of iron into the circulation is diminished (Vulpe *et al*., 2001). It is of interest that during the process of absorption, iron undergoes at least two changes in its oxidation status: reduction at the brush border and oxidation at the basolateral membrane.

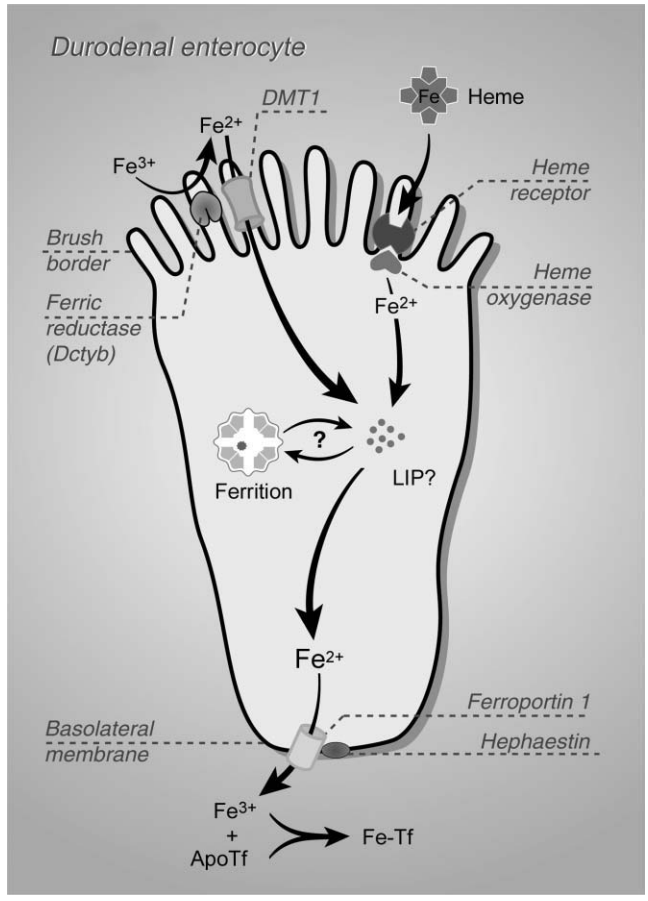

FIGURE 3 Iron transport across the intestinal epithelium. Iron must cross two membranes to be transferred across the absorptive epithelium. The apical transporter has been identified as DMT1/Nramp2. It acts in concert with Dcytb, which reduces ferric iron to more malleable Fe^{2+} ions. The basolateral transporter, ferroprotein 1, requires ferroxidase activity of hephaestin (ceruloplasmin-like molecule) for the transfer of iron to the plasma. Hephaestin is depicted here at the basolateral surface of the cell, but it is not known whether it functions at this location. Heme iron is taken up by a separate process that is not well characterized. Excess iron within enterocytes is stored as ferritin.

Physiologically, the two major factors affecting iron absorption are the amount of body iron stores and the rate of erythropoiesis (Miret *et al*., 2003). The uptake of iron by mucosal cells is inversely proportional to total body iron content but seems to be independent of changes in plasma iron or transferrin concentration. The 3′ untranslated region (UTR) of mRNA for DMT1 expressed in intestinal cells contains the IRE (Canonne-Hergeaux *et al*., 1999); hence, on the basis of the IRE/IRP paradigm (see later), diminished iron levels would be expected to increase DMT1 expression and vice versa. It is unclear how increased erythropoietic activity (increased plasma iron turnover?) enhances iron absorption. Hypoxia can directly stimulate iron absorption, independent of changes in erythroid activity. Interestingly, the gene for DMT1 seems to contain regulatory elements that can be responsible for its increased transcription under hypoxic conditions (Lee *et al*., 1998).

Recent research, on the basis of genetic studies, revealed that hepcidin, a peptide synthesized and secreted by the liver, plays an important role in iron metabolism. In its presumed active form, hepcidin is a 25-amino acid peptide that has intrinsic antimicrobial activity (Ganz, 2005; Park *et al*., 2001). Mice that are unable to express hepcidin develop iron overload associated with decreased iron in macrophages (Nicolas *et al*., 2001; 2002), whereas animals that overexpress hepcidin develop lethal iron deficiency anemia (Nicolas *et al*., 2002). Hence, it seems (Ganz, 2005) that hepcidin may be a long-sought hormone mediating communication between the sites of iron storage (hepatocytes and macrophages) and iron release from duodenal enterocytes or macrophages. Indeed, high iron levels were shown to stimulate hepcidin production, whereas iron deficiency suppresses hepcidin synthesis (Ganz, 2005). Recently, hepcidin binding to ferroportin overexpressed in HEK293 cells was demonstrated. After binding,

ferroportin was internalized and degraded, leading to decreased export of cellular iron (Nemeth *et al*., 2004). Hence, iron regulates the secretion of hepcidin that, in turn, controls ferroportin levels on the cell surface.

5.6 Control of Cellular Iron Homeostasis

Cells are equipped with a remarkable regulatory system that tightly controls iron levels in the "labile iron pool" (LIP), presumably representing iron-in-transit among various intracellular compartments. Sensitive control mechanisms exist that monitor iron levels in the LIP and prevent its expansion, while still making the metal available for iron-dependent proteins and enzymes. In general, enlargement of the LIP leads to a stimulation of ferritin synthesis and to a decrease in the expression of transferrin receptors, but the opposite scenario develops when this pool is depleted of iron. Pivotal players in this regulation are iron regulatory proteins (IRP1 and IRP2), which "sense" iron levels in the LIP.

Iron-dependent regulation of both ferritin and transferrin receptors occurs posttranscriptionally (i.e., it does not involve mRNA synthesis) and is mediated by virtually identical iron-responsive elements (IREs). IREs present in the 5′ UTRs of mRNAs, as in ferritin and erythroid-specific 5-aminolevulinic acid synthase (ALA-S2, the first enzyme of heme biosynthesis), mediate inhibition of translation of the respective mRNAs in iron-deprived cells. Five similar IREs (versus a single IRE in ferritin and erythroid ALA-S2 mRNAs) are also present in the 3′ UTR of transferrin receptor mRNAs. These IREs increase the stability of transferrin receptor mRNAs as a function of cellular iron levels. The IREs are nucleotide sequences that are recognized by specific cytosolic RNA-binding proteins known as IRP1 and IRP2. An IRE-binding form of each IRP accumulates in iron-depleted cells, but the mechanism of accumulation differs. When cellular iron is low, IRP1 is in a form that can bind to IREs, and IRP2 (which has constitutive RNA binding activity) is stable. Binding of IRPs to IREs found in the 5′ end of mRNA (ferritin, erythroid-specific ALA-S) inhibits translation of these transcripts, whereas binding to IREs in the 3′ UTR of the TfR (and probably also in the intestinal form of mRNA for DMT1) stabilizes the transcripts. Hence, iron deficiency promotes cellular iron acquisition, and possibly intestinal iron absorption, while it decreases levels of the cellular iron-storing protein ferritin. On the other hand, the expansion of the LIP inactivates IRP1 and leads to a degradation of IRP2, resulting in efficient translation of ferritin mRNA (and ALA-S2 mRNA in erythroid cells) and rapid degradation of transferrin receptor mRNAs (Hentze *et al*., 2004; Mikulits *et al*., 1999; Richardson and Ponka, 1997; Rouault and Klausner, 1997).

Some cells and tissues with specific requirements for iron have probably evolved mechanisms that can override the IRE/IRP-dependent control of transferrin receptor formation. Erythroid cells, which are the most avid consumers of iron in organisms, use a transcriptional mechanism (i.e., actively synthesize receptor mRNA) to maintain very high transferrin receptor levels (Lok and Ponka, 2000; Ponka, 1997). Moreover, erythroid cells are equipped with an important regulatory mechanism that coordinates protoporphyrin IX formation with iron supply (Ponka, 1997). Because the 5′ UTR of mRNA for erythroid-specific ALAS contains the IRE (Cox *et al*., 1991; Dandekar *et al*., 1991; Melefors *et al*., 1993), the formation of ALA-S2 (the rate-limiting enzyme of porphyrin biosynthesis) and, consequently, protoporphyrin IX depends on the availability of iron.

Although the IRE/IRP system has probably evolved to sense iron-in-transit and to maintain it at appropriate levels, iron is not the only factor that modulates IRPs. Recently, nitric oxide (NO) and oxidative stress were shown to dramatically affect the binding of IRPs to IREs and, consequently, cellular iron metabolism (Hentze and Kühn, 1996; Kim and Ponka, 1999; 2000; 2002a,b; Pantopoulos *et al*., 1995; 1996). We should note, however, that this modulation of the IRE/IRP system might also involve indirect, oxidant-mediated changes in intracellular iron availability. As will be discussed later, NO-mediated changes in iron metabolism in "inflammatory" macrophages provide important clues to the pathogenesis of anemia of chronic disease.

6 PATHOPHYSIOLOGY OF IRON METABOLISM

6.1 Diseases of Iron Deficiency

Iron deficiency anemia is the most prevalent cause of anemia, affecting more than a half billion people worldwide. The anemia of iron deficiency is caused by a decreased supply of iron for heme synthesis and, consequently, hemoglobin formation in developing erythroid cells. Defective hemoglobinization leads to the production of red blood cells that are smaller than normal (microcytosis) and contain reduced amounts of hemoglobin (hypochromia). Because iron is essential for numerous hemoproteins besides hemoglobin, as well as for many nonheme iron-containing proteins, severe iron deficiency leads to a significant compromise in the cellular function of many organs. However, iron deficiency is not the only condition causing defective hemoglobinization. Decreased production of hemoglobin and, consequently, microcytic anemia can also be caused by a defect in recycling hemoglobin iron

(anemia of chronic disease), a defect in the production of protoporphyrin, or a decreased synthesis of globin.

In infants and toddlers, dietary insufficiency is the most common cause of iron deficiency. In older children and adults, blood loss is the most common cause. One milliliter of blood contains approximately 0.5 mg of iron and, hence, a steady blood loss of as little as 3–4 mL/day (1.5–2 mg of iron) can result in a negative iron balance. In men and postmenopausal women, unexplained iron deficiency is nearly always due to occult bleeding from the gastrointestinal (GI) tract. Bleeding from the upper GI tract, even of a significant degree, easily escapes the attention of both the patient and the physician. Moreover, because such bleeding is frequently intermittent, a negative result on testing for occult blood in stool does not exclude GI bleeding. Sources of GI bleeding include hiatal hernia; peptic ulceration; diverticulosis; tumors of the stomach and intestine or adenomatous polyps; colitis; esophageal varices and ingestion of salicylates; steroids; nonsteroidal antiinflammatory agents; and even heavy endurance exercise ("runners anemia"). Importantly, iron deficiency is often the first sign of an occult GI malignancy. In premenopausal women, menstrual blood loss is the most common cause of iron deficiency. Worldwide, the leading cause of GI blood loss is hookworm infection, which is very common in tropical and subtropical areas (Ponka, 2001).

Anemia of chronic disease, which is also very common, has complex pathophysiology (Weiss and Goodnough, 2005) but shares some similar features with iron deficiency anemia. Iron-deficient erythropoiesis results from a defect in the recycling of hemoglobin iron in the reticuloendothelial system. As a result, iron is plentiful in macrophages, but this iron is not available to erythroid precursors. Characteristic laboratory findings include low serum iron levels, low serum iron-binding capacity, increased serum ferritin, and normocytic or slightly microcytic erythrocytes. In patients with anemia of chronic inflammation, there seems to be a defect in the release of iron from macrophages that can be caused by several independent mechanisms.

One of us recently demonstrated that, in macrophages treated with inflammatory cytokines, increased NO production leads to rapid IRP2 degradation resulting in a dramatic upregulation of ferritin synthesis (Kim and Ponka, 2002a). Moreover, an NO-mediated increase in ferritin synthesis leads to increased sequestration of intracellular iron in ferritin. Hence, increased sequestration of iron in ferritin, when its synthesis is stimulated in macrophages of patients with chronic inflammation, provides one possible explanation for hypoferremia and, consequently, anemia (Kim and Ponka, 2002a). Moreover, inflammation is associated with increased production of hepcidin (Ganz, 2005), which, as already discussed, acts by inhibiting the efflux of iron through ferroportin (Nemeth *et al*., 2004).

6.2 Diseases of Iron Overload

Too much of a good thing can be harmful, and this is exemplified by iron overload. Iron metabolism in humans is characterized by a limited external exchange and by an efficient reutilization from internal sources. Inhalation of iron fumes (mainly iron oxides) can give rise to deposition of iron in the lungs with related roentgenological changes. The picture seen on X-ray is similar to the one in pneumoconioses like silicosis, but the deposition of iron particles in the lung does not lead to fibrosis. The roentgenological changes attributable to iron oxide exposure have been named siderosis, iron pneumoconiosis, hematite pneumoconiosis, iron pigmentation of the lung, and arc welder lung. The total amount of magnetic dust can be determined and is less that 4 mg in unexposed individuals. Arc welders exposed to iron oxide for 18 years had between 30 and 2000 mg in their lungs (Kalliomäki *et al*., 1978). As mentioned in the following, the roentgenological pulmonary changes after inhalation of iron dust (i.e., siderosis) is a benign condition, considered not to progress to fibrosis (Stokinger, 1984).

Because of the body's limited ability to excrete excess iron, iron overload may develop as a consequence of administering excess iron parenterally (e.g., by blood transfusions). Accumulation of iron caused by chronic ingestion has been reported only in those with genetic predisposition to increased iron absorption (Moyo *et al*., 1998). This is an extremely rare condition. Patients with iron overload accumulate excessive amounts of iron in various organs, including the liver, pancreas, and heart, and, consequently, they have conditions that include cirrhosis, diabetes, and heart dysfunction. Without treatment, iron overload leads to death. Secondary iron overload develops in transfusion-dependent patients, such as those with thalassemia, aplastic anemia, and myelodysplastic syndrome. Repeated transfusions lead to rapid iron loading because each unit of blood contains 200–250 mg of iron (1 mL of red blood cells contains approximately 1 mg of iron) that cannot be excreted. Because this iron is derived from red cells, reticuloendothelial macrophages become iron loaded before hepatocytes and other parenchymal cells do. The only treatment for secondary iron overload is administration of iron-chelating agents, such as desferrioxamine.

Hereditary hemochromatosis (HH) is an autosomal-recessive disorder of iron metabolism that is one of the most prevalent genetic diseases in people of Northern European descent (Adams, 2004; Feder

et al., 1996; Fleming *et al*., 2004; Pietrangelo, 2004). In this population, HH occurs in 1 in 300 people, with a remarkable carrier frequency of 1 in 10 individuals. The primary defect in HH is the inability to down-regulate intestinal Fe absorption when iron stores are high. The gene responsible for HH, which is localized on chromosome 6, has been cloned (Feder *et al*., 1996). The gene, termed HFE, encodes a predicted protein of 343 amino acids, including several cysteines that seem to be required for maintaining proper structure of the protein. Two missense mutations have been reported in the gene, both causing amino acid substitutions (C282Y and H63D), but to date only the C282Y mutation has been found to clearly correlate with HH in most affected patients. HFE is highly homologous to genes in the major histocompatibility complex (MHC) class I family. The HFE gene, like other MCH class I genes, encodes a heterodimeric protein that is complexed to β_2-microglobulin, and such coupling is essential for cell surface expression of the functional molecule. The cysteine-to-tyrosine mutation is very close to the expected site of β_2-microglobulin association and, hence, the association of HFE with β_2-microglobulin is disrupted in HH. Although the exact function of HFE gene product is unclear, it is known that there is a physical interaction of the transferrin receptor and HFE (Lebron *et al*., 1998). However, the relevance of this phenomenon to the pathogenesis of HH is not clear.

Unlike many other genetic disorders, HH is treatable. If the disease is diagnosed early enough and the patients are subjected to regular phlebotomies, their life expectancy is normal. Ideally, therapy should begin before symptoms develop, when the serum ferritin levels exceed 200 μg/L in nonpregnant, premenopausal women or 300 μg/L in men and postmenopausal women. Typically, phlebotomy is performed at a rate of 1 unit of blood per week until the patient has mild hypoferritinemia. Thereafter, it is continued as needed to keep the serum ferritin <50 μg/L. On average, men require phlebotomy three or four times per year, and women require it one or two times per year.

Table 2 shows that genetic iron overload can have causes other than the mutation of the HFE gene. Although non-HFE iron-overloading conditions are rare, analyses of the pathological effects caused by specific gene mutations offer important insights into the functions of the products of these genes.

6.3 Mechanisms of Tissue Damage in Iron Overload

Damage to cells and organs arising from chronic iron overload is remarkable in its ability to affect a wide range of tissues (both replicative and nonreplicative) and often causes a slow and insidious onset of organ dysfunction. Foremost among the organs and cell types affected by iron overload are the liver, heart, and pancreatic beta cells. Hepatic disease (e.g., cirrhosis and hepatoma) causes a large number of premature deaths among patients with primary hemochromatosis (Niederau, 1985), whereas heart failure is most frequent in patients with secondary hemochromatosis (Aldouri *et al*., 1990; Gabutti and Borgna-Pitnatti, 1994). Both groups share a substantially increased risk for development of diabetes compared with the normal population (Gabutti and Borga-Pitnatti, 1994).

In the etiology of this organ damage, it may be important that the liver and heart have a high steady-state production of superoxide [O_2^-] and hydrogen peroxide [H_2O_2] (Chance *et al*., 1979), largely derived from mitochondrial activity (Harman, 1983). The pancreatic beta

TABLE 2 Genetic Iron Overload

Disease	Inheritance	Mutated Gene	Population Frequency	Main Clinical Features	Reference
Hereditary hemochromatosis (HH type 1)	AR*	HFE	1:250	Liver disease	Feder *et al*., 1996; Pietrangelo, 2004
HH type 2	AR	a) Hemojuvelin	Rare	Heart disease	Papanikolaou, 2004
(Juvenile H)	AR	b) Hepcidin	Rare	Heart disease	Roetto *et al*., 2003
HH type 3	AR	TfR2	Rare	Liver disease	Camaschella *et al*., 2000
HH type 4	AD	Ferroportin 1	Rare	Liver disease (↑ Fe in macrophages)	Pietrangello, 2004
Atransferrinemia	AR	Transferrin	Rare	Liver disease	Ponka, 2002
African Fe overload	AR	Unknown	Common	Liver disease	Gordeuk, 2002

* AR, autosomal recessive; AD, autosomal dominant

cell is similarly rich in mitochondria and quite sensitive to oxidant-generating xenobiotics (such as alloxan and streptozotocin) and exogenous oxidants (Olejnicka *et al*., 1997; Zhang *et al*., 1995). Affected tissues of patients with iron overload contain swollen mitochondria with an electron-dense matrix and ruptured mitochondrial membranes (Thakerngpol *et al*., 1996). It is highly likely that the pathologies caused by iron overload involve iron-driven oxidation reactions, but the slow onset implies some kind of biological "memory" by which the cumulative damage is ultimately translated into organ failure. Interestingly, ultrastructural abnormalities similar to those observed in iron overload, including mitochondrial swelling and disintegrating cristae, have been observed in the hearts of rats treated with mitoxantrone (an anthracycline analog that is thought to have an iron-dependent toxicity) (Herman *et al*., 1997), in the pancreas of rats poisoned with dietary carbonyl iron and ethanol (Tandler *et al*., 1996), and in the hearts of mice iron-loaded by repetitive injections of iron-dextran (Bartfay, 1999).

In the heart, an early accumulation of iron occurs predominantly in the epicardium (Olson *et al*., 1987). Later on, stainable iron assumes a sarcoplasmic localization, but the precise subcellular distribution of iron has not been well defined. Diastolic dysfunction appears early in the course of iron overload, whereas systolic dysfunction occurs very late (Liu and Olivier, 1994). The possibility that redox reactions involving mitochondria may be important is supported by observations that other transition metals, such as copper, may engender similar cardiac damage. In Wilson's disease—and in copper-overloaded dogs—mitochondria accumulate copper (Sokol *et al*., 1994) and ~30% of these patients develop cardiac problems such as left ventricular hypertrophy (Kuan, 1987).

Somewhat paradoxically, the cardiac effects of iron *deficiency* show a similarity to those of iron overload. In iron-deficient rats, there is a marked cardiac hypertrophy (especially, left ventricular) along with enlarged mitochondria with abnormal matrices and deficient succinate dehydrogenase activity (Tanne *et al*., 1994). Furthermore, it seems that damage to mitochondrial DNA (mtDNA) may accumulate in both iron-deficient and iron-overloaded rats (Walter *et al*., 2002). Thus, some of the cellular abnormalities at both extremes of abnormal iron balance *may* reflect mitochondrial dysfunction, and progressive damage to mitochondrial DNA *may* provide a clue as to the nature of the biological "memory" involved in the slow organ damage in iron overload.

6.3.1 Compensatory Responses to Oxidation/Iron Overload May Limit Early Damage

Early in the course of iron overload, numerous homeostatic mechanisms likely prevent damage from the accumulating iron. These include ferritin induction (as discussed previously), which limits the availability of redox active iron and suppresses iron-catalyzed oxidant damage to cells (Balla *et al*., 1992; 1993; Eaton and Qian, 2002; Richardson and Ponka, 1997). Similarly, certain antioxidant enzymes may be induced when cells are challenged with "free" iron coupled with endogenous redox agents. However, as discussed elsewhere (Scott and Eaton, 1997), increments in antioxidants and/or antioxidant enzymes may be of minimal importance in prevention of oxidant damage promoted by transition metals such as iron. In part, this is because, paradoxically, cellular-reducing species such as reduced glutathione (GSH) can actually promote iron-mediated oxidative reactions (Scott and Eaton, 1995). As a possible example of this, H_2O_2-mediated damage to the DNA of isolated mitochondria is actually *decreased* by prior GSH depletion and, in isolated nuclear DNA, *increased* by added GSH (Giulivi and Cadenes, 1998). Thus, although GSH and other cellular-reducing species can act as antioxidants, these same reducing agents can have prooxidant effects when acting as part of a metal-catalyzed redox couple.

There is, however, at least one exception to the rule that antioxidant enzymes are of minor import in protection against iron damage: mitochondrial manganese superoxide dismutase (MnSOD). Rats fed carbonyl iron show significant increases in hepatic MnSOD activity similar to those that occur in a variety of experimental settings wherein mitochondrial oxidation is increased (Brown *et al*., 1998). Furthermore, mice heterozygous for deficiency of MnSOD with normal iron balance exhibit progressive mitochondrial damage as reflected by deficiencies in iron-sulfur enzymes, an interesting parallel with the abnormalities found in the iron-loaded mitochondria of patients with Friedreich ataxia (*vide infra*) (Li *et al*., 1995; Melov *et al*., 1998; 1999). Interestingly, MnSOD-deficient mice also have an accumulation of oxidative damage to mtDNA (Melov *et al*., 1999), and homozygotes die very early with dilated cardiomyopathy (Li *et al*., 1995; Williams *et al*., 1998).

6.3.2 Iron-Driven Cellular Damage Involves Oxidative Reactions

Because iron amplifies oxidant damage, the preferential damage to organs with greater aerobic metabolism in iron overload disorders is probably no coincidence. This is because iron per se is relatively nontoxic in the absence of agents that affect the active oxidation and reduction of the metal. Chelators that keep iron in one valence (such as Fe(III)) effectively block iron-mediated oxidant damage (Graf *et al*., 1984) as do those

that occlude all six coordination positions of the metal (Graf *et al*., 1987).

However, the simple invocation of the potent hydroxyl radical (·OH) in iron-mediated damage may be overly simplistic. Given the evanescent and highly reactive nature of this radical, it would be unlikely to do substantial damage to any single cellular component. Rather, the fundament of iron-mediated oxidant damage may more likely involve the tendency of "free" iron to associate with oxidizable targets within cells (e.g., polyunsaturated fatty acids, proteins, or DNA) and ensuing site-specific oxidation reactions. These latter reactions probably represent tripartite reactions arising from complexes of iron bound to particular target molecules and redox equivalents (oxidants/reductants) that, on reaction with the iron, form highly oxidizing intermediates (ferryl or perferryl) that directly attack the substrate (Bielski, 1992). But which substrates?

6.3.3 Polyunsaturated Fatty Acids

In acute *in vitro* models of iron damage, peroxidation of polyunsaturated fatty acids (PUFA) within membrane phospholipids may be a crucial event, because antioxidants that rather specifically prevent PUFA oxidation will also block cell death (Balla *et al*., 1990a; 1990b; 1991). In *in vivo* models of iron overload, greatly increased oxidation of PUFA within hepatic mitochondria—accompanied by substantial decrements in mitochondrial metabolism—have been reported (Bacon *et al*., 1983a; 1983b; 1985; 1986; 1988; Britton *et al*., 1994). In an *in vitro* system involving iron loading of primary cultures of newborn rat cardiomyocytes, similar iron-mediated PUFA oxidation and mitochondrial dysfunction have been observed (Link *et al*., 1999). However, most products arising from PUFA oxidation (with the possible exceptions of DNA adducts and lipofuscin) are readily cleared, suggesting that oxidation of fatty acids may not explain the long-term cumulative damage done by iron overload.

6.3.4 DNA

It was shown many years ago that oxidant-induced damage to naked DNA and intracellular DNA is greatly enhanced by iron (Jackson *et al.,* 1987; Schraufstatter *et al*., 1988), and in the absence of transition metals (such as iron and copper), DNA is quite unreactive with oxidants such as H_2O_2. Interestingly, in the presence of added iron, the scission of DNA still packaged in nucleosomes occurs preferentially in internucleosomal linker regions (Enright *et al.,* 1992), producing "ladders" resembling those of classical apoptosis (Enright *et al*., 1994). Iron-mediated DNA damage can be decreased or completely prevented by iron chelators, which fill all six coordination positions and make the iron chemically nonreactive (Graf *et al*., 1987; Hermes-Lima *et al*., 1998).

The products of iron-mediated DNA damage are not fully characterized but include strand breaks, oxidatively modified bases, DNA-protein cross-links (Altman *et al*., 1995; Zastawny *et al*., 1995), and covalent addition products involving lipid peroxidation products (Vaca and Harms-Ringdahl, 1989) and other structurally uncharacterized bulky DNA lesions (these latter detected using ^{32}P after labeling in hepatic DNA from patients with hereditary hemochromatosis [Carmichael *et al*., 1995]). The relative importance of all these products in long-term or irreversible oxidative DNA damage is not yet known. Possibly relevant to the theory that DNA damage may provide the biological "memory" required to explain slow organ damage caused by iron overload, increased damage to mtDNA (estimated as changes in the ratio of "fragmented" [form I] to supercoiled [form II] mtDNA) has been reported to occur in iron-supplemented rats (Walter *et al*., 2002).

6.3.5 Proteins

As is true of PUFA and DNA, proteins also are generally resistant to oxidation by simple oxidants such as H_2O_2 in the absence of transition metals. However, in the presence of such metals, oxidative damage to proteins includes loss of histidine residues, bityrosine cross links, oxidative scission, the introduction of carbonyl groups, the formation of protein-centered alkyl, alkylperoxyl, and alkoxyl radicals, and—in the case of metalloproteins—oxidative destruction of heme prosthetic groups (e.g., Berlet and Stadtman, 1997). Sohal and colleagues have made the interesting observation that two mitochondrial proteins (aconitase and adenine nucleotide translocase) may be important targets of long-term oxidative damage (Yan and Sohal, 1998; Yan *et al*., 1997). However, with a few exceptions, protein damage is likely to be a reparable and nonlethal event for a cell and, therefore, by itself may be insufficient to explain long-term organ damage arising from chronic iron overload.

6.3.6 Iron-Mediated Damage to Mitochondria

Once again, the question is: How might iron-catalyzed oxidation of DNA, PUFA, and/or proteins eventuate in the progressive organ damage characteristic of chronic iron overload? A number of considerations raise the possibility that iron overload selectively targets mitochondria and, perhaps, the mitochondrial genome. As reviewed previously, damage caused by transition metals such as iron requires the conspiracy of cellular oxidizing and reducing substances. In this regard, it is probably important that the mitochondrial

electron transport chain "leaks" 1–2% of its electrons into O_2^-/H_2O_2 (Turrens *et al*., 1985); however, more recent work (Hansford *et al*., 1997; St-Pierre *et al*., 2002) suggests lower estimates. This is the source of ~90% of the activated oxygen generated by most cell types. Consequently, assuming that mitochondria within cells of iron-overloaded animals are also exposed to elevated iron, it would seem likely that these organelles might be severely damaged.

Indeed, there is some evidence that damage arising from elevated intracellular iron preferentially targets mitochondria. Iron loading of cultured rat myocardial cells is accompanied by a decrease in membrane PUFA content, inhibition of thiol-dependent enzyme activities, lowered ATP, and enhanced lysosomal fragility (Link *et al*., 1993a; 1993b; 1994). Similar changes in mitochondrial energy production with iron loading have also been observed in the livers of rats with chronic iron overload (Bacon *et al*., 1993c). In addition, in a cultured rat myocardial cell model there is a substantial inhibition of NADH-cytochrome *c* oxidoreductase (complex I–III) and succinate dehydrogenase (Link *et al*., 1998; 1999).

One analog of chronic iron overload may be Friedreich ataxia, which is thought to involve an abnormal accumulation of iron within mitochondria. As is the case in chronic iron overload, many of these patients develop diabetes and hypertrophic cardiomyopathy (Harding, 1981). This congenital disorder arises from a mutation of the protein frataxin, normally targeted to the mitochondrial inner membrane and apparently responsible for maintenance of intramitochondrial iron balance (Babcock, 1997; Becker and Richardson, 2001; Napier *et al*., 1995; Radisky *et al*., 1999; Rotig *et al*., 1997), perhaps through enhancing Fe-S cluster biosynthesis (Chen *et al*., 2003). Affected mitochondria show not only iron accumulation but also inactivation of iron-S enzymes—complexes I, II, and III and aconitase (Napier *et al*., 2005). Importantly, similar damage to mitochondrial metabolism results from iron loading of normal myocytes *in vitro* (Link *et al*., 1998) and hepatocytes *in vivo* (Bacon *et al*., 1993c).

If the slow appearance of organ damage in chronic iron overload does arise from cumulative mitochondrial dysfunction, it would seem reasonable that in patients with this disorder the progression of iron-mediated damage might take a period of years, but the consequences could become manifest precipitously. In fact, patients with chronic iron overload can undergo a rapid deterioration of systolic function, leading to heart failure and death within a few days (with an ejection fraction of >50% falling to <30% over this brief period) (Liu and Olivieri, 1994). It is possible (but far from certain) that such a cataclysmic event represents acute "mitochondrial failure" arising from years of damage to the mtDNA of a sort that cannot be reversed even by successful chelation therapy (e.g., Westra *et al*., 1993). If there is anything to this pathogenic mechanism, it suggests the possibility of a feed-forward mechanism in which mitochondria with sufficiently damaged genomes may become factories for the production of increased amounts of activated oxygen species that, in turn, further accelerate organ damage.

There are certain links, albeit tentative, to a possible involvement of iron accumulation and mitochondrial DNA damage in the general process of aging (Beckman and Ames, 1997; 1998). For instance, the prolongation of lifespan by dietary restriction (Sohal and Weindruch, 1996) is accompanied by decreased age-dependent iron accumulation in rats (Cook and Yu, 1998) and probably other species. More directly, inhibition of iron absorption in *Drosophila melanogaster* has been reported to prolong life expectancy by >20% (Massie *et al*., 1993), and similar longevous effects in house flies were achieved by administration of the chelator, diethyldithiocarbamate (Sohal *et al*., 1984). These considerations raise the possibility that organ damage in chronic iron overload may be, in a sense, iron-driven progeria.

6.3.7 Iron-Mediated Destabilization of Lysosomal Membranes

Iron-mediated destabilization of lysosomal membranes represents an alternative and perhaps additive mechanism of damage involving accumulation of iron within the cellular lysosomal compartment, which sensitizes lysosomes to damage by endogenous oxidants such as H_2O_2. The resultant destabilization of the lysosomal membrane can lead to the release of damaging lysosomal digestive enzymes and iron into the cytoplasm of the cell (Brunk *et al*., 2001; Persson *et al*., 2003; Yu *et al*., 2003). In fact, it has recently been reported that lysosomal iron, released by oxidant stress, leads to extensive damage to nuclear DNA, but it is not yet known whether this extends to mtDNA (Kurz *et al*., 2004; Tenopolou *et al*., 2004). The potential importance of lysosomal instability has also been suggested by others (Link *et al*., 1993b), and iron-mediated lysosomal instability and enhanced lipid peroxidation do occur in animal models of iron overload (Peters *et al*., 1986). *In vitro* experiments also indicate that iron loading leads to increased fragility of lysosomal membranes (Link *et al*., 1993b; Mak and Weglicki, 1985), a proposition further supported by early observations made on hepatic biopsy material from hemochromatotic patients (Seymour and Peters, 1978). In fact, Stal and colleagues (1990) suggest that the changes in hepatic lysosomal volume density

in idiopathic hemochromatosis correlate very well with the extent of iron overload and are effectively reversed on iron removal. However, it is not established whether, *in vivo*, these lysosomal abnormalities are an important pathophysiological factor.

7 "CARCINOGENIC" EFFECTS

7.1 Role of Iron in DNA Synthesis and Cell Proliferation

The requirement for iron in tissue culture medium has long been known (Bomford *et al*., 1986; Hoffbrand *et al*., 1976; Lederman *et al*., 1984; Morgan *et al*., 1950; White, 1955), but probably the first convincing experimental evidence demonstrating that iron plays a crucial role in the initiation and maintenance of DNA synthesis was provided by Robbins and Pederson (1970). They showed the presence of iron in a nuclear fraction, perhaps associated with a polysaccharide. Even more importantly, these authors reported significant inhibition of DNA synthesis in HeLa cells by the iron chelator desferrioxamine. This has been confirmed and elaborated in various cell types, using not only desferrioxamine but also other iron-chelating agents such as 3,4-dihydroxypyridine (Tsai and Ling, 1971), picolinic acid (Fernandez-Pol *et al*., 1977), aroylhydrazones of pyridoxal and salicylaldehyde (Laskey *et al*., 1988; Richardson *et al*., 1995), and numerous others (Kontoghiorges *et al*., 1986). These, and many studies that followed, have shown that iron limitation arrests cells in the G1 phase of the cell cycle, but there are reports indicating that the progression of cells through S phase and perhaps beyond is iron-dependent (Kühn *et al*., 1990; Le and Richardson, 2002).

One plausible link between iron and cell proliferation is the enzyme ribonucleotide reductase, which produces the four deoxyribonucleotides from the corresponding ribonucleotides (the rate-limiting reaction in the synthesis of DNA precursors and, therefore, a key control point in DNA synthesis). In mammalian cells, the enzyme is composed of two nonidentical protein subunits: R1, the catalytic subunit that binds ribonucleotides; and R2, which requires Fe(III) for the stabilization of a tyrosyl radical. Inhibitors of ribonucleotide reductase block DNA synthesis and cell replication; one such inhibitor is the iron chelator desferrioxamine (Hershko, 1994), which is thought to act by withholding iron from the nonheme iron subunit of the enzyme. In addition to this "nutritional" requirement of proliferating cells for iron, this metal seems to play some kind of "signaling" role in the cell cycle: iron chelators were shown to decrease levels of cyclins in various cell lines (Le and Richardson, 2002).

7.2 Evidence That Iron Promotes Carcinogenesis in Humans Is Lacking

The aforementioned observations are likely responsible for a myth, expressed implicitly or explicitly in numerous review articles, that iron in excess promotes carcinogenesis. Two strong arguments against this idea exist. First, the fact that iron is essential for DNA synthesis and cell proliferation provides no support for the conclusion that iron is involved in carcinogenesis. Second, clinical evidence that iron in excess plays a role in pathogenesis of cancers is lacking. The only iron overloading disease, "classical" HH (see Section 6.2), is the sole condition that can lead to one type of cancer, hepatocellular carcinoma (Harrison and Bacon, 2005), which occurs in 19–24% of HH (Kew *et al*., 1990). However, iron overload alone is unlikely to cause hepatocellular carcinoma, as documented by the absence of hepatocellular carcinoma in patients with iron overload caused by repetitive transfusions (Kew and Popper, 1984). In addition, the presence of cirrhosis caused by iron overload seems to be an important factor in hepatocarcinogenesis. This view is supported by reports documenting that the risk of hepatocellular carcinoma persists in cirrhotic patients depleted of excess iron stores (Fargion *et al*., 1992; Niederau *et al*., 1985). There is no evidence that patients with HH would have a higher incidence of extrahepatic tumors (Bradbear *et al*., 1985; Elmberg *et al*., 2003).

There are occasional reports that repeated injections of iron dextran has induced sarcomas in animals, leading to speculation that at least some sarcomas in humans could be caused by intramuscular injections of iron. However, Fielding (1977) analyzed nine human cases reported in a period of approximately 30 years (since 1945) and concluded that there was no causal relationship between sarcomas and iron injections in humans (see also Chapter 10). Similarly, some reports claimed that hematite miners and iron foundry workers had a higher incidence of lung cancer mortality than the rest of the population. However, Stokinger (1984), who reviewed the world literature, has concluded that no existing evidence supports the notion that inhaled iron could play a pathogenic role in lung cancer in humans. (The carcinogenic effects of concomitant exposure to agents such as radon and polyaromatic hydrocarbons are discussed in Chapter 10.) That said, the jury is still out on this question, and we have recently reported that iron complexed with free fatty acids (found abundantly in cigarette smoke) readily enters cells. Furthermore, the imported iron leads

to mutations (in the gene for hypoxanthine/guanine phosphoribosyl transferase) and to transformation of cells into an anchorage-independent phenotype generally equated with tumorigenesis (Gao *et al*., 2005).

8 IRON POISONING

8.1 Introduction

Despite being available without a prescription, iron is more hazardous than most prescription drugs and is the most common cause of poisoning death in young children (Litovitz and Manoguerra, 1992). However, iron poisoning is not confined to this age group, because purposeful overdoses occur in both adolescents and adults that result in significant morbidity and mortality (Watson *et al*., 2004). Within the realm of acute poisonings, iron is unique in that it is not a xenobiotic. Because iron is highly reactive, there is a need for complex mechanisms for its absorption, transport, and storage. These mechanisms are reviewed elsewhere in this chapter. However, the pharmacokinetics of iron has not been fully characterized, and its toxicokinetics are even less well understood. Because of this, there is not uniform agreement regarding the toxic dose, the optimal method of gastrointestinal decontamination, the role of intragastric complexation therapies, or the use of the specific iron chelator, deferoxamine. More specifically, the indications, dose, duration of therapy, and efficacy of this antidote are not precise.

8.2 Iron Preparations

Iron, either alone or in combination with vitamins and other minerals, is available as ferrous salts. These are ferrous gluconate (12% elemental iron), ferrous sulfate (20% elemental iron), ferrous fumarate (33% elemental iron), and ferrous succinate (35% elemental iron). The proportion of iron in each salt is important, because the toxicity of iron is a function of the dosage of elemental iron ingested. Carbonyl iron is highly purified metallic iron that is uncharged and not a salt. There have been no reports of significant morbidity or of mortality from this type of iron (Madiwale and Liebelt, 2006).

The absorption, transport, and storage of iron has been discussed previously and is reviewed elsewhere (Bezkorovainy, 1989a,b; Finch and Huebers, 1986; Harju, 1989). Typically 10% of the ingested dose is absorbed; however, the proportion is highly variable and is a function of iron stores and the ingested dose. Because iron absorption is an active, saturable process, as the dose increases, the percentage absorbed decreases. In massive overdose, a relatively small proportion (as little as 15%) is absorbed (Reissman *et al*., 1955). A failure to appreciate this factor has contributed to the controversies regarding antidotal therapy. Peak serum iron concentrations occur within 4–6 hours and half-life is approximately 6 hours (Harju, 1989). This decline is due to tissue redistribution, with the liver being the prime site. Within the vascular compartment, iron is tightly bound to transferrin. For toxicity to occur, the capacity of transferrin binding must be exceeded. However, free plasma iron does not occur; it complexes with other plasma proteins and organic ligands. This substance, known as nontransferrin-bound iron, is loosely bound and readily causes toxicity (Arouma *et al*., 1988; Gutteridge *et al*., 1985; Hershko and Peto, 1987).

8.3 Pathophysiology

Iron toxicity is best thought of in terms of local and systemic effects. The former is a result of local irritation of the gastrointestinal tract. The latter consists of tissue damage and organ dysfunction. Gastrointestinal irritation manifests as nausea, vomiting, diarrhea, and abdominal pain. As little as 10–20 mg/kg of elemental iron may produce these symptoms; however, this dose is too small to cause significant systemic toxicity, which requires at least 40 mg/kg but typically >60 mg/kg of elemental iron (Banner and Tong, 1986; Hernretig and Temple, 1984). A lethal dose is estimated to be 200–250 mg/kg (Henretig and Temple, 1984; Robotham and Leitman, 1980). However, deaths have been documented with doses as low as 75–125 mg/kg of elemental iron (Olenmark *et al*., 1987; Smith *et al*., 1950; Spencer, 1951; Thompson, 1947).

Toxicity is a consequence of free radicals generated by iron catalysis (Halliwell and Gutteridge, 1986). Target organs and tissues are sites of high iron concentration and metabolic activity. The gastrointestinal tract is a prime site, because this is where the highest concentration of iron occurs. Necrosis and hemorrhage can be significant (Tenenbein *et al*., 1990). Significant gastrointestinal toxicity may occur without systemic toxicity, but it may not occur in patients who succumb to iron poisoning (Reissman *et al*., 1955). The chief sites of systemic toxicity are the heart (Tenenbein *et al*., 1988) and the liver. The liver is susceptible because, unlike other tissues and organs, it is capable of clearing nontransferrin-bound iron (Craven *et al*., 1987; Wright *et al*., 1986). Hepatoxicity in iron poisoning has been well described (Robertson and Tenenbein, 2005; Tenenbein, 2001).

8.4 Clinical Presentation

Iron poisoning is divided into five clinical stages (Banner and Tong, 1986). These include gastrointesti-

nal toxicity, relative stability, circulatory shock, hepatotoxicity, and gastrointestinal scarring. Patients will not necessarily experience all five stages, but onset is a function of time because ingestion.

8.4.1 Gastrointestinal Toxicity

Onset of gastrointestinal symptoms and signs occurs within a few hours of iron ingestion and includes abdominal pain, vomiting, diarrhea with hematemesis, and hematochezia. The gastrointestinal hemorrhage may be life-threatening, and intestinal infarction has been reported (Tenenbein *et al*., 1990).

8.4.2 Relative Stability

The gastrointestinal symptoms and signs typically abate after a few hours, and there is often a period of several hours before the onset of circulatory shock and acidosis. During this period, a stage of relative stability, the patient's condition seems improved. However, with careful assessment of the patient, abnormalities are found. There will be subtle evidence of hypovolemia (tachycardia, decreased peripheral perfusion) and likely a mild metabolic acidosis. It is important to differentiate this stage from that of the patient who has had gastrointestinal toxicity but is not destined to have systemic toxicity.

8.4.3 Circulatory Shock and Metabolic Acidosis

Toxic shock is the most common cause of death in iron poisoning. Its pathogenesis is multifactorial. Early on, during the first 12 hours or so, it is hypovolemic because of fluid and blood loss from the gastrointestinal tract. This may be aggravated by a coagulopathy that is short term and hyperferremia related (Tenenbein and Israels, 1988). However, the most common type of shock is distributive, a key component of systemic toxicity. Its onset occurs within 6–24 hours of iron poisoning. Later on, typically >24 hours after ingestion, cardiogenic shock may occur (Tenenbein *et al*., 1988). This is a consequence of the direct effect of iron on the heart and of the profound metabolic acidosis associated with iron poisoning.

Hypoperfusion is a contributor to the metabolic acidosis of iron poisoning; however, it is primarily caused by the hyperferremia. After the binding for iron in the plasma is exceeded, the final remaining defense is simple hydration:

$$Fe^{+++} + 3H_2O \rightarrow Fe(OH)_3 + 3H^+$$

Therefore, one ferric ion generates three protons. Hence, a large amount of bicarbonate is required to treat this acidosis (Vernon *et al*., 1989).

8.4.4 Hepatotoxicity

Acute hepatic necrosis is the second most common cause of death caused by iron poisoning. It is characterized by hypertransaminasemia with enzyme concentrations in the several thousands of international units per liter (Robertson and Tenenbein, 2005; Tenenbein, 2001). When this occurs, the prognosis is ominous, because the site of damage is zone 1 of the hepatic acinus. These are the hepatocytes possessing the capacity for hepatic regeneration, which differs from acetaminophen poisoning, despite similar transaminase concentrations. The prognosis for the latter is less severe because the site of damage is zone 3 of the hepatic acinus, the daughter cells.

8.4.5 Gastrointestinal Scarring

Patients who have reached the stage of gastrointestinal scarring present with symptoms and signs of gastrointestinal obstruction. The classic lesion is pyloric stenosis, but obstruction may develop anywhere within the gut (Tenenbein *et al*., 1990). The condition is due to ongoing inflammation and healing with contracture and stenosis. Patients who develop this complication typically have persistent abdominal pain during its development. The abdominal pain that accompanies iron poisoning is otherwise self-limited and resolves within the first 24 hours.

8.5 Iron Overdose During Pregnancy

Iron overdose during pregnancy is not an uncommon event (Rayburn *et al*., 1984) because pregnant women are often treated with iron. The consequences for the mother are no different than those in the nonpregnant patient. However, the fetus is protected from hyperferremia, because transplacental iron passage is an active, saturable process. Thus, the risk to the fetus is secondary to the maternal toxicity and not to a direct effect from the iron. The presence of a fetus does not alter the treatment for the mother (Tenenbein, 1990).

8.6 Laboratory Evaluation

The key laboratory tests for iron overdose are serum iron and blood gas determinations. The serum iron verifies that there has been an iron overdose, and it prognosticates severity. Peak serum concentrations occur within 4–6 hours. Often, serial measurements, every 1 or 2 hours, are required to define the peak. Values less than 90 µmol/L (500 µg/dL) are not associated with systemic toxicity. Moderate toxicity is associated with 90–180 µmol/L (500–1000 µg/dL) and values >180 µmol/L (1000 µg/dL) are associated with severe systemic toxicity. Serum iron >1800 µmol/

L (10,000 μg/dL) is usually fatal, although survivors with this concentration have been reported. Serum iron declines rapidly after 12 hours, although the patient may remain very ill during this time. Therefore, serum iron concentrations are of no value in following the clinical course. However, the arterial pH is of great importance during this time, because it is an indicator of ongoing toxicity. Other laboratory tests of value include the complete blood count, coagulation profile, and clinical chemistry renal and hepatic profiles. The total iron-binding capacity is no longer considered helpful in the management of iron poisoning (Tenenbein and Yatscoff, 1991).

8.7 Treatment

Treatment consists of initial assessment, gastrointestinal decontamination, supportive care, and the administration of the specific antidote, deferoxamine. Most young children are seen soon after overdose and before the onset of significant toxicity. Adolescents and adults may be seen later and with symptoms of obvious iron poisoning. With any acute presentation, the patient should be assessed for stability, with emphasis on the status of the blood circulation, and then stabilized as necessary. The abdominal radiograph is a key intervention that guides the early management. Iron tablets are easily seen on X-ray (Hosking, 1969; Ng *et al*., 1979). A negative X-ray in a patient with no symptoms indicates a patient who will not develop significant iron poisoning. A positive X-ray is an indication for gastrointestinal decontamination.

The gastrointestinal decontamination procedure of choice for iron overdose is whole-bowel irrigation (Tenenbein, 1987; 1997). Iron is not adsorbed by activated charcoal (Decker *et al*., 1968), and induced emesis and gastric lavage are an ineffective intervention for iron overdose (Tenenbein, 1985). The indication for whole-bowel irrigation is a positive X-ray. Chelating or complexing iron within the gut using deferoxamine, sodium bicarbonate, phosphate, or magnesium is not recommended, because this approach is ineffective and is associated with significant adverse effects.

Supportive care is paramount in the management of iron poisoning. Emphasis should be focused on management of hypoperfusion and metabolic acidosis. Very large amounts of crystalloid and bicarbonate are needed, and severe cases will require pressor therapy. Occasionally, a blood transfusion is also required. A critical care environment is typically essential, coupled with invasive circulatory monitoring to define the etiology of the shock and to guide its management. Should acute hepatic failure ensue, it is managed with routine protocols. A liver transplant should be considered early on, because the prognosis for survival with this complication is grim. Enhanced elimination (hemodialysis) is not indicated.

Deferoxamine is the specific chelator for iron. It was originally described more than 40 years ago (Keberle, 1964; Moeschlin and Schnider, 1963) and is a very potent chelator with a binding constant exceeding that of transferrin. Indications for deferoxamine therapy may arise from either clinical examinations or laboratory tests. The presence of systemic toxicity, such as shock or acidosis, is an obvious clinical indicator. A peak serum iron concentration greater than 90 μmol/L (500 μg/dL) is a laboratory indicator. Deferoxamine should be given intravenously at a rate of 15 mg/kg/hour. A bolus of crystalloid should be administered to all patients before deferoxamine therapy, because the administration of this antidote in a patient with a contracted intravascular volume carries with it a risk of acute renal failure (Koren *et al*., 1989). Duration of therapy is problematic. Serial serum iron concentrations are unhelpful, because there is no correlation of systemic toxicity with serum concentrations during this time and the presence of deferoxamine confounds its accurate measurement (Gevirtz and Wasserman, 1966; Helfer and Rodgerson, 1966). Indications are the presence of ongoing symptoms and signs of systemic toxicity and the presence of metabolic acidosis. Prolonged infusion of deferoxamine beyond 24–48 hours risks respiratory failure because of the possible development of acute respiratory distress syndrome (Tenenbein *et al*., 1992). Fortunately, therapy beyond 24 hours is rarely indicated.

8.8 Prevention

For young children, routine poison prevention advice is indicated for all other potentially poisonous substances. This includes keeping iron out of their reach and having child-resistant closures for the containers. This safeguard is mandated by legislation in the United States (Tenenbein, 2005). The use of blister packs affords additional protection for young children and may also have a protective effect for adolescents and adults (Tenenbein, 2005). Carbonyl iron does not seem to have the potential for iron toxicity (Madiwale and Liebelt, 2006). Therefore, prescribing carbonyl iron rather than iron salts has the potential to prevent iron poisoning for all age groups.

9 CONCLUSIONS

As described previously, the metabolism of iron is a carefully regulated process. Absorption of iron into the

body, and thence into various metalloproteins, is done in a way that guards against the major danger of iron: incidental metal-catalyzed oxidation reactions. Unfortunately, mammals are not equipped with mechanisms for the excretion of excess iron, and chronic iron overload is associated with slowly progressing failure of various organs including heart, liver, and pancreas, which in the absence of effective chelation therapy (in secondary iron overload) or phlebotomy (in patients with hereditary hemochromatosis) can bring on early death. Similarly, acute overdose of large amounts of medicinal iron is potentially life threatening. The mechanisms involved in this iron-mediated damage remain incompletely understood, although iron-catalyzed oxidation reactions are almost certainly at the core of the disease process. An improved understanding of these mechanisms may lead to the design of more effective therapies for this devastating disorder.

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CHAPTER

31

Lead

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ABSTRACT

Inorganic lead is certainly the most extensively studied of all toxic agents. Occupational exposure occurs in a wide variety of settings. There is also widespread exposure in the general environment. However, after the ban of lead addition to petrol, the exposure has decreased dramatically in several parts of the world. Exposure and risk are usually assessed by biological monitoring, mainly by blood-lead concentration (B-Pb). However, B-Pb has limitations, because there is saturation at high exposure. Lead accumulates in teeth and in the skeleton, where it may be determined by *in vivo* methods, which reflect long-term uptake. Toxic effects may occur in the central and peripheral nervous systems, blood (including inhibition of heme synthesis, which also affects other cells), kidney, and cardiovascular, endocrine and immune systems, gastrointestinal tract, and male reproduction (sperm quality). Lead causes increase of blood pressure; slight effects may occur in adults with a mean B-Pb of 0.4 μmol/L. Furthermore, lead passes the placenta and may cause effects on the nervous system of the fetus. Lead in the skeleton is mobilized during pregnancy and lactation and is transferred to both the fetus and the lactating infant. Slight (but adverse) effects on the mental development of infants and children have repeatedly been reported at a mean B-Pb of 0.5 μmol/L, or even less, in the pregnant woman or the child. Lead is carcinogenic in animal experiments, but there is only limited evidence for carcinogenicity in humans.

The most important organolead compounds are tetraethyl and tetramethyl lead, which have been used in enormous quantities in leaded petrol. They are easily absorbed through inhalation and through the skin and may cause acute encephalopathia.

1 BACKGROUND

Lead poisoning was described in antiquity (Nriagu, 1996), and has followed man ever since (Markowitz and Rosner, 2002; Nriagu, 1998; Wedeen, 1984). The history of lead is not only technological, but also touches on our view of safety and the connection between toxicology and politics, which is sometimes controversial. That makes the history of lead an interesting topic, from which many lessons can be learned, although we do not attempt to review it here.

The literature on lead is enormous. Lead is certainly the most extensively studied toxic agent. Hence, this chapter often has to refer to reviews. Several extensive reviews of the toxicology of lead have occurred during the last decades (e.g., Claudio *et al*., 2003; Deutsche Forschungsgemeinschaft, 2005; Goyer, 1996; RTECS, 2003; 2005; Skerfving, 1993; 2005; Tsuchiya, 1986; U.S. ATSDR, 2005; U.S. CDC, 2002; 2005; WHO, 2000b; WHO/ICPS, 1995). Referring to specific articles has mainly been made to those published after 1990.

In this chapter, concentrations will generally be given as presented in the publications, with the exception that B-Pbs, which were presented as μg/dL (very common in the United States) have here been changed to μg/L. The numbers of decimals given for information on concentrations in the publications have often been kept, despite the fact that they are not always warranted by the analytical technique. For bone lead, negative values are sometimes obtained (i.e., lower

than the standard) and reported in the publications (not to skew the distribution). Also, the original numbers of decimals for effect estimates have been kept, despite the sometimes large uncertainties.

2 INORGANIC LEAD

2.1 Physical and Chemical Properties

Lead (Pb: CAS 7439-92-1); atomic weight, 207.19 (1 μg = 0.004826 μmol).

Only one of the stable, naturally occurring lead isotopes, ^{204}Pb, is nonradiogenic, whereas other lead isotopes are end products of one of three series of radioactive decay: the uranium series (end product: ^{206}Pb), thorium series (^{208}Pb), and actinium series (^{207}Pb). As a consequence, the abundance of its four stable isotopes (^{204}Pb, ^{206}Pb, ^{207}Pb, and ^{208}Pb) varies between different lead samples. Therefore, lead has the unusual feature of not having a fixed natural ratio between its isotopes; it depends on the geological source of the lead.

Density, 11.3 g/cm^3; melting point, 327.5°C; boiling point, 1740°C; oxidation state, lead in inorganic compounds usually has the oxidation state II, but IV also occurs.

Solubility: Metallic lead is hard to dissolve in water but will dissolve in nitric acid and concentrated sulfuric acid. Most lead (II) salts are hard to dissolve (e.g., lead sulfide and lead oxides), but exceptions are found in, for example, lead nitrate, lead chlorate and—to some extent—lead sulfate and lead chloride. In addition, some salts with organic acids are insoluble (e.g. lead oxalate).

Coordination chemistry: Lead (II) has electronic properties resulting in a rich coordination chemistry, giving it the ability to mimic both zinc and calcium ions in biological systems. On the scale between hard and soft acids, it is considered an intermediate. It has the ability to bind to different donor atoms (e.g., O, N, S, and P). Claudio *et al*. (2003) made a fascinating review of the coordination chemistry of lead.

Further information on physical and chemical properties of lead compounds may be obtained from, for example, *CRC Handbook of Chemistry and Physics* (CRC, 2004).

2.2 Methods and Problems of Analysis

A variety of methods are available for determination of lead levels in air, soil, water, foods, biological samples, cosmetics, paint, and other matrices. Instrumentation ranges from hand-held X-ray fluorescence (XRF) instruments for direct determination of lead in paint, soil, and other environmental samples (Kuruvilla *et al*., 2004), over anodic stripping voltammetry (ASV) instruments for determination of lead in, for example, blood and water samples, to larger stationary instruments, such as atomic absorption spectrometry (AAS) and inductively coupled plasma mass spectrometry (ICP-MS). This section gives a brief overview of lead determination in the most common matrices, blood, water, soil, and sediments, followed by some examples of more specialized analytical techniques. An overview of analytical methods is given in Chapter 2.

2.2.1 Blood Analysis

The most common biological matrix for lead determination is whole blood. Generally, venous blood, sampled from the cubital vein of the arm, is analyzed. The alternative of using capillary samples leads to some falsely high results because of contamination even when the personnel collecting the samples are well instructed (Parsons *et al*., 1997). Capillary sampling may, however, be useful for screening purposes, but its use may be hard to justify in epidemiological studies.

There are three commonly used principles for determination of the concentration of lead in blood. These are ASV, electro thermal AAS (ETAAS; sometimes also referred to as graphite furnace AAS, GFAAS), and ICP-MS. All of these can perform very well in determination of B-Pbs (Bannon and Chisholm Jr., 2001; Parsons *et al*., 2001), but significant differences have been noted between ASV and ETAAS in samples collected at high altitudes, possibly because of affected blood chemistry (Taylor *et al*., 2004). All these techniques often reach detection limits on the order of 1 μg/L and variations below 5% relative standard deviation in routine determination of lead concentration in blood. Potentials for lower detection limits exist but are of little importance for blood analyses.

The method of ASV is fairly simple, both as regards sample treatment and instrumentation, whereas ETAAS and ICP-MS are more expensive and complex instruments, but also with greater potential. While ASV and ETAAS have been on the market for many years, new modifications of the ICP-MS technique are still developing. Cubadda (2004) made a review of its limitations and modifications.

Another method that is not as sensitive, but may be useful for screening purposes or determination of blood-lead concentrations at elevated levels, are a portable testing system developed for screening purposes (Pineau *et al*., 2002) and flame AAS (FAAS). The FAAS used to be the "workhorse" of trace element

laboratories during the 1960s and 1970s. It does not reach as low detection limits as ETAAS, but fairly simple FAAS methods exist, based on complexation of lead followed by extraction into an organic phase, that allow detection limits of 10 µg/L in blood and 3 µg/L in urine (Schütz and Skerfving, 1976). Considering the simplicity and worldwide availability of FAAS instruments, these methods may be of value in lead exposure investigations in regions where costs limit the availability of newer instruments with higher performance. Further back in history lie insensitive colorimetric methods, which require even simpler instrumentation.

Regardless of the analytical method used, the issues of contamination and calibration are crucial for success in the determination of lead, especially at low levels, as found in most biological samples. Contamination always occurs in all steps from sampling to determination. The question is not whether contamination occurs, but how large it is and how much it varies between samples.

Plastic materials are often preferred in containers for samples and chemicals. Chemicals, as well as components of the analytical instrumentation, such as tubing and pumps, may need to be checked for any lead contamination. Also, the atmosphere can be a significant source of contamination, with lead-containing dust particles contaminating samples. This problem may be overcome by special filtering systems for the air in the laboratory or workbenches or minimizing the time samples are exposed to open air. To prepare and analyze samples in duplicate or triplicate is a good way of assuring that occasional contaminations do not pass unnoticed.

For some methods, it has become evident that calibration needs to be made in the same matrix as the samples. Thus, when determining lead in human blood, it may be necessary to calibrate the method by using the method of standard addition or calibration with spiked human blood samples.

Internal quality control (QC) within the laboratory by inclusion of QC samples in each analytical run is essential. Intercalibration between laboratories by use of external QC samples is widespread in lead determination, and there are several national and international intercalibration programs for lead in blood, for example, from United Kingdom National External Quality Assessment Service, UKNEQAS; Centre de Toxicologie du Quebec, CTQ; and the German External Quality Assessment Scheme, G-EQUAS. Also, accreditation systems and/or approval of laboratories by governmental bodies are available in several countries. Finally, reference blood samples with different lead levels are commercially available (e.g., from the International Atomic Energy Agency, IAEA; National Institute of Standards and Technology, NIST, US; and the commercial company Sero, Norway).

2.2.2 *Air, Water, Soil, and Sediments*

Inorganic lead in air of normal temperatures is present as particles. In work environments, standard hygienic methods are used with collection of air particles on filter, usually followed by wet ashing. Several different analytical techniques are available for the quantification of lead on filter, such as ASV, ETAAS, ICP-MS, and FAAS, but other methods are also available (US ATSDR, 1999).

Water may be analyzed for dissolved or particulate lead. Different extraction or preconcentration methods may be used for dissolved lead to increase the concentration before analysis. Particulate lead may be collected on a filter and is then usually wet ashed. Also, samples of dusts, sediments, and soils are usually wet ashed before analysis.

2.2.3 *Specialized Techniques*

In addition to the analyses mentioned previously, there are numerous other analytical techniques available for the determination of lead in different matrices. Some examples will be mentioned in the following, especially techniques that cannot be regarded as routine, but have provided (and may also in the future provide) new insights in lead toxicology.

XRF may be used for determination of lead in a variety of matrices. Because of its nondestructive character, one of its uses is for noninvasive measurements of the lead concentration in bone *in vivo* (Ahlgren *et al*., 1976; Somervaille *et al*., 1985).

The mass spectrometric techniques, mainly ICP-MS and thermal ionization mass spectrometry (TIMS) but also others, make it possible to determine the abundance of the different lead isotopes. As mentioned previously (Section 2.1), the ratio between the different lead isotopes depends on the geological source of the lead. These differences in isotopic profiles can be used to evaluate the contribution to lead in blood from different sources or "fingerprinting" the lead (Gulson *et al*., 2003; Naeher *et al*., 2003).

With its low detection limits, ICP-MS has also recently made it possible to determine lead in blood plasma, serum (Schütz *et al*., 1996), and seminal plasma (Apostoli *et al*., 1997), for example. The lead concentration in these matrices are usually so low that they were previously very difficult to determine. Saliva (Koh *et al*., 2003) and sweat (Omokhodion and Crockford, 1991) from occupationally exposed workers have been analyzed for lead by ETAAS, and analyses of urine and hair have been carried out with different techniques, such as ETAAS, ICP-MS, or ASV.

There are also techniques for speciation analysis of lead (i.e., identifying or measuring the quantities

of chemical species, such as various alkyl lead species and inorganic lead). Speciation is often made through the hyphenation of a chromatographic system with a lead detector (e.g., an ICP-MS instrument), but other detectors may also be used. Most of the analytical reports on speciation of lead concern the determination of organic lead species, which is further discussed later (Section 3). Another application is the coupling of liquid chromatography to ICP-MS, which has been used for studies of protein binding of lead in blood (Bergdahl *et al*., 1996; 1997b; Gercken and Barnes, 1991). In these applications, a chromatographic column that separates proteins according to the size of the molecule was connected to the ICP-MS, giving information on the molecular mass of the proteins that bind lead. Such information can be used as a first step in identification of the principal lead-binding proteins in a tissue (Bergdahl *et al*., 1997b).

2.3 Production and Uses

The annual world mine production of lead is approximately 3 million metric tons. More than two thirds of the lead mined is produced in four countries: China, Australia, the United States, and Peru (U.S. GS, 2005). The world lead consumption is significantly larger, because it also includes recycled lead. The overall lead consumption increased during the years 2001–2004 from 6.5 to 7.0 million tons (ILZSG, 2005).

Today, the predominant use (71%) of lead is in batteries, mainly for vehicles, but also for electricity backup systems and industrial batteries. Other uses are as pigment (12%), ammunition (6%), and cable sheeting (3%; ILZSG, 2005). Lead is also used in solders, alloys (brass and bronze), weights, crystal, and as stabilizer in polyvinyl chloride (PVC).

Lead has had a widespread use in paint, both for corrosion protection of steel constructions and products (e.g., bridges, ships, locomotives) and for houses. In some countries, especially the United States, Australia, and New Zealand, white lead (lead carbonate hydroxide, $[PbCO_3]_2Pb[OH]_2$) became common not only for exterior painting, but also for use in interior painting, furniture, and even toys. In such paint, lead may constitute up to 40% of the final dried solid. Lead has also been frequently used in water piping and in fitting of water pipes. Such water pipes, as well as lead-painted surfaces, may still be in use and continuously release lead.

Another large-scale use of lead during the 20th century was in the form of organic lead compounds used as antiknocking agent in gasoline (Section 3.3). Until the 1970s, the addition was approximately 1 g/L.

Annual global lead emissions to the environment were in the order of 400,000 metric tons during the 1960s–1980s, but have decreased since, as a consequence of the phaseout of lead in gasoline (Nriagu, 1996). However, leaded petrol is still used in many countries.

The history of lead pollution is very long: around 3500 BC, a method for extracting silver from lead ores appeared. With the subsequent popularity of silver in jewelry and coins followed an increasing release of the by-product lead into the environment, peaking during the age of the Roman Empire (Nriagu, 1998). Then there was a widespread lead technology (aqueducts, etc.), and lead acetate was used as a sweetener in wine. In the 19th century, tin-containing lead was widely used in household utensils. With the start of the industrial revolution came a new rapid increase of lead production and emission, which peaked around 1970–1980.

2.4 Exposure

The body burden of lead in the general population has been estimated to be in the order of 1000 times higher than that of the prehistoric human (Patterson *et al*., 1991). Main sources of this general lead exposure are lead added to gasoline, industrial emissions, and lead paint. In many countries, especially industrialized wealthy ones, these exposures of the general population are now significantly decreasing, mainly as a consequence of elimination of lead additives to gasoline. In contrast, high environmental exposures to lead are still common in developing countries. Even higher exposure to lead is prevalent in many occupations.

Clinical lead poisoning is reported mainly from occupational exposures and as a consequence of ingestion of deteriorated lead paint or use of lead-glazed ceramics for food storage or cooking. However, with its widespread use, there are several other possibilities for a human to ingest, inhale, or by other means experience a toxic dose of lead (see later). Apart from the routes described in the following, it should be mentioned that lead bullets retained in a tissue might release significant amounts of lead into the body (Gerhardsson *et al*., 2002).

2.4.1. General Environment

2.4.1.1 Sources

Lead is a multimedia pollutant, with several sources and media contributing to the exposure. Any attempt to picture all major sources and exposure routes for lead ends up in a complex pattern (Figure 1). Such a scheme is, however, a valuable tool for identifying the significant sources and routes in any particular environment or clinical case.

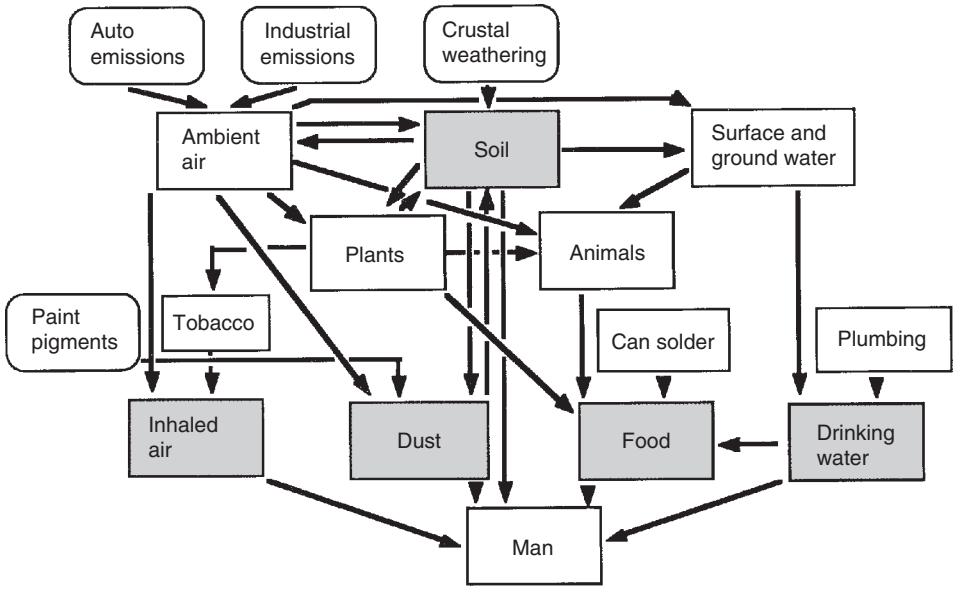

FIGURE 1 Sources and routes of lead exposure in the general population. Modified from Skerfving (1993).

Lead carbonate hydroxide has had a widespread use as pigment in house paint in some countries, and weathering, chalking, and peeling paint may cause heavy exposure (Rosner *et al*., 2005). Another large-scale source of exposure is organolead added to gasoline. At combustion in the engine, organic lead is transformed into the inorganic lead oxide and is emitted almost entirely as such. This causes exposure to inorganic lead, in particular in people living in areas with heavy traffic. Also, industrial emissions, both from large- and small-scale industries and producers as well as recycling activities, may cause exposure in neighborhood populations, and exposed workers may bring the exposure home to their families in the form of lead dust carried in clothing, hair, or on the skin.

2.4.1.2 Inhalation

The exposure through ambient air largely depends on any use of leaded gasoline. In areas where the air levels of Pb are low, food is the dominating source of lead uptake, whereas high air concentration can lead to a situation where inhalation is of significance, or even a dominating source. Ikeda *et al*. (2000) studied the contribution of lead in air at levels in the order of 75 ng/m^3 and estimated that air then contributed approximately half of the absorbed lead in women living in Tokyo and Kyoto. In cities where leaded gasoline is still in use, or has only recently been phased out, mean air-lead levels in the order of 200–400 ng/m^3 is common in residential areas (He *et al*., 2004; Lu *et al*., 2003). Levels are higher in areas with heavy traffic. For example, Kaul *et al*. (2003) reported 2000–3900 ng/m^3 in such areas in the city of Lucknow, India, and Hashisho and El-Fadel (2004) an average of 2860 ng/m^3 in urban Beirut. Similar levels (yearly mean, 2800 ng/m^3) were observed in the Valley of Mexico in 1987, when lead was still added to gasoline in relatively high concentrations. Until 1997, lead in gasoline was reduced by >98.5%, and in 2002, the yearly mean air-lead concentration had dropped to 70 ng/m^3 (Schnaas *et al*., 2004).

Lead exposure through inhalation, and also ingestion, is increased near lead-emitting industries. The variation in air-lead levels is, of course, great. For example, air-lead concentrations near lead acid battery recycling plants in Brazil varied from 70 to 18,300 ng/m^3 (Paoliello and De Capitani, 2005). Near an Australian lead smelter (in Port Pirie) a mean of 2150 ng/m^3 was obtained approximately 600 m from the blast furnace (Esterman and Maynard, 1998), and in British Columbia a mean level of 300 ng/m^3 was found at two stations within 2 km from the smelter, in contrast to 1100 ng/m^3 during a previous period when an older lead smelter was in use (Hilts, 2003).

Additional inhalation exposure occurs through cigarette smoking, although the association between smoking and blood-lead concentration may, to some extent, be confounded by alcohol intake (Grandjean *et al*., 1981). The lead content in a cigarette is 3–12 µg. Approximately 2% of this is inhaled into the active smoker, leaving most of the lead in the environmental tobacco smoke. As a consequence, there is an association between children's lead exposure and environmental tobacco smoke (Baghurst *et al*., 1992; Willers *et al*., 1992). Moreover, cigarette smoking in workplaces

with lead exposure may add to the exposure (e.g., from touching cigarettes with unwashed hands).

Some hobbies may cause lead exposure. Examples are indoor shooting, tin-soldier moulding, ceramic work, application of lead-containing glazes, and motor sports involving work with the exhaust system from cars run on leaded gasoline. These activities give exposure through inhalation, but in addition, oral intake may occur. Finally, all particulate airborne lead sooner or later ends up in dust or soil.

2.4.1.3 Ingestion

In infants and small children, ingested lead objects (e.g., fishing sinkers, curtain weights) may cause massive exposure, in particular if large enough (diameter >20 mm) not to pass the pylorus, but are retained in the stomach, where lead is solubilized and absorbed further downstream. An ingested lead object may also poison an adult (Gustavsson and Gerhardsson, 2005).

Dust (in homes as well as in streets) and soil may contain high lead concentrations and are significant sources of children's exposure. In particular, dust in homes painted with paint containing lead pigment, and soil around lead-emitting industries may contain very high lead levels (U.S. CDC, 2002; 2005; WHO, 1995). The maximum uptake in children seems to occur around 2 years of age and is higher in the summer than in the winter (Baghurst *et al.*, 1992; Yiin *et al.*, 2000). The hand-to-mouth behavior of children is important for lead intake (Lanphear *et al.*, 1998), and even small babies unable to grasp objects receive much of their lead exposure from mouthing their own fingers (Kranz *et al.*, 2004). The solubility of the lead ingested is of great importance for the uptake of lead (Section 2.5.1). When comparing inhalation and ingestion intakes, the limited uptake of lead in the gastrointestinal tract should be borne in mind.

The lead content of drinking water may vary considerably. As an illustration of the contrast, intakes of approximately 1 μg/day or less have been reported from Sweden (Svensson *et al.*, 1987), whereas a study in Hamburg, Germany, in an area where lead pipes are common in old plumbing systems, showed a large variation in the lead concentration in tapwater: from <5 μg/L up to 330 μg/L (Fertmann *et al.*, 2004). Among the samples, a mean of 15 μg/L was found. High concentrations of lead in tapwater are of special concern for bottle-fed babies when formula feeding is prepared from the tapwater.

Of course, any lead contamination of the water reservoir is of importance. Such may occur from industrial discharges or highway runoff. Also, acidity may cause an increase in the lead concentration of water, for example, from private wells. In the next link of the chain, at distribution of drinking water, it may be contaminated with lead from lead pipes, lead-soldered copper pipes, lead-containing brass joints for plastic pipes, or from other parts of the water system. In particular, acidic and soft water has the potential for dissolving lead from the distribution system. The level then depends on the time during which the water did dwell in the pipe. Thus, the lead content is often higher in the first flush than a later one. In the study in Hamburg mentioned previously, it was shown that flushing water before drinking it could significantly lower blood-lead levels. Moreover, in households without tap water, intake of contaminated rainwater may cause exposure.

Also, lead intake occurs through foods. Lead is present in vegetables mainly as a result of deposition from air; uptake by roots is less important (De Temmerman and Hoenig, 2004). Lead is further transferred from plants to animal foods.

There is a large variation of lead exposure through foods between countries. For example, in the United States an average intake in adults of 3 μg/day has been reported (WHO, 2000b), whereas higher values have been given for Denmark (18 μg/day) and the United Kingdom (27 μg/day; EU SCOOP, 2004). Countries in East and Southeast Asia ranged from 7 to 32 μg/day in women in the general population (Ikeda *et al.*, 2000). However, because of the changes in the pollution (Section 2.4.1.6), such figures may change rapidly. Children have a considerably higher dietary intake of lead in relation to body weight than adults (EU SCOOP, 2004; WHO, 2000b).

In the diet, fruits and vegetables, cereals, bakery wares, and beverages are major sources of lead, together supplying most of the intake (EU SCOOP, 2004).

Alcoholic beverages cause lead exposure (Grandjean *et al.*, 1981). Earlier, lead acetate was used as a sweetener in wines. Today, potentially lethal doses of lead may be taken in through illegally produced alcohol ("moonshine"), distilled in apparatuses with automobile radiators, containing lead solders as condensators. High copper intakes may also occur, depending on the composition of the cooler. Lead intoxications caused by moonshine run the risk of being attributed to other causes, leaving the lead toxicity untreated (Morgan *et al.*, 2003). Even less spectacular alcoholic beverages, especially wines, may contain considerable lead concentrations, partly because of use of lead arsenate as a fungicide in vineyards and contamination from containers, including crystal decanters and glasses (Graziano and Blum, 1991). Further, intake of herbal medicine products (ayurvedic) may cause lead exposure (Saper *et al.*, 2004; Sjöstrand *et al.*, 2007).

Lead-glazed or lead-painted pottery is a significant source of lead. In particular, storage of acidic foods, such as fruit juice, in lead-glazed ceramics may lead to a large lead intake. Although such ceramics are most common in certain parts of the world (e.g., Mexico [Téllez-Rojo *et al*., 2004] and Greece), single cases of toxic intake may occur anywhere (Hellström-Lindberg *et al*., 2006). When used for storing or cooking, they contaminate the food with lead. Moreover, some foods take up lead from the water during cooking (Moore *et al*., 1979). Lead-soldered tin cans used to contribute to the general lead intake but today, lead soldering of tin cans is uncommon, although it is uncertain whether the technique is completely abandoned worldwide.

2.4.1.4 Skin Exposure

Skin exposure to inorganic lead occurs, and there is an uptake through the skin (Section 2.5.1). However, the major influence of skin exposure seems to be that lead contamination of the hands contributes to the oral intake. This is certainly of importance in children (Kranz *et al*., 2004), but also in occupationally exposed adults (Askin and Volkmann, 1997). Exposure may occur from cosmetics (kohl, surma; Al-Ashban *et al*., 2004). In occupationally lead-exposed workers, contamination of cigarettes by the hands seems to cause inhalation exposure to smokers (Dykeman *et al*., 2002).

2.4.1.5 Geographical Differences

Because leaded gasoline has for a long time been a significant source of environmental lead, living close to a road with heavy traffic is a determinant of exposure level, as well as living close to a lead-emitting industry (Osman *et al*., 1992; 1994) or in an area with lead-painted houses. People living in city centers have higher blood-lead concentrations than people living in rural areas.

There are large variations in lead exposure between geographical regions (Skerfving *et al*., 1999). On the global scale, it is evident that the highest exposure levels do not occur in the Western world. Fewtrell *et al*. (2004) assessed mean blood-lead concentrations in different parts of the world, based on published studies (Table 1). They estimated that regions with approximately 25% or more of the children having a blood-lead concentration of $>100\,\mu g/L$ were South and Central America, the Middle East, and parts of east Europe and the former Soviet Union. The percentage was <10% in Australia, North America, and western Europe.

2.4.1.6 Time Patterns

Historically, lead emissions peaked during the 1970s, with annual emissions estimated at 400,000 metric tons (Nriagu, 1996). Maybe human exposure peaked at the same time, but considering the geographical differences mentioned previously and the population density of the different regions, the peak may come later. It must anyway be assumed that the global exposure to lead is now decreasing, with the recent phase out of leaded gasoline in Sub-Saharan Africa as a recent example.

There has been a significant, and well-documented, decrease of B-Pb in the Western world during recent decades. For example, the mean B-Pb in a sample of

TABLE 1 Blood Lead Concentrations (B-Pb) in Urban Children and Adults in Different Areas (Fewtrell *et al*., 2004). The Areas Correspond to the World Health Organization's Regions.

Area	Surveyed countries	B-Pb (µg/Liter)	
		Children	Adults
Africa	Nigeria	111	116
	South Africa	98	104
Americas	Canada, USA,	22	17
	Argentina, Brazil, Chile, Jamaica, Mexico, Uruguay, Venezuela,	70	85
	Ecuador, Nicaragua, Peru	90	108
Eastern Mediterranean	Saudi Arabia	68	68
	Egypt, Morocco, Pakistan	154	154
Europe	Denmark, France, Germany, Greece, Israel, Sweden,	35	37
	Turkey, Yugoslavia,	58	92
	Hungary, Russia	67	67
South East Asia	Indonesia, Thailand,	74	74
	Bangladesh, India	74	98
Western Pacific	Australia, Japan, New Zealand, Singapore,	27	27
	China, Philippines, Korea	66	36

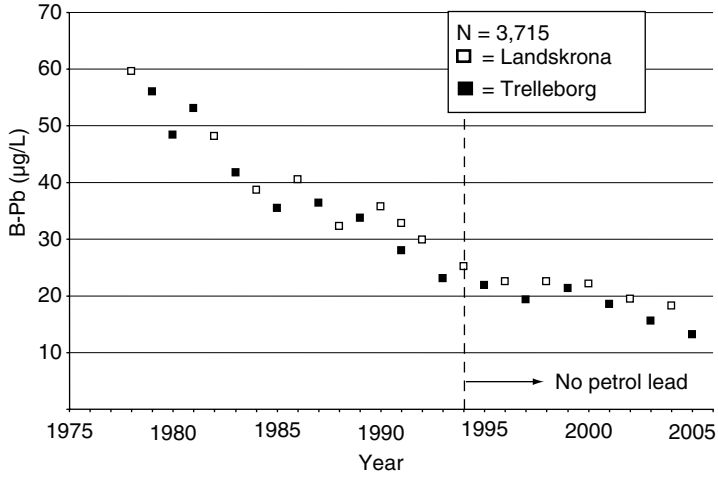

FIGURE 2 Blood lead levels (B-Pb; geometric means) in Swedish children in the towns of Landskrona and Trelleborg, 1978–2005. Strömberg *et al*. (2003), and to be published.

adults living in the United States dropped 78% (from 128 to 28 µg/L) between 1976 and 1991, and a similar decline was seen among children (Pirkle *et al*., 1994). In Turin, Italy, the mean B-Pb in adults dropped 58% (from 153–64 µg/L) between 1985/1986 and 1993/1994 (Bono *et al*., 1995), and in Swedish children a similar decline has been observed between 1978 and 2005 (Figure 2). Erythrocyte samples from adults indicated that there was still a decline in Sweden throughout the 1990s; approximately 4% annual decrease (Wennberg *et al*., 2006). In all these cases, the decrease or removal of lead from gasoline is certainly the main reason for the decline, although the removal of lead from soldered cans probably also plays a role. There is less lead contamination of foods (Larsen *et al*., 2002).

The phaseout of leaded gasoline has been successful in several parts of the world, including recently the nations in Sub-Saharan Africa. As of January 1, 2006, leaded gasoline was still being used in parts of the former Soviet Union (though not Russia), some countries in Southeast Asia, a few countries in eastern Eurpoe and Northwest Africa and a few other countries (UNEP, 2006).

2.4.2 Occupational Environments

In addition to the exposure from the general environment, many work environments imply exposure to lead. Between 100 and 200 different lead-exposing occupations have been listed (Table 2).

Lead paint for anticorrosion purposes often contains much more lead than house paint, up to 70–80% lead. Flame cutting in metal painted with such paint (e.g., in scrapping of ships) causes a considerable risk. Other occupational environments well known for being lead exposing are lead smelters and storage battery manufacturing. In the work environment, exposure occurs both through inhalation (air and contaminated tobacco smoking) and through ingestion of contaminated foods, drink, and snuff. As examples of air-lead levels, average concentrations in the ranges 0.05–0.2 mg/m^3 (Richter *et al*., 1979) and 0.01–0.03 mg/m^3 (Hodgkins *et al*., 1992) were found in different parts of a U.S. storage battery plant. This issue is further discussed in Section 2.5.1.

2.5 Toxicokinetics

A simple model of the toxicokinetics of lead is shown in Figure 3.

2.5.1 Absorption

2.5.1.1 Inhalation

Lead may be inhaled as an aerosol. The pattern of deposition of inhaled lead in the respiratory tract depends on the particle size. Particles with an aerodynamic diameter >5 µm are mainly deposited in the upper- and middle-sized airways, cleared by the mucociliary mechanism, and swallowed. Some of this lead is then absorbed from the gastrointestinal tract (see later).

TABLE 2 Work Tasks Which Cause, or May Cause, Risk for Lead Exposure (Skerfving, 1993)

High risk	Moderate/low risk
Primary and secondary lead smelting	Lead mining
Production of lead paint	Plumbing
Spray-painting with lead paint	Cable industry
Flame welding and cutting in lead-painted metal	Type casting in printing shops
Blasting or scrapping of lead-painted metal	Stereo-type composing
Ship breaking	Lead casting (tin casting)
Brass foundry work (including bronze)	Lead soldering (tin soldering)
Storage battery manufacturing	Car repairing
Addition of lead stabilisator or lead pigment to polyvinyl chloride	Porcelain manufacturing
Car radiator repairing	Earthenware manufacturing
Annealing of enamel	Crystal-glass manufacturing
Ammunition work	Glass painting
Indoors shooting	Electric welding in lead-painted metal
Aluminium forging	Sulphuric acid production
	Application of lead-arsenate pesticides
	Wire-drawing
	Rubber-tube drawing
	Tool hardening

For particles inhaled by mouth, and with a size in the range 0.01–5 μm, 10–60% are deposited in the alveolar tract; for particles inhaled through the nose, the fraction is lower. Most of the lead deposited in the alveolar part of the lung is absorbed. The rate of absorption depends on the solubility of the chemical species of lead. In human radiotracer experiments, the absorption has generally been completed within 24 hours (Chamberlain, 1985; Hursh and Suomela, 1968). Such a rapid absorption is in accordance with the low pulmonary lead content in lead workers (Barry, 1975). On the other hand, increased levels of lead have been found in lung tissue of lead workers who had been exposed to a lead compound with low solubility (lead sulfide; Gerhardsson *et al.*, 1988; Section 2.6.1; Figure 4).

2.5.1.2 Gastrointestinal Tract

Lead is absorbed from the gastrointestinal tract. In radiotracer experiments in fasting subjects, the absorbed fraction was 37–70% (average approximately

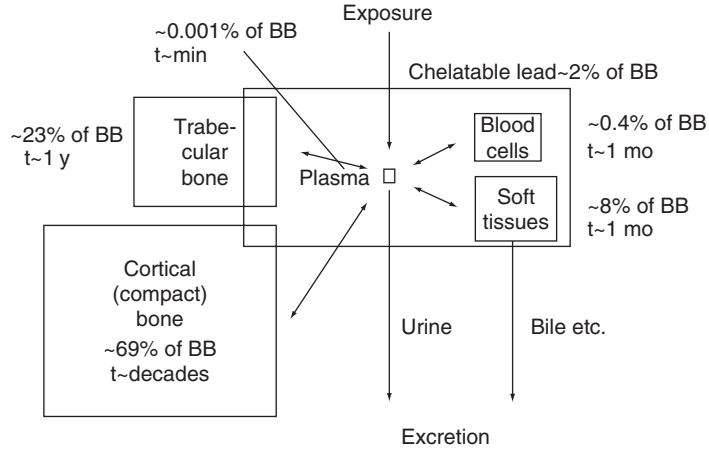

FIGURE 3 Metabolic compartment model for lead in an adult human (Skerfving *et al.*, 1995). The figures shown for the percentage of body burden in different compartments are those corresponding to approximately steady-state conditions (i.e., the situation after regular exposure during long time). The areas of the boxes are not proportional to the sizes of the compartments. "Chelatable lead" denotes the amount available for binding to a chelating agent in a mobilization test. BB, total body burden; t, biological half-time; y, year; mo, month; min, minute.

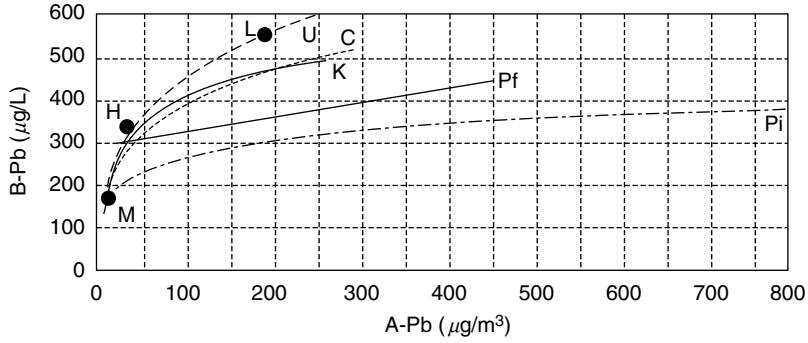

FIGURE 4 Relationships between lead (Pb) concentrations in blood (B-Pb) and air (A-Pb) in different studies. *Pb battery workers*: C, Chavalitnikul *et al*. (1984); logB-Pb (μg/dL) = 1.042 + 0.273 × logA-Pb (μg/m^3). U, Ulenbelt *et al*. (1990); logB-Pb (μg/L) = 2.045 + 0.305 × logA-Pb (μg/m^3); "background" B-Pb not given. H, Hodgkin *et al*. (1992); mean B-Pb approximately 340 μg/L at an air-Pb of 30 μg/m^3; "background B-Pb not given. K, Kentner and Fischer (1994); B-Pb (μg/dL) = 62.183 + 21.242 × logA-Pb (mg/m^3); "background" B-Pb not given. L, Lai *et al*. (1997); mean B-Pb 569 μg/L , air-Pb 190 (μg/m^3); "background" B-Pb not given. *Copper smelter workers*: Pf, Pfister *et al*. (1994); "control group": B-Pb 56 + 13 μg/L. *Crystal industry workers*: Pi, Pierre *et al*. (2002); logB-Pb (μg/L) = 2.13 + 0.161 × logA-Pb (μg/m^3); unexposed referents: B-Pb: GM = 92 (range, 55–178) μg/L. *Pb/tin soldering*: M, Masci *et al*. (1998); mean B-Pb 190 μg/L, mode air-Pb approximately 10 μg/m^3; "general population": B-Pb: GM 82 (range, 50–160) μg/L. GM, geometric mean. Modified from Kentner and Fischer (1994), Pierre *et al*. (2002), and Skerfving (2005).

60%) according to different studies (James *et al*., 1985; Rabinowitz *et al*., 1980). Of soluble lead salts taken with meals, 4–21% (average approximately 8%) have been absorbed. From studies of the uptakes of stable lead in adults, an average absorption of 15–20% may be estimated. There is an interindividual variation in absorption (Hursh and Suomela, 1968). There are indications of a higher gastrointestinal absorption in children.

The nutritional status affects the fractional uptake of lead. Hence, in humans, simultaneous intake of lead, on the one hand, and calcium or phosphate, on the other, may cause a reduction of gastrointestinal lead absorption (Chamberlain, 1985; James *et al*., 1983; 1985). Milk is a major source of these nutrients. Hence, for more than a century, milk was recommended as a prophylactic for lead toxicity in industry. However, milk increases the uptake of lead (James *et al*., 1985). Lead salts and milk lead are absorbed in different modes (Henning and Cooper, 1988). Lactose has a limited effect (James *et al*., 1983), whereas lactoferrin may cause an increase (Quarterman, 1983).

A low iron intake (Cheng *et al*., 1998b) and deficient iron status (Bárány *et al*., 2005; Berglund *et al*., 1994; Osman *et al*., 1998b) was associated with increased B-Pb, although the causality is not clear (US CDC, 2002). The effect of selenium status seems to be limited in man (Gustafsson *et al*., 1987). Vitamin D increases the absorption of lead. Other agents may also affect lead absorption. Hence, phytate causes a decrease, alcohol an increase (James *et al*., 1985).

2.5.1.3 Skin

A fraction of soluble inorganic lead salt applied on the skin is absorbed (Stauber *et al*., 1994). In one study, the absorption was only 0.06% during 1 month (Moore *et al*., 1980). Probably, the absorption of lead soaps (lead naphtenate and lead stearate) is considerably higher (Ong *et al*., 1990).

2.5.2 Distribution

2.5.2.1 Blood

Within blood most of the lead is present in the erythrocytes (red cells), leaving only a fraction of less than 1% in plasma. At high blood-lead concentrations the fraction of lead in plasma increases, giving the relation between blood and plasma lead a curvilinear shape (Figure 5).

The reason for the lead binding in erythrocytes seems to be lead's high affinity for the protein δ-aminolevulinic acid dehydratase (ALAD, equivalent to porphobilinogen synthase, PBGS, EC 4.2.1.24; Figure 6). It is an enzyme present in all cells, including the erythrocytes (Kelada *et al*., 2001). It is the second enzyme in the heme pathway, promoting the asymmetrical addition of two molecules of δ-aminolevulinic acid (ALA) to form the monopyrrole porphobilinogen. It is a 250-kDa homo-octameric enzyme, containing four active sites, reactive cysteines, and two different types of zinc-binding sites (Jaffe *et al*., 2000).

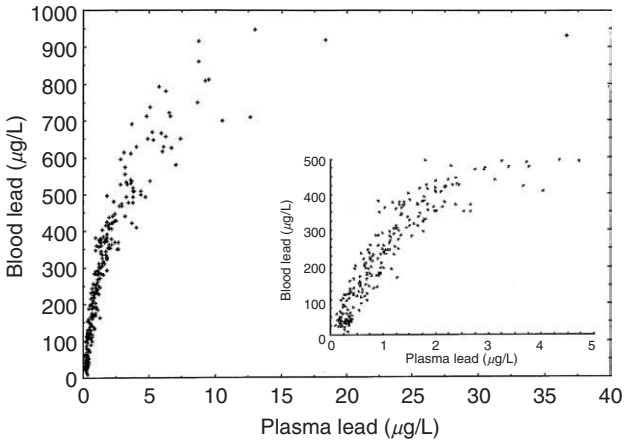

FIGURE 5 Blood- and plasma-lead concentrations in 271 samples from active and retired lead workers, lead-exposed children, and referents (Bergdahl, 1997). The insert shows an enlargement of results from samples with a blood-lead concentration <500 μg/L.

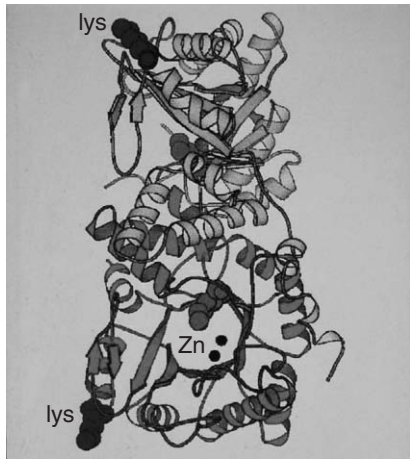

FIGURE 6 Structure of the crystal enzyme δ-aminolevulinic acid dehydratase (ALAD; porphobilinogen synthetase), on the basis of the structure in yeast and *P. aeruginosa* (Jaffe *et al.*, 2000). A dimer part of the human homo-octamer is shown. The two active-site Zn(II) are shown in the lower monomer. Amino acid 59 is shown as lysine, which is the case in ALAD-1; in ALAD-2 it is asparagine.

Lead can replace some of the zinc (Jaffe *et al.*, 2001), and has approximately 20 times higher affinity for the protein than zinc (Simons, 1995). Such binding causes inhibition of the enzyme activity.

In older literature, it is sometimes stated that lead in erythrocytes is mainly bound to hemoglobin, but that has been demonstrated not to be the case (Bergdahl *et al.*, 1997b). Instead, ALAD binds approximately 80% of the lead in erythrocytes (Bergdahl *et al.*, 1996; 1998b). However, the amount of ALAD in the cell is limited, and thus also the lead-binding capacity. Then, there is a shift of lead binding to what seems as the next best binding site, which is a 45-kDa protein (probably pyrimidine-5-nucleotidase; Bergdahl *et al.*, 1998b). Therefore, the fraction of ALAD-bound lead decreases with increasing blood-lead concentration. As a consequence of the limited number of high-affinity lead-binding sites in erythrocytes, the fraction of lead present in plasma increases with increasing blood-lead concentrations, which explains the curvilinear relation between blood and plasma lead. It is worth noting that the protein binding of lead, at least in erythrocytes, is remarkably specific. It binds with high affinity to certain protein sites.

Also, there seems to be a small lead-binding protein of approximately 10 kDa in erythrocytes (Bergdahl *et al.*, 1996; Raghavan and Gonick, 1977), but its properties in chromatographic separation systems make studies of it into a tough challenge to the analytical chemist (Bergdahl *et al.*, 1998b), and, therefore, its role has not been clarified.

The genetic polymorphism of ALAD (Section 2.5.6) may influence the distribution of lead between erythrocytes and plasma: A higher P-Pb/B-Pb ratio has been observed among carriers of the ALAD-2 gene (Montenegro *et al.*, 2006), although an earlier study failed to find the same difference (Bergdahl *et al.*, 1997c).

In plasma, most of the lead has been claimed to be present in a low-molecular-weight fraction, supposed to represent an ionic form (Sakai *et al.*, 1998). Possibly, some of the lead is bound to cysteine (Al-Modhefer *et al.*, 1991). However, the low concentrations present in plasma and the probably low affinity of binding sites causes methodological problems in studies of lead binding in blood plasma. Thus, firm conclusions are difficult to draw, and only little is known.

It has been hypothesized that lead released from bone would distribute different than, for example, inhaled or ingested lead (Cake *et al.*, 1996), and, indeed, there is an association between the lead concentrations in plasma and bone, even when adjusting for B-Pb in whole blood (Tsaih *et al.*, 1999). However, studies of retired lead workers with high lead deposits in bone (Bergdahl and Skerfving, 1997), and lead isotope ratios in urine from women with different isotope ratios in their bone as compared with those in external lead (Gulson *et al.*, 1998a), have failed to confirm the hypothesis. Nevertheless, the association between lead in plasma and bone indicates that they are both relevant biomarkers (Section 2.6).

2.5.2.2 Soft Tissues

From the blood plasma, the absorbed lead is distributed to other organs. Among the soft tissues, the liver and the kidney attain the highest concentrations (Baheman-Hoofmeister *et al.*, 1988; Barry, 1975; Skerfving *et al.*, 1985). In those organs, lead occurs as intranuclear inclusion bodies (Fowler and Du Val, 1991).

Lead does, to some extent, pass the blood–brain barrier (Barry, 1975; Skerfving *et al*., 1985). The distribution within the nervous system is uneven, with high levels in the hippocampus and the amygdala (Grandjean, 1978) and the choroids plexus (Manton and Cook, 1984). The degree of passage of lead into the nervous system is probably higher in infants/children than in adults. The lead level in cerebrospinal fluid is very low (Conradi *et al*., 1980; Manton and Cook, 1984); it is correlated with plasma lead (although even lower).

In animal experiments, there was no constant relationship between concentrations in blood and soft tissues (Larmo and Savolainen, 1981). Thus, the accumulation in liver and kidney was higher than in blood, whereas it was lower in the central nervous system (CNS). The peripheral nervous system (PNS) may accumulate considerably more lead than the CNS.

Lead is distributed to the gonads and other parts of the male reproductive system (see later).

2.5.2.3 Calcified Tissues

A large proportion of the absorbed lead is incorporated into the skeleton (Barry, 1975; Gusserow, 1861; McNeill *et al*., 1997; Silbergeld *et al*., 1993; Wittmers *et al*., 1988). The skeleton contains >90% of the body burden of lead; in lead workers, that fraction may be even higher (Barry, 1975).

The lead content in the skeleton is not a homogenous pool. By analogy with calcium, there is probably a small, but rapidly exchangeable, skeletal lead pool. In addition, there are at least two other pools: one is contained in trabecular bone (the spongy bone [e.g., in vertebrae and in the heads of the long bones]; Barry, 1975; Schütz *et al*., 1987a). In addition, there is a pool in cortical bone (the compact bone, e.g., in the hollow cylinder in the long bones; Gerhardsson *et al*., 1998; Rabinowitz *et al*., 1977; Skerfving *et al*., 1985; Somervaille *et al*., 1989). The skeleton contains approximately 20% trabecular and approximately 80% cortical bone, but the surface areas of the two types of bones are similar. Thus, the turnover of the trabecular bone lead pool is much faster than that of the cortical one (Schütz *et al*., 1987a). The turnover rate of lead in the skeleton is higher in infants than in adults (Chamberlain, 1985).

The lead content in the skeleton in subjects without occupational exposure varies in different areas of the world. It was very low, probably only a few milligrams, in prehistoric subjects living in a world without traffic and industries (Ericson *et al*., 1979), approximately 10 mg in contemporary Scandinavians (Erkkilä *et al*., 1992; Schütz *et al*., 1987a), and approximately 100 mg in subjects in the United Kingdom (Barry, 1975) and the United States (Ericson *et al*., 1979), although the amounts have most probably decreased lately (Section 2.4.1.6).

In lead workers, the lead levels in bone are high (Barry, 1975; Gerhardsson *et al*., 2005; Nilsson *et al*., 1991; Schütz *et al*., 1987a). The total bone lead burden averaged approximately 100 mg in Finnish lead workers (Erkkilä *et al*., 1992). High levels have also been found in subjects with extreme nonoccupational lead exposure from other sources (Flood *et al*., 1988). In heavily exposed subjects, the skeletal lead content may be in the order of magnitude of 1 g.

There is a continuous turnover of the skeleton. This causes a release of lead from the skeleton, and an endogenous lead exposure (Section 2.5.5).

Lead is incorporated into teeth. The turnover of lead in teeth is slow (Gulson and Gillings, 1997). Lead in teeth has been widely used for biomonitoring (Section 2.6.1.4).

2.5.3 Biotransformation

There is only circumstantial evidence that microorganisms may methylate inorganic lead. It is not known whether this may occur in the gastrointestinal tract. There are no indications of methylation or any other biotransformation in the tissues.

2.5.4 Elimination

Lead is excreted from the body mainly through the urine and feces; there are also other, minor routes of elimination, which have a practical importance. At low exposures, the excretion in the feces is approximately half that in the urine, at higher levels probably less.

2.5.4.1 Kidneys

The excretion into urine occurs through glomerular filtration, although the filtration is probably followed by partial tubular reabsorption (Araki *et al*., 1986).

There is a circadian rhythm in the urinary lead excretion rate, with a decrease during the night (Yokoyama *et al*., 2000). Furthermore, the excretion rate is affected by the urinary flow (Section 2.6.1.3).

There is a correlation between lead levels in urine and whole blood (Bergdahl *et al*., 1997c; Erkilä *et al*., 1992; Schütz and Skerfving, 1976). However, the variation is large. Also, the association is not linear; the urinary lead level increases relatively more than the B-Pb, probably exponentially (Fukui *et al*., 1999; Tsaih *et al*., 1999). This is explained by the dependence of urinary lead mainly on plasma lead, which increases relatively more than the B-Pb in whole blood. Indeed, there seems to be a linear relationship between plasma lead and urinary lead (Bergdahl *et al*., 1997c; Gerhardsson *et al*., 1998).

Lead concentration in urine has been used widely for biomonitoring (Section 2.6.1.3).

2.5.4.2 Gastrointestinal Tract

Lead is also excreted through bile (Ishihara and Matsushiro, 1986) and pancreatic juice (Ishihara *et al*., 1987) into the feces (Rabinowitz *et al*., 1980). Possibly, the excretion in bile is in the form of a lead–glutathione complex (Alexander *et al*., 1986).

2.5.4.3 Other Routes of Elimination

Lead is also, to some extent, excreted in saliva (Koh *et al*., 2003) and sweat (Kehoe, 1987; Omokhodion and Cockford, 1991; Rabinowitz *et al*., 1977). Amounts without practical importance (besides, possibly, for biological monitoring; Section 2.6.1.4) are excreted in nails and hair (Foo *et al*., 1993; Rabinowitz *et al*., 1977).

Lead is also incorporated into semen, the placenta, the fetus, and milk (Section 2.11).

2.5.5 Biokinetics

The toxicokinetics of lead may be described by compartment (U.S. EPA, 1994; Skerfving *et al*., 1995) and physiologically based pharmacokinetic (Legett, 1993; O'Flaherty, 1993) models.

A simple compartment model is presented in Figure 3. There are very fast (blood plasma), rather fast (blood cells and soft tissues), and slow (skeleton) compartments. The U.S. Environmental Protection Agency has designed an "Integrated Exposure Uptake Biokinetic Model for Lead in Children" (IEUBK), a detailed, classical compartment model (U.S. EPA, 1994; 2002; White *et al*., 1998). In a four-step process, it mathematically and statistically links several sources (soil, house dust, drinking water, air, and food) of environmental lead exposure to distributions of B-Pbs in populations of children, 0–84 months of age. It takes into account indoor and outdoor air lead, time spent outdoors, ventilation rate, and lung absorption. The gastrointestinal bioavailability is expressed in relation to lead acetate in pigs. The *in utero* transfer is estimated from maternal B-Pb. The body compartments are lungs, gastrointestinal tract, plasma/extracellular fluid, red blood cells, kidney, liver, other soft tissues, and trabecular and cortical bone. The elimination occurs through urine, feces, and skin/hair/nails. The transfer rates are based in part on kinetic data in baboons. Certain nonlinearities, specifically capacity-limited binding in the red cell and absorption from the gastrointestinal tract, are built into the model.

The accuracy of the model in prediction of B-Pb has been verified (Choudhury *et al*., 1992). Furthermore, a probabilistic (of exposure parameters) version has been developed (Goodrum *et al*., 1996). The use of a high gastrointestinal absorption rate (40–50%, even at age 7) has been criticized (Gulson *et al*., 1997).

Another model of lead kinetics has been developed and validated for adults with a wide range of exposures from a variety of sources (O'Flaherty, 1993). It has been supplemented with a probabilistic module (Beck *et al*., 2001). The model has been tested in children (O'Flaherty, 1995) and lead workers (Fleming *et al*., 1999). Both B-Pb and bone lead are very labile in early childhood; they respond rapidly to increases in exposure, and decrease almost as rapidly to near-preexposure concentrations when exposure returns to background levels. From the peak in adolescence and into early adulthood, the rate of bone turnover drops dramatically, and, hence, the ability to reverse bone-lead accumulation rapidly decreases.

Despite some qualitative and quantitative differences in lead uptake, the O'Flaherty (1993 and 1995) and IEUBK (U.S. EPA, 1994) models give predictions of B-Pb that are not greatly dissimilar (O'Flaherty, 1998).

After a rise of the exposure intensity, the B-Pb increases gradually, usually to reach a steady state after weeks to months (Christoffersson *et al*., 1984). However, after a heavy inhalation exposure, the B-Pb may rise by five times aleady within a few hours (Schütz and Skerfving, 1976).

After a decrease of exposure, there is a decay of B-Pb concentrations, which shows several components (Figure 7). In adults, the decline rate is compatible with an initial phase with a half-time of approximately 1 month, if slow phases are taken into consideration (Nilsson *et al*., 1991; Rabinowitz *et al*., 1977; Schütz *et al*., 1987b). The interindividual variation is large. There are some indications that the rate may be slower in infancy (Ryu *et al*., 1978), at older age (Hryhorczuk *et al*., 1985; Schütz *et al*., 1987b), and in subjects with renal impairment (Hryhorczuk *et al*., 1985). During CaNaEDTA treatment, the half-time is approximately 1 week (Hryhorczuk *et al*., 1985), in combination with hemodialysis only a few hours (Martegani *et al*., 1989).

Slow phases of lead elimination from blood reflect elimination of bone pools. Again, there is an interindividual variation (Schütz *et al*., 1987b). Lead is lost from the bone by diffusion (heteroionic exchange), as well as by resorption. The trabecular (spongy, e.g., in calcaneus, patella, and vertebrae) bone lead has a more rapid turnover than the cortical (Schütz *et al*., 1987a). From analyses of B-Pb elimination curves, the half-time in trabecular bone was estimated at approximately 1 year (Nilsson *et al*., 1991). On the other hand, by XRF measurements, the half-times in the mainly trabecular calcaneus (Gerhardsson *et al*., 1992; Hu *et al*., 1998),

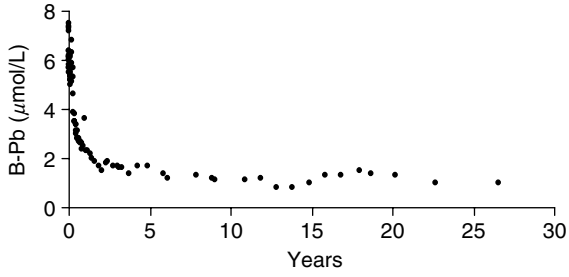

FIGURE 7 Blood-lead level (B-Pb) in a worker during 28 years after end of heavy exposure for years. His initial B-Pb was 7.4 μmol/L. It is possible to identify three different compartments, representing soft tissues (half-time approximately 1 month), trabecular bone (approximately 1 year), and cortical bone (decade) Note: At the end of the period, the mean B-Pb in the "background population" was approximately 0.15 μmol/L. Skervfing *et al.*, to be published.

the mainly cortical finger bone (Börjesson *et al.*, 1997b; Nilsson *et al.*, 1991), and the cortical tibia (Brito *et al.*, 2001) have been estimated to be 1–2 decades.

Evidence suggests increased mobilization of lead from the skeleton during bone demineralization. Women loose as much as half of the trabecular bone and a third of cortical peak bone mass during their later lifetime. Estrogen supplementation may decrease the lead mobilization (Korrick *et al.*, 2003; Latorre *et al.*, 2003). Increased B-Pb has been observed during pregnancy and lactation (Section 2.11.1), menopause (Berglund *et al.*, 2000a; Grandjean *et al.*, 1992; Hernandez-Avila *et al.*, 2000; Korrick *et al.*, 2003; Lagerqvist *et al.*, 1993; Latorre *et al.*, 2003), old age (Tsaih *et al.*, 2001), thyrotoxicosis (Goldman *et al.*, 1994; Osterode *et al.*, 2000), and hyperparathyroidism (Osterloh and Clark, 1993; Osterode *et al.*, 2004). Because of the large deposit of lead in the skeleton, bone tumor (Guijarro *et al.*, 1988) and progressive osteoporosis (Shannon *et al.*, 1987; Berlin *et al.*, 1995) may also cause "endogenous" lead toxicity in previously exposed subjects.

There are associations between bone lead, on the one hand, and both B-Pb (Börjesson *et al.*, 1997a; 1997b; Erkkilä *et al.*, 1992; Gerhardsson *et al.*, 1998; Wasserman *et al.*, 2003) and serum/plasma lead (Bergdahl *et al.*, 1998a; Gerhardsson *et al.*, 1998; Hernandez-Avila *et al.*, 1998), on the other. Studies in women immigrating to Australia, from areas where the exposure was to lead of a different $^{206}Pb/^{204}Pb$ isotopic ratio, confirmed that release from bone causes an endogenous exposure, making up 45–70% of total B-Pb (Gulson *et al.*, 1995). The relationships between exposure time, B-Pb and bone lead are nonrectilinear (Brito *et al.*, 2002; Fleming *et al.*, 1997). In lead workers, approximately 1.8 μmol/L (Schütz *et al.*, 1987b), or 1.7 μg/L per μg/g bone mass in tibia (Bleecker *et al.*, 1995), seemed to originate from endogenous exposure. The association between bone lead and B-Pb is particularly close in retired workers.

The bone lead exerted a greater influence on B-Pb during the winter months than in the summer (Oliviera *et al.*, 2002). The explanation was supposed to be enhanced bone resorption because of decreased levels of activated vitamin D as a result of lower exposure to sunlight.

2.5.6 Gene–Environment Interaction

For centuries it has been known that there is a large interindividual variation in sensitivity to lead, but the mechanism behind this has been largely unknown. Genetics seems to play a role, because there was an association of B-Pb in twins (Björkman *et al.*, 2000).

A single gene on chromosome 9q34 encodes ALAD. Human ALAD is a polymorphic enzyme. Eight ALAD variants have been described. The allele contains a site-directed mutagenesis, a G→C transversion at position 177 of the coding region, resulting in the substitution of asparagine for lysine at amino acid 59 (proteins K59 and N59, respectively; Figure 6).

The enzyme is codominant, in that both these alleles are expressed if a copy is present. Hence, there are three distinct isoenzyme phenotypes: K59-K59 (ALAD 1-1), K59-N59 (ALAD 1-2), and N59-N59 (ALAD 2-2). The first one will be denoted $ALAD^1$, and the latter two $ALAD^2$. In Caucasian populations, approximately 80% of the individuals have ALAD 1-1, 19% ALAD 1-2, and 1% ALAD 2-2 (Kelada *et al.*, 2001). Asian and African populations have lower frequencies of $ALAD^2$ (approximately 10%; Lee *et al.*, 2001a; Schwartz *et al.*, 2000b; Theppeang *et al.*, 2005; see also Chapter 4).

As mentioned previously (Section 2.5.2), ALAD is the major binding site for lead in red cells and is probably also important in other tissues. Hence, genetic polymorphism in *ALAD* may affect the metabolism of lead. Several studies have shown higher B-Pb in $ALAD^2$ subjects than in $ALAD^1$ (Wetmur *et al.*, 1991; Alexander *et al.*, 1998; Fleming *et al.*, 1998; Schwartz *et al.*, 2000c); this has led to the hypothesis that the $ALAD^2$ gene product may bind lead more tightly than the $ALAD^1$ one. However, that is not easily explaining the higher P-Pb/B-Pb radio observed among $ALAD^2$ subjects, as compared to $ALAD^1$ subjects (Montengegro *et al.*, 2006).

Furthermore, in $ALAD^1$ subjects, the urinary excretion of lead, both unprovoked (Süzen *et al.*, 2003) and after chelation (Gerhardsson *et al.*, 1999; Schwartz *et al.*, 1997a), was higher than in $ALAD^2$ subjects.

The *ALAD* genotype also seems to affect the lead kinetics in calcified tissues. In several studies, $ALAD^2$ subjects had lower bone (Fleming *et al.*, 1998; 1999; Hu *et al.*, 2001; Kamel *et al.*, 2003; Schwartz *et al.*, 2000b)

and dentine (Bellinger *et al*., 1994a) lead, although all the information is not consistent (Bergdahl *et al*., 1997a; Theppeang *et al*., 2005).

Modification by ALAD polymorphism on various toxic effects will be discussed later (Section 2.7). Hereditary *ALAD* deficiency and lead is discussed elsewhere (Section 2.6.2.1). Gene–environment interactions are also discussed in Chapter 4.

Calcium status affects lead absorption (Section 2.5.1). The effect is mediated through Calcium-binding proteins, which are, in turn, affected by the blood-borne form of vitamin D (calcitrol), which binds to the vitamin D receptor (VDR), a major determinant of bone density. *VDR* genotypes may affect blood (Haynes *et al*., 2003; Lee *et al*., 2001b; Schwartz *et al*., 2000a) and bone (Theppeang *et al*., 2005) lead in adults, although the information is not consistent (Chuang *et al*., 2004; Kamel *et al*., 2003). In children, the VDR genotype modified the relationship between exposure from floor dust and B-Pb (Haynes *et al*., 2003).

Polymorphism in the endothelial nitric oxide synthase (eNOS) gene was not associated with bone lead (Theppeang *et al*., 2005).

Hereditary hemochromatosis (with increased absorption of iron causing overload) was associated with B-Pb in one study (Barton *et al*., 1994), but not in another (Åkesson *et al*., 2000).

2.6 Biological Monitoring

Lead is the foremost example of a toxic agent for which biological monitoring of exposure and risk has proven to be successful, and much of the concepts and basic principles of biological monitoring derive from studies on lead. The biomarkers (see also Chapter 4) can be grouped into those based on a direct measurement of the lead concentration in blood, urine (with or without chelation), bone or other tissues or secreta, and biomarkers of effects, such as the inhibition of certain enzymes (Section 2.6.3); for effects that are specific for lead, these can also be used as secondary biomarkers of exposure and risk.

2.6.1 Biomarkers of Exposure

The most commonly used marker of lead exposure is the lead concentration in whole blood, but the concentration in bone is also frequently being used in epidemiological studies. Lead concentration in whole blood serves as an indicator of recent exposure, whereas bone lead is an indicator of long-term exposure. Lately, improved analytical techniques have also made it possible to determine lead at the low concentrations present in plasma, although still only few laboratories in the world do this analysis. Possibly, plasma and also chelatable lead may serve as indicators of "effective" internal dose.

2.6.1.1 Blood Lead

Lead in blood has at least two compartments, one with a half-time of approximately 1 month, and another with a half-time of decade(s) (Section 2.5.5; Skerfving *et al*., 1995); there might also be an intermediate one with a half-time of one year. Thus, blood reflects a combination of the exposure during the last months and several years back in time. The kinetics of plasma-lead concentration is not as well characterized, but can be expected to reflect the same exposure period as lead in whole blood, because equilibration of lead between erythrocytes and plasma has been shown to be very rapid (Simons, 1993).

2.6.1.1.1 Erythrocytes/Whole Blood Most of the information on human exposure to and health effects of lead is based on the concentration of lead in whole blood. Therefore, a B-Pb result can be related to almost the whole body of toxicological knowledge. This is a major advantage for B-Pb versus any other biomarker for lead, especially in the clinic and in prevention, including occupational health services.

B-Pb levels are usually determined from analysis of venous blood, although sometimes capillary blood has been used. It is necessary to use sampling tubes with sufficiently low lead contamination. Guidelines for sampling of blood and urine for trace element analysis have been published (Cornelis *et al*., 1996).

The level in capillary blood may be higher than in venous blood, because of higher packed cell fraction. There is also a risk that contamination causes falsely high levels in capillary samples (Parsons *et al*., 1997); it is important that the skin is always carefully cleaned before sampling, especially at capillary sampling.

Most of the lead in blood is present in the cells. Therefore, the concentration in erythrocytes would be a suitable measure, but for practical reasons, levels in whole blood are usually used. The packed cell volume may, however, vary. It is thus advisable to consider determining the packed cell fraction (hematocrit) or hemoglobin level in connection with the blood-lead analysis, especially when a very good precision is required, or in conditions with affected packed cell volume, such as in anemia, or heart or lung disease; otherwise too low or too high readings may be obtained (Thomson *et al*., 1983).

A long series of studies, both experimental and epidemiological, have been devoted to the relationship between lead levels in blood and exposure, intake, or uptake of lead.

The relationship between lead uptake and B-Pb cannot be described by a straight line; it is curvilinear. Thus, at low lead levels there is a steady increase in B-Pb with increasing lead uptake, but at high lead levels, the curve flattens out, and B-Pb then changes only marginally at an increase in lead uptake. This is most probably an effect of the saturation of lead-binding sites in erythrocytes. Also, there is a wide interindividual variation in the attained blood-lead concentration at a given daily lead uptake, so on an individual basis, one has to be cautious when using blood-lead concentrations for assessment of individual lead exposure.

A consequence of the combination of curvilinearity and interindividual differences in attained blood-lead concentrations is that although the analytical precision in determining a blood-lead concentration at a high level may be very good, often with a coefficient of variation of 5%, the accuracy in assessment of lead exposure will be very poor, even at high blood-lead concentrations. For example, an uptake of 100 µg/day, corresponding to 1.5 µmol/L of lead in blood had an individual range of approximately 65–190 µg/day, whereas the corresponding range at 3 µmol/L covers from 250 up to several thousands µg/day (UK Royal Commission, 1983). The risk of toxicity is probably closer related to the uptake than to B-Pb. Therefore, although lead concentration in whole blood has proven to be a very useful marker of exposure, it may be argued that biological monitoring of lead exposure and risk through the analysis of lead in whole blood is probably best suited for moderate and low levels.

So far, this section has only dealt with the relationship between blood-lead concentration and *uptake* of lead. It is more complicated to discuss the relationship with *intake/exposure* to lead, since then the absorption has to be taken into consideration. For airborne lead it has been shown that the relationship between B-Pb and airborne lead varies between different workplaces (Section 2.5.1; Figure 4). From the figure it seems that workers in lead battery factories, where the exposure is to soluble lead salts, have higher blood-lead concentrations than crystal glass industry workers, who are exposed to less soluble lead particles. Also, the particle size is of importance, because it determines the fraction deposited in the lung, and also the hygienic standard of the worker (in particular eating and smoking at work) influences the blood-lead concentration. Hence, there may be a wide variation in the relationship between B-Pb and air lead, even within the same factory.

2.6.1.1.2 Plasma/Serum Lead is present in blood plasma, generally making up less than 1% of the total blood-lead concentration (Bergdahl *et al*., 1997c; Schütz *et al*., 1996; Smith *et al*., 2002). Plasma is readily transported to target organs and has a very rapid turnover. This should make lead concentration in plasma or serum a good measure of lead uptake and risk for health effects but, because of the low concentrations, determination of lead in plasma or serum was for a long time difficult and with doubtful accuracy. However, the use of ICP-MS has made these analyses much simpler and the practical problems around sampling seem manageable (Bergdahl *et al*., 2006).

The relationship between lead concentration in plasma and whole blood is curvilinear (Section 2.5.2.1; Figure 5; Bergdahl *et al*., 1998b). As a consequence, the fraction of lead in plasma rises with increasing lead levels. At high blood-lead levels (at approximately 4–5 µmol/L and above) the concentration of lead in plasma is more than 1/100 of the concentration in whole blood compared with less than 1/100, as is the case at lower levels (Bergdahl *et al*., 1998b).

The distribution of lead between erythrocytes and plasma shows a two- to fourfold inter-individual variation (Bergdahl *et al*., 1997c; Smith *et al*., 2002). The reason is unknown; it may be due to interindividual differences in the concentration of ALAD (porphobilinogen synthetase; PBGS) and other lead-binding proteins in erythrocytes, but could also be due to other differences, interindividual or on a day-to-day basis.

Whether serum or plasma is the most suitable medium is not clarified. The lead concentrations are similar (Bergdahl *et al*., 2006). In favor of serum is the absence of anticoagulants, which could potentially contribute to lead contamination. In favor of plasma is the absence of a coagulation process in the blood, which potentially could cause some release of lead from erythrocytes.

There may be a rectilinear relationship between plasma-lead concentration and lead uptake, but little data to support this have been published. Although there are indications that the lead concentration in plasma or serum could be an alternative for biological monitoring, there are very few epidemiological studies in which plasma or serum lead have been used in exposure assessment. It is, therefore, impossible to tell whether plasma or serum lead is a better marker of exposure or risk than B-Pb. However, closer correlations have been reported for plasma lead than for lead in whole blood both in a study of hemoglobin levels in children (Bergdahl *et al*., 1999) and in a study of the effect of maternal lead levels during pregnancy on infant neurocognitive development (Tellez-Rojo *et al*., 2004).

2.6.1.2 Skeletal/Bone Lead

The lead concentration in bone can be determined *in vivo* by noninvasive methods based on XRF. With its slow turnover, bone lead slows long-term lead

exposure. It also reflects the total body burden, because the dominating fraction of the body burden of lead is in the skeleton. Lead determination has been carried out in different bones, mainly finger bone (Nilsson and Skerfving, 1993; Skerfving and Nilsson, 1992; Schütz *et al*., 2005), patella (Hu *et al*., 1998; Watanabe *et al*., 1994), tibia, and calcaneus (Erkkilä *et al*., 1992; Todd and Chettle, 1994). The trabecular bones, such as calcaneus and patella, have a faster turnover than the cortical ones, such as the tibia. Therefore, the trabecular bones reflect a shorter time span than the cortical ones (Hu *et al*., 1998). There is a reasonably good correlation between the lead concentrations in the different bones (Erkkilä *et al*., 1992). Occasionally, determinations have been reported also for the ulna and sternum, but the measurements were less precise (Erkkilä *et al*., 1992).

The lead concentrations in bone are much higher in lead workers than in the general population. Usually, the highest concentrations have been recorded in retired workers, where levels on the order of 100 µg/g bone mineral may be found (Gerhardsson *et al*., 1993), the reasons being the long exposure duration and high exposure intensity in the past, in combination with the slow elimination of lead from bone. In an old lead worker, the skeleton may contain 1 g of lead. This causes an endogenous exposure, which may make up half of the B-Pb.

Several studies have been carried out on bone lead in the general population. Determinations are possible for tibia, calcaneus, and patella, at least in populations with a relatively high exposure. However, for finger bone, the sensitivity has not been sufficient. Because bone lead reflects long-term exposure to lead, it is attractive in epidemiological studies where retrospective exposure assessment is required, such as in studies of the long-term effects on the developing brain (Hu *et al*., 1998). Indeed, stronger associations to neurological outcome have been shown for bone lead compared with B-Pb in a study in Kosovo (Wasserman *et al*., 2003).

Bone-lead concentrations are associated with the lead concentration in both whole blood and plasma. The associations are particularly close in retired workers, but less in active ones, in whom the current exposure is superimposed on the endogenous one from bone (Börjesson *et al*., 1997b; Christoffersson *et al*., 1984; Erkkilä *et al*., 1992). There is an increase in bone lead with age (Lin *et al*., 2004).

2.6.1.3 Urinary Lead and Chelatable Lead

Urinary lead has been used in biological monitoring of lead, but only to a limited extent. Urinary lead excretion after administration of chelating agents (chelatable lead) has, however, been fairly widely used as an index of risk and the body burden of lead.

There is a clear association between the lead concentrations in urine and blood (Bergdahl *et al*., 1997c; Fukui *et al*., 1999; Gulson *et al*., 1998a), but the variation is too large to allow a prediction of an individual blood-lead concentration from a urinary lead concentration. Partly, this is caused by the difficulties in handling the necessary adjustment for variations in dilution of spot samples (Sata and Araki, 1996). For example, the creatinine excretion, which is often used, depends on muscle mass and meat intake (Suwazono *et al*., 2005). This makes comparisons between subjects of different gender and age doubtful. Also, there is some diurnal variation in the excretion, with the lowest concentrations in the night (Yokoyama *et al*., 2000). Moreover, the association between urinary and blood-lead concentrations has a curved shape because of the saturation of lead-binding sites in the erythrocytes (Section 2.5.2.1). In contrast, plasma-lead concentration seems to be rectilinearly related to urinary lead (Bergdahl *et al*., 1997c), as well as to the urinary excretion of lead after chelation (Gerhardsson *et al*., 1999). In line with this, urinary lead has been suggested as a possible surrogate for plasma lead (Fukui *et al*., 1999; Tsaih *et al*., 1999), but data indicate that, just as in the case of whole blood, the variation is too large to allow a prediction of an individual plasma-lead concentration based on an analysis of urine (Bergdahl *et al*., 1997c). Nevertheless, it may still be that urinary lead can work as a surrogate for the filterable fraction of lead in plasma.

Urinary excretion of lead after administration of a chelating agent has often been used as an index of risk and total body burden. After administration of calcium disodium ethylenediamine tetraacetic acid (EDTA), the concentration of lead increases in plasma because of the presence of a Pb-EDTA complex, which is filtrated into urine (Sakai *et al*., 1998). An alternative to EDTA is dimercaptosuccinic acid (DMSA); there are differences between these two chelators in how they affect lead excretion (Lee *et al*., 1995).

Chelatable lead has been used as an index of the total body burden, but it has been shown not to be a good measure. It mainly reflects lead concentrations in blood and soft tissues (Gerhardsson *et al*., 1998; Tell *et al*., 1992), and possibly trabecular bone (Tell *et al*., 1992; Section 2.5.5), whereas it is not a good index of total body burden, and thus not of long-term accumulation, which mainly occurs in cortical bone. In accord with this, chelation did not cause any decrease of either tibia or calcaneus lead (Tell *et al*., 1992).

2.6.1.4 Other Indices

Lead is excreted in the saliva, which is probably the explanation of the black gingival lead seam sometimes

seen in lead workers. The saliva lead is <1% of the B-Pb (Koh *et al*., 2003). Saliva lead should not be used for biomonitoring.

Lead grows out into hair. A correlation has been demonstrated between the lead concentration in hair and that in blood (Foo *et al*., 1993). Lead levels along a hair strand may be used to map the time pattern of lead uptake in forensic medicine. However, there is an obvious risk of contamination, and analyses of hair segments have shown that, at least in unwashed hair, most of the lead in a hair sample may be a result of external deposition (Martin *et al*., 2005). Then the hair would merely be a personal sampler for dust.

The lead level in teeth has been used as an index of lead exposure, and especially the shedded deciduous teeth in children seem attractive for this purpose, because it reflects the uptake from formation of the tooth up to the time of shedding. However, there are problems: Within one tooth, there is a considerable variation of lead level (Arora *et al*., 2004), and it is difficult to make a homogenate from a tooth. Associations between dentine and bone lead in children have been shown (Kim *et al*., 1996a). Deciduous teeth lead levels have been rather widely used in epidemiological studies of neurobehavioral effects in children.

Feces analyses may be useful for assessing per oral intake (Vahter *et al*., 1991). They may also be useful in clinical cases when a large oral intake of lead is suspected.

2.6.2 Biomarkers of Effects

2.6.2.1 Heme Metabolism

Lead inhibits several enzymes in the heme synthesis, primarily ALAD (Figure 8). The ALAD inhibition can be restored to normal by addition of reduced glutathione or dithiothreitol *in vitro*. Lead is also widely believed to inhibit heme chelatase, although the observed lead-induced effect on insertion of iron to form the heme unit may merely be an effect of lead affecting iron transport (Claudio *et al*., 2003).

Enzymatic determination of the ALAD activity in erythrocytes has been used as an index of lead exposure/absorption and risk (Section 2.7). However, determination of ALAD activity has considerable method problems (Jaffe *et al*., 1991). ALAD activity (especially the fraction of the activity restored by zinc or dithiothreitol) is more sensitive than disturbances in concentrations of its substrate δ-aminolevulinic acid (ALA; Sakai and Morita, 1996).

The effect of lead on heme synthesis causes accumulation of ALA and coproporphyrins (Section 2.7.2), which both increase in serum/plasma and are also excreted into urine. ALA and coproporphyrins, as determined by colorimetry, or better liquid chromatography, have often been used as indices of lead exposure/absorption and risk of other toxic effects. In particular, urinary ALA was used before B-Pb could be determined in a simple and accurate way.

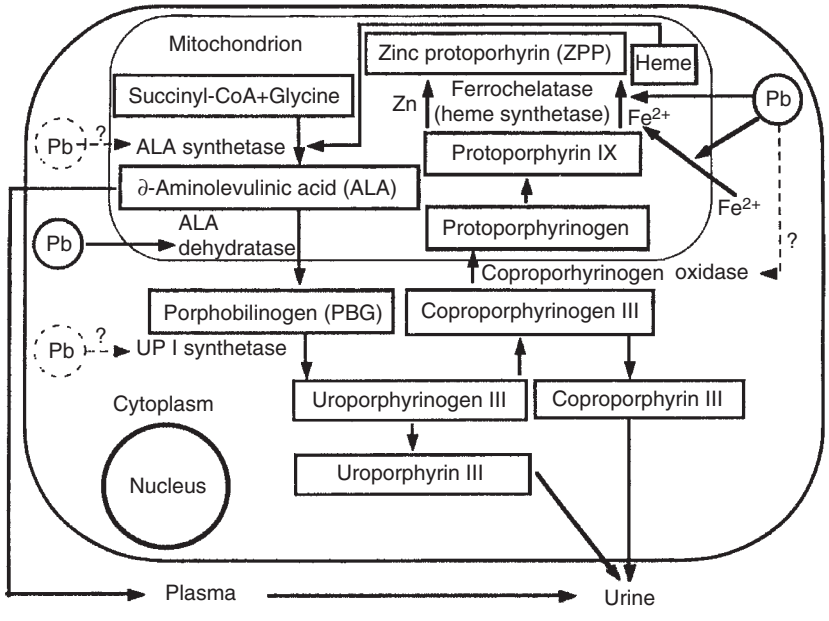

FIGURE 8 Heme metabolism. Known and suspected interactions by lead (Pb) are indicated (Skerfving, 1993).

In lead workers, ALA increases earlier in plasma (but not in red cells) than in urine; there is an exponential increase of plasma ALA with rising B-Pb (Morita *et al*., 1994; Sakai and Morita, 1996; Sakai *et al*., 1998). Plasma lead is associated with ALA concentrations in plasma and urine and coproporphyrin in urine; it displays closer correlations than B-Pb (Hirata *et al*., 1995; Sakai *et al*., 1998).

In Japanese workers with B-Pb <400 μg/L, the "benchmark dose" (excess risk 5%) was calculated to B-Pb of 27 μg/L for erythrocyte ALAD activity, 33 μg/L for ALA in plasma, and 88 μg/L for ALA in urine (Murata *et al*., 2003). Probably, effects on the enzymes ALAD in red cells are proportional to the B-Pb, right down to the B-Pb in subjects without particular lead exposure, even at the low B-Pb seen in many geographical regions (mean B-Pb approximately 0.10 μmol/L; Table 3; Skerfving, 2005). Disturbance of heme metabolism is more pronounced in women than in men, and more in children than in adults.

Lead exposure causes accumulation of free erythroporphyrin/zinc (FEP) protoporphyrin (ZPP) in

TABLE 3 Overall Summary of Information on at Which Lowest Blood Concentrations (average in studied populations; mol/L[1]) Various Adverse, Slight Effects of Lead Have Been Reported with Some Consistency

		Population		
			General	
Organ	**Effect**	**Occupational**	**Adults**	**Children**
Nervous system				
Central	Encephalopathia[2]	>4.0	>4.0	>4.0
	Slight symptoms	1.5–2.0	–	–
	Neurobehavioural	1.5–2.0	–	<0.5[3]
Peripheral	Symptoms	1.5	–	–
	Neurophysiological	1.5	–	–
Complex effects	Evoked potentials	1.5	–	–
	Posture	1.5	–	–
	Hearing	–	–	0.5
Autonomous	Heart rate variability	1.5	–	–
Blood	Anemia[2]	>3.0	>3.0	>3.0
	Hemoglobin concentration	2.0–2.5	–	–
	Heme metabolism	0.1–0.3	–	–
	Nucleotide metabolism	≈0.3	–	–
Kidneys	Tubular	1.5	–	0.5?
	Glomerular	2.0?	0.5?	0.5?
Cardiovascular	Blood pressure	1.5–2.0?	0.4	1.8?
	Heart rate variability	1.5	–	–
Endocrine system[4]	Hypothalamus/pituitary/thyroid/adrenal axes	1.5–2.0	–	–
Immune system	Immunosuppression	2.0	–	–
Mutagenicity	Chromosome aberrations, micronuclei, SCEs	1.5–2.0	–	–
Cancer	Kidney, lung[5]	?	–	–
Reproduction				
Female	Abortion	?[6]	0.5?	–
	Fetal growth	–	0.1?	–
	Neurobehavioural	–	–	<0.5[3]
Male	Endocrine function	1.5	–	–
	Sperm quality	2.0	–	–
	Fertility	2.0?	–	–
Gastro-intestinal tract[2]	Constipation, abdominal pain	>3.0	>3.0	>3.0

?=Limited data, inconsistent results and/or possible/probable confounding

–= Not relevant or not sufficiently studied. From Skerfving (2005)

[1] 1 μmol/L = 207 μg/L. 100 μg/L= 0.48 μmol/L.

[2] See Skerfving, 1993.

[3] Unceratinty whether effects are mainly due to exposure *in utero* or after birth.

[4] Except for reproduction.

[5] Uncertain.

[6] Levels not clear, probably high.

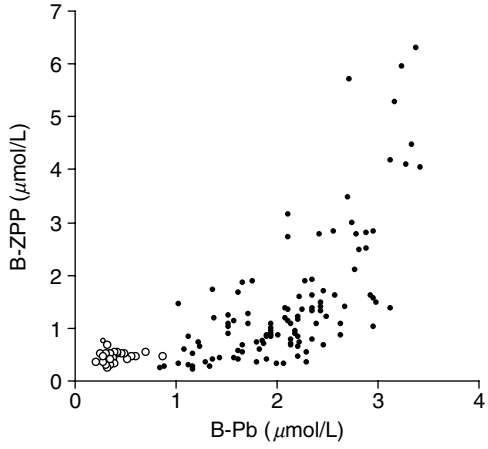

FIGURE 9 Blood lead (B-Pb) and zinc protoporphyrin (B-ZPP) concentrations in 112 male lead workers and 29 occupationally unexposed men. Data from Haeger-Aronsen and Schütz (1978).

erythrocytes (Section 2.7.2). Hence, the blood concentration of ZPP, which may be determined by a simple fluorimetric method, has been used as an index of lead exposure/absorption and risk. Partly because of the saturation of lead binding in erythrocytes (Section 2.5.2.1), there is an exponential increase of ZPP with rising B-Pb (Figure 9). Erythrocyte ZPP reflects the bone marrow lead concentration at the time of cell formation and remains during the life of the blood cell (4 months); hence, the time pattern may differ from B-Pb (half-time 1 month). Blood ZPP is less sensitive to lead exposure than plasma ALA (Sakai and Morita, 1996).

There are indications of a gene–environment interaction: Contrary to what might be expected if assuming a higher affinity of lead to $ALAD^2$ than to $ALAD^1$, more pronounced effects on ALA (Sakai *et al*., 2000; Schwartz *et al*., 1997b; Sithisarankul *et al*., 1997; Süzen *et al*., 2003) and ZPP (Alexander *et al*., 1998; Sakai *et al*., 2000; Schwartz *et al*., 1995) metabolism were shown in lead workers with homozygous $ALAD^1$ genotype, compared with $ALAD^2$ subjects. Because there seems to be no clear genotype-dependent difference in basic ALAD activity in erythrocytes (Sakai *et al*., 2000; Süzen *et al*., 2003; Zhang *et al*., 1998), the effect on ALA may be caused by an induction of ALA synthetase, caused by negative feedback regulation because of lack of heme, which is a result of inhibition of ferrochelatase-mediated incorporation of Fe^{3+} into heme in the mitochondria, because lead is not as efficiently sequestered by the ALAD protein in $ALAD^1$ subjects, compared with $ALAD^2$ subjects.

2.6.2.2 Nucleotide Metabolism

The enzyme pyrimidine 5′-nucleotidase (P5N) is present in the cytosol of erythrocytes. It catalyses, as a step in degradation of ribosomal RNA, hydrolytic dephosphorylation of pyrimidine-5′-monophosphates, but is ineffective on purine nucleotides, which are kept as a source of ATP. Lead inhibits P5N activity. This leads to accumulation of pyrimidine nucleotides in red cells (Ichiba *et al*., 1992; Kim *et al*., 1995a), which is believed to shorten the life span of the cells.

Concentrations of pyrimidine nucleotides have been used for biomonitoring of lead exposure/absorption and risks. However, the inhibitory effect of lead on P5N is lower than on ALAD (Kim *et al*., 1995a). Nicotinamide adenine dinucleotide synthetase (NADS) activity is also inhibited by lead (Morita *et al*., 1997), but this has only occasionally been used for monitoring.

2.6.2.3 Other

There are other possibilities of biomonitoring of effects. In particular, they are used in health surveillance of lead workers to detect early signs of toxicity (as well as to identify subjects with particular susceptibility; Section 2.13). Hence, examination of blood pressure and the nervous system is usually made together with determination of blood hemoglobin (or hematocrit) and proteins in urine. Also, the long series of subclinical findings related to effects on different organs described in the following (Section 2.7) may be regarded as biomonitoring. But they are nonspecific and seldom used and will not be further discussed here.

2.6.3 Summary

The lead concentration in whole blood is the traditional and dominant tool for biomonitoring of lead. A major advantage is the wealth of information it can be linked to. However, it has a problem: its relationship to uptake (as well as effects) is not rectilinear. Hence, at high exposure, there is an underestimate of the exposure and risk; in the low range, this is less of a problem. The B-Pb has a fairly rapid turnover (half-time approximately 1 month) and may thus vary parallel to variations in exposure. However, endogenous exposure from the slow skeletal lead pool may dampen this.

Serum or plasma lead may probably not have such a nonrectilinearity and may also reflect a more toxicologically relevant fraction of the lead in blood. On the other hand, there is still too limited information, and although the analytical problems are no longer unsurpassable, only few laboratories offer the analyses.

Determinations of bone lead by *in vivo* XRF reflect the long-term uptake and the body burden. Determinations in finger bone, tibia, calcaneus, and patella have been fairly widely used in recent epidemiological studies. However, the body of information still is limited to allow conclusions on dose-response relation-

ships for toxic effects. Another biomonitoring tool in children is analyses of shedded deciduous teeth, which reflects long-term uptake. Lead levels in hair have limited value as an index of uptake, because of the contamination problem.

The inhibition by lead of the heme metabolism has been widely used as a tool for biomonitoring of uptake and risk. Hence, inhibition of ALAD activity in blood cells occurs at a very low lead uptake. Also, levels of ALA in serum and urine, as well as the level of ZPP (FEP) in erythrocytes, are sensitive indicators of lead uptake, although with a considerable interindividual variation. However, they have been used less frequently lately, mainly because of the focus on effects of low lead exposures and the improvement of lead determinations. Inhibition of nucleotide metabolism has only seldom been used in biomonitoring.

2.7 Organ Effects

Lead can cause toxic effects on a long series of organs and tissues. There is little information on the relationship between lead exposure and effects. Instead, almost all dose/exposure response data refer to concentrations of lead in blood or bone. In some cases, biomonitoring of effects on the formation of heme has been related to toxicity on other functions.

2.7.1 Nervous System

2.7.1.1 Central Nervous System

2.7.1.1.1 Symptoms and Signs Exposure to inorganic lead may cause encephalopathy, especially in children, but also in adults. The classical signs in severe toxicity are ataxia, coma, and convulsions. After elimination of exposure and treatment by chelating agents, the signs of acute encephalopathy may improve, but residual symptoms may remain.

At less severe exposure, there are symptoms indicating less dramatic CNS effects. Hence, lead workers report higher prevalences of irritability, hostility, anxiety, fatigue, tension, depressed mood, interpersonal problems, and difficulties in concentrating (Ehle and McKee, 1990; Maizlish *et al.*, 1995).

2.7.1.1.2 Neuropsychological Tests A long series of studies of neuropsychological performance in lead workers have been published. Several meta-analyses have been made (Goodman *et al.*, 2002; Seeber *et al.*, 2002). The interpretation of the results is far from unanimous, in particular at low exposures. There are several problems. Hence, the results may be sensitive to the selection of studies, inclusion or exclusion of tests, multiple inferences because of many tests, adjustment for reliability, and choice of statistical methods. Furthermore, the choice of index of exposure is not obvious. Thus, sometimes, current B-Pb has been used, in other cases time-integrated exposure indices, such as time-weighted B-Pb or bone lead. Also, potential confounders (factors associated with both exposure and effects), such as age, education, and alcohol use are problematic. Furthermore, the referent groups often had relatively high exposures.

The main focus has been on effects on the attention/concentration/memory, visuospatial and visuomotor skills, and speed of learning and problem-solving ability. For example, in a study of 467 male current and former Canadian lead workers from a lead smelter, which had operated for 26 years. Because of hygienic measures and personal protection, the exposure had decreased significantly 12 years ago. The time-integrated B-Pb (mean, 7,52 µg/L × year) was associated with impairment of five tests (visuomotor skill, psychomotor speed, and dexterity and verbal memory) of 13 neuropsychological tests, after adjustment for age, education, alcohol use, and—less obviously why—time of employment and depressive symptoms. There were no associations with the current B-Pb (mean, 275 µg/L; Lindgren *et al.*, 1996).

Furthermore, in a Finnish study of 54 storage battery workers with well-defined exposure to lead (mean recent B-Pb, 1.3 µmol/L; tibia lead, 20 µg/g body mass), those who had never exceeded 2.4 µmol/L still had a decrement in visuospatial and visuomotor function, attention, and verbal comprehension (Hänninen *et al.*, 1998).

In a study of 803 Korean workers (mean B-Pb, 320 µg/L; tibia-lead, 37 µg/g body mass) and 135 controls (mean, B-Pb 53 µg/L; tibia-lead, 5.8 µg/g body mass), there were associations between B-Pb and DMSA-chelatable lead on the one hand and some neurobehavioral core battery tests on the other (Schwartz *et al.*, 2001). Tibia-lead was associated with decline in test score during the next year (Schwartz *et al.*, 2005).

Neuropsychological effects have also been reported in subjects from the general population without occupational lead exposure (B-Pb, 37 ± 23 µg/L; Nordberg *et al.*, 2000), but including young adults living close to a lead-emitting industry (mean B-Pb had decreased from 493 to 29 µg/L; referents 16 µg/L; Stokes *et al.*, 1998). However, the causality is not clear.

In a recent evaluation of all available information, it was concluded that neurobehavioral effects may occur at B-Pb of 1.5–2.0 µmol/L (tibia lead, 40 µg/g bone material) and higher (Table 3; Skerfving, 2005).

In lead workers, there were indications of a gene–environment interaction, with some protection by ALAD-2 for motor dexterity function (Chia *et al*., 2006).

There is some evidence of reversibility of the neurobehavioral deficit with decreasing lead exposure (Chuang *et al*., 2005; Winker *et al*., 2005; 2006).

Effects on the CNS of the fetus, newborn, infant, and child will be discussed together later (Section 2.11.1), because they are difficult to separate.

2.7.1.1.3 Other Effects There are some indications that lead workers have changes in their electroencephalogram (EEG; Kovala *et al*., 1997), indicating an effect on the CNS.

Similarities between the primary motor neuropathy caused by lead and motor neuron disease/amyotrophic lateral sclerosis (ALS) have been noted. In a U.S. case-referent study of ALS, the cases more often reported occupational lead exposure (Kamel *et al*., 2002). Also, there was a rise of the risk with increasing B-Pb (over the interval, <10–140 µg/L). However, such an effect may be spurious, because low physical activity may mobilize bone lead to the blood. However, the risk was also related to bone lead. But the matter may be more complicated, because there were indications that the *ALAD* polymorphism may confound the picture (Kamel *et al*., 2003).

2.7.1.2 Peripheral Nervous System

2.7.1.2.1 Symptoms and Signs Exposure to inorganic lead may damage the PNS. At severe exposure, the main clinical disorder is peripheral motor neuropathy with paralysis ("wrist drop" and "ankle drop"). In particular, the dominant hand is affected.

At lower exposures, there are motor symptoms in terms of mild distal weakness (decreased pinch and grip strength) of the upper limb (Schwartz *et al*., 2001; Yeh *et al*., 1995) and sensory effects in term of tingling or numbness in arms or legs, muscle pain, affected sensory and pain perceptions thresholds in fingers, and decreased vibration thresholds in hands and toes (Bleecker *et al*., 1997a; Chuang *et al*., 2000; Kovala *et al*., 1997; Lee *et al*., 2000; Rubens *et al*., 2001). The neuropathy is reversible, if adequately handled.

The effects are probably caused by demyelinization, axonal degeneration, and possibly also presynaptic block. It seems that the large, fast sensory fibers are particularly sensitive to lead.

In a recent review, it was concluded that slight sensory symptoms and signs have been noted at mean B-Pb of approximately 1.5 µmol/L and higher (Table 3; Skerfving, 2005).

2.7.1.2.2 Nerve Conduction At exposures that do not cause clinical disease, there are subclinical effects on the function of the PNS. At lower exposures, there were lead-associated impairment of sensory and motor nerve conduction velocities, distal latencies, and decreased amplitudes in median, radial, tibial, or sural nerves (Araki *et al*., 1993; Chia *et al*., 1996b,c; Kovala *et al*., 1997; Yeh *et al*., 1995; Yokoyama *et al*., 1998).

In recent reviews, it was concluded that neurophysiological disturbances of motor and sensory nerve conduction velocities and of vibration sense have repeatedly been associated with mean B-Pb of approximately 1.5 µmol/L (tibia-lead, 30–40 µg/g bone mineral), and higher (Table 3; Araki *et al*., 2000; Skerfving, 2005).

It is not known whether the reduced conduction velocities are really subclinical signs of the clinical neuropathy—it might be that they signify a more harmless disturbance of the ion transport over the cell membrane of the nerve cell. Also, there are some indications of reversibility at decreased exposure. However, in light of the severe neuropathy that may affect heavily lead-exposed subjects, the conduction velocity disturbances should be considered adverse.

2.7.1.3 Autonomous Nervous System

Occupational lead exposure affects the autonomous nervous system. Hence, associations between decreased electrocardiographic (ECG) heart rate variability (HRV) and B-Pb have been reported in several studies (Murata *et al*., 1995; Murata and Araki, 1991; Teruya *et al*., 1991). This indicates effects on the sympathetic and (less) parasympathetic nervous systems, possibly at the brain stem level.

In recent reviews, it was concluded that such effects may occur at B-Pb of 1.5 µmol/L and higher (Table 3; Araki *et al*., 2000; Skerfving, 2005).

2.7.1.4 Other Effects

2.7.1.4.1 Evoked Potentials In a series of studies, different varieties of evoked EEG potentials have been assessed in relation to lead exposure. Hence, associations between occupational lead exposure and auditory brain stem and event-related, visual and visual event-related and somatosensory evoked potentials have been reported (Araki *et al*., 1992; Discalzi *et al*., 1992; Hirata and Kosaka, 1993; Murata *et al*., 1993; Solliway *et al*., 1994). In one study, the findings correlated with urinary ALA; the authors suspected that this was due to ALA-induced inhibition of δ-aminobutyric acid (GABA) release, or competitive binding of ALA to GABA receptors, which has been shown experimentally (Solliway *et al*., 1995a,b).

In recent reviews, it was concluded that effects on evoked potentials in adults may occur at B-Pb of 1.5 µmol/L and higher (Table 3; Araki *et al*., 2000; Skerfving, 2005).

In a study of children in a lead-polluted area in Poland, some aspects of the auditory brain stem evoked potential differed between children with B-Pb above and below 100 µg/L (0.48 µmol/L; Osman *et al*., 1999a).

Disturbances in evoked potentials may be the result of effects in different areas of the nervous system. Hence, visual evoked potentials involve both the visual nerve and the cerebral cortex, auditory brain stem evoked potentials the auditory nerve, the brain stem, and the cortex, whereas somatosensory evoked potentials involve the afferent sensory nerves of the PNS, the plexus, the brain stem, and the cortex. Auditory or visual event-related evoked potentials also assess cognitive functions. Thus, it is difficult to know which part(s) of the nervous system is affected.

2.7.1.4.2 Postural Stability Subclinical effects in terms of increased postural sway have been recorded in several studies of lead workers (Chia *et al*., 1994c; 1996a; Yokoyama *et al*., 1997; 2002a,b) and children (Bhattacharya *et al*., 1993). Postural stability is a complex function. It involves exteroceptors and proprioceptors, sensory nerves of the PNS, the spinal medulla, the brain stem, the visual nerves, the cerebellum, and the cerebral cortex. There are some indications that the effect is due to toxicity on the anterior cerebellar lobe and the vestibulocerebellar and spinocerebellar afferent systems.

In recent reviews, it was concluded that slight effects on posture stability in adults may occur at B-Pb of 1.5 µmol/L or higher (Table 3; Araki *et al*., 2000; Skerfving, 2005).

2.7.1.4.3 Other Lead also affects auditory and visual functions (Otto and Fox, 1993). For example, in lead workers and referents, there was an association between impaired visual contrast and B-Pb (Lucchini *et al*., 2000). This was considered to be a sensitive indicator of neurotoxicity. Furthermore, in children from a lead-contaminated region of Poland, the hearing threshold was associated with B-Pb, even below 100 µg/L (0.48 µmol/L; Osman *et al*., 1999a).

2.7.1.5 Mechanisms

The mechanism behind lead-induced neurotoxicity is not clear (Rice and Silbergeld, 1996). However, several possibilities have been proposed. Hence, lead interferes with heme synthesis, which may impair the energy metabolism in the nervous system. Furthermore, ALA, which accumulates when ALAD is inhibited, is neurotoxic. However, lead binds to proteins in brain tissue (Quintilla-Vega *et al*., 1995); such binding seems to alleviate inhibition of ALAD (Goering *et al*., 1986).

Also, lead interferes with calcium-dependent processes (i.e., such as related to neuronal signaling and intracellular signal transduction) (Rice and Silbergeld, 1996). Hence, lead perturbs intracellular calcium cycling, altering releasability of organelle stores, such as endoplasmic reticulum and mitochondria. In some cases, lead inhibits calcium-dependent events (e.g., release of several neurotransmitters and receptor-coupled ionophores in glutamatergic neurons); in other cases, lead seems to augment calcium-dependent events, such as protein kinase C, calmodulin, and calcium-dependent ion channels.

Experimentally, lead can induce significant function impairment *in vivo* in the nervous system at doses below cytotoxicity (Rice and Silbergeld, 1996).

2.7.2 Blood and Blood-Forming Organs

Heavy lead exposure can cause anemia. Lead-induced anemia is characterized by basophilic stippling of peripheral erythrocytes, because the P5N inhibition causes an accumulation of pyrimidine nucleotides; normal depolymerization of reticulocyte ribosomal RNA does not occur, and granulae are formed. Lead inhibits enzymes in the heme synthesis and maybe also interfere with iron transport (ALAD and heme chelatase; Figure 8**;** Section 2.6.3). In the bone marrow, there are sometimes sideroblasts, probably because iron fails to be incorporated into heme. Moreover, lead inhibits the synthesis of a- and ß-chains of globulin. Also, there might be an anemic effect mediated by erythropoietin deficiency as a result of tubular kidney toxicity with impaired stimulation of erythroid progenitors (Osterode *et al*., 2006).

Importantly, lead shortens the life span of circulating erythrocytes, probably because of inhibition of the red cell membrane Na^+,K^+-ATPase and changes of membrane structural proteins, which may cause hemolysis.

In a series of studies in lead workers, associations have been observed between blood hemoglobin concentration, packed cell volume/cell count, on the one hand, and lead concentrations in blood or bone, on the other, although the explained variance is only a small percent (Gennart *et al*., 1992a; Solliway *et al*., 1996).

In a recent review, it was concluded that anemia might be caused by heavy lead exposure (B-Pb approximately 3.0 µmol/L) and that slight effects on blood hemoglobin concentrations and hematocrit have been reported at mean B-Pb of 2.0–2.5 µmol/L (Table 3; Skerfving, 2005).

However, B-Pb has particular limitations as an index of exposure when effects on the blood are assessed, because a reduced volume of red cells will limit the availability of lead-binding sites in blood. Plasma lead may then have advantages. Hence, in lead-smelter workers, there was an inverse relationship between blood-hemoglobin level and plasma-lead concentration, which might indicate an effect on heme synthesis (Bergdahl *et al*., 2006).

Furthermore, some data indicate that bone lead levels may be more closely associated with blood effects than is B-Pb (Hu *et al*., 1994; Lee *et al*., 2001b; Smith *et al*., 1995b), perhaps through a local effect on the bone marrow.

The main target by lead in the heme synthesis is ALAD, the activity of which seems to be inhibited at B-Pb right down to those in general populations with low exposure (approximately 0.10 µmol/L; Table 3; Skerfving, 2005). This results in increased ALA concentrations. It is not known whether such slight effects have health consequences. It is not known whether a corresponding inhibition occurs in other tissues at similarly low exposure. However, considering the central position of heme in the energy metabolism (CNS inclusive), and in handling of organic xenobiotics by the tissues (e.g., by cytochrome P450), the effect on the heme metabolism is potentially adverse. Also, it must be considered that ALA is neurotoxic and induces formation of free radicals.

There is a rare hereditary ALAD deficiency (for some reason denoted plumb porphyria by some authors), which may constitute an inborn error of lead metabolism, making the subject vulnerable, and predispose to severe toxic effects at relatively low exposure (Doss *et al*., 1984). Also, other acute porphyrias may predispose (Battle *et al*., 1987).

2.7.3 Kidneys

Heavy lead exposure may cause renal dysfunction characterized by glomerular and tubulointerstitial changes, resulting in hypertension, hyperuricemia and gout ("saturnine gouty arthritis"), and chronic renal failure.

There are indications that environmental lead exposure in the general population plays a role in the etiology and/or progression of kidney disease, at least in populations with high exposure. Hence, among Taiwanese patients with chronic renal insufficiency, those with gouty arthritis had higher "body lead stores" (lead excretion after chelation with EDTA) than the other cases (Lin *et al*., 2001). Furthermore, among patients with chronic renal disease of varying etiology, those with high lead burdens had a worse progression of their disease during 24 months (Lin *et al*., 2003). Moreover, when patients with high lead burdens were treated with EDTA for 27 months to mobilize lead, their glomerular filtration rate increased, whereas it decreased in untreated patients with similar lead burdens (Lin *et al*., 2003).

There are several reports of increased mortality from kidney disease in lead workers (Steenland *et al*., 1992; Cocco *et al*., 1997), but confounding by cadmium often precludes firm conclusions on a causal relationship with lead.

In Australians who had been treated for lead poisoning as children, there was increased mortality from kidney disease (Emmerson, 1973). However, no such effect was seen in the U.S. (McDonald and Potter, 1996), and no evidence of impaired renal function as adults (Hu, 1991a; Moel and Sachs, 1992).

At lower lead exposures in the occupational and general environments, there were associations between blood- and bone-lead concentrations, on the one hand, and increased serum levels of urate, as well as urinary excretion of low molecular weight proteins (in particular α_1-microglobulin and retinol binding protein) and lysosomal enzymes (*N*-acetyl-ß-D-glucosaminidase), on the other (Chia *et al*., 1994a, 1994b; 1995; Endo *et al*., 1993; Santos *et al*., 1994; Shadick *et al*., 2000; Weaver *et al*., 2003a; 2005a,b). The effect on serum urate may be more pronounced at old age (Weaver *et al*., 2005b). It has been proposed that a urate increase may be the mechanism behind lead-induced tubulointerstitial changes, although other mechanisms may also operate (Weaver *et al*., 2005b). Slight tubular proteinuria has also been reported to be associated with lead exposure in children (Bernard *et al*., 1995; Fels *et al*., 1998; Osman *et al*., 1999b; Verberk *et al*., 1996). The findings indicate an effect on the *proximal tubuli*, with deficiencies in excretion of urate and reabsorption of proteins, which have been filtered in the glomeruli, and shedding of tubular cells (although hyperuricemia in itself may also reflect oxidative stress; Waring *et al*., 2001). The effects on α_1-microglobulin and retinol binding protein were more evident than on ß$_2$-microglobulin, which may be due to the methodological problem of destruction of the latter at low urinary pH, but may also be a result of the fact that it has a lower molecular weight, which might make it less sensitive to inefficient reabsorption in the proximal tubuli. Possible interference by lead of renal hydroxylation of vitamin D is discussed later (Section 2.7.5).

In a recent review, tubular effects have been seen in occupational groups with mean B-Pb of approximately 1.5 µmol/L and higher (Table 3; Skerfving, 2005). In a few studies, changes have been seen in workers with mean bone lead levels of approximately 40 µg/g bone

mineral and higher. Limited data indicate that corresponding effects may occur in general populations of children with B-Pb of approximately 0.5 µmol/L and higher; in one study even lower (Osman *et al*., 1999b).

However, these slight effects are sensitive to confounding. Then, a particular problem is cadmium, which is known to cause proximal tubular effects, even at very low exposures (Åkesson *et al*., 2005; de Burbure *et al*., 2003). Hence, smelter workers—one of the most frequently studied categories—are often exposed to both lead and cadmium. This may cause a bias, overestimating any lead effect. Also, there is a possibility of a lead/cadmium interaction/sum effect. On the other hand, effects have also been seen in workers in work environments (mainly battery factories) with less likelihood of cadmium exposure.

As to *glomerular* effects, the picture is less clear. Associations between blood- and bone-lead concentrations, on the one hand, and serum concentrations of creatinine, blood urea nitrogen, cystatin C (an index of glomerular filtration rate), urate, and estimated and measured creatinine clearance, on the other, have been reported in general populations (Åkesson *et al*., 2005; Payton *et al*., 1994; Shadick *et al*., 2000; Staessen *et al*., 1992), including children (Osman *et al*., 1999b).

Glomerular effects (blood urea nitrogen, serum creatinine, creatinine clearance) have to a limited extent been studied in lead workers (Ehrlich *et al*., 1998; Roels *et al*., 1994) and have then sometimes indicated hyperfiltration at low exposure, decrease at higher (Ehrlich *et al*., 1998; Roels *et al*., 1994; Weaver *et al*., 2003a; 2005a,b). The hyperfiltration might be due to effects on the eicosanoid metabolism, affecting renal hemodynamics (Cárdenas *et al*., 1993). The glomerular effect may be more pronounced at old age (Weaver *et al*., 2005a).

A problem in the interpretation of data from occupational studies is the health surveillance that lead workers are often subject to, meaning that subjects with history or signs of kidney disorder or hypertension are rejected before and during employment, which, in a cross-sectional study, would cause an underestimate of the risk, in particular if the role of lead is an aggravation of other kidney disease.

Based on what is referred previously, there are indications of an effect on the glomerular filtration rate. However, because urate and creatinine are also excreted by tubular secretion, this may—at least partly—be due to tubular damage. Also, there is a possibility that a decrease of glomerular filtration rate may result in an accumulation of lead (reverse causality). This possibility has not been conclusively addressed. There are no conclusive data showing lead-induced albuminuria.

In a recent review, it was concluded that potential glomerular effects have been seen in adult general populations with mean B-Pb of 0.5 µmol/L and higher; in a couple of studies even lower (Table 3; Skerfving, 2005). In lead workers, effects have been recorded at B-Pb of approximately 1.5 µmol/L (Weaver *et al*., 2005a,b).

There are indications of interactions between lead retention, on the one hand, and age (Kim *et al*., 1996b; Weaver *et al*., 2005a; greater effect in older subjects), hypertension (Muntner *et al*., 2003; Weaver *et al*., 2005b), and diabetes (Tsaih *et al*., 2004), on the other, as regards serum creatinine.

It is not known whether recent or long-term (continuous or peaks) lead exposure is the most relevant determinant of risk. Furthermore, the relationship between the sensitive urinary tests for kidney function and later clinical chronic renal disease is not clear.

The mechanism(s) behind the nephrotoxicity of lead is not understood; inhibition of renal mitochondrial respiratory function is one possibility (Goyer, 1996). High-affinity lead-binding proteins are formed (Smith *et al*., 1998) with intranuclear inclusions in proximal tubule cells (Oskarsson and Fowler, 1985). Metallothionein may be involved in this process (Walker *et al*., 2004). The protein binding may modulate the bioavailablity of lead (e.g., alleviate lead inhibition of the heme pathway enzyme ALAD) (Oskarsson and Fowler, 1985).

There are some indications of a gene–environment interaction. Hence, the *ALAD* genotype may interact, although the picture is not consistent (Bergdahl *et al*., 1997a; Chia *et al*., 2006; Smith *et al*., 1995a; Weaver *et al*., 2003b). Also, *eNOS* genotype may modify the effect, whereas no such interaction was seen for *VDR*.

2.7.4 Cardiovascular System

2.7.4.1 Blood Pressure

Lead causes an increase of blood pressure in experimental animals. The exposure-response relationship seems to be unusual: the effect is probably relatively more pronounced at low exposures than at high ones.

Also, there is considerable evidence that lead absorption, at least in predisposed individuals, may lead to hypertension. Hence, several studies indicated a blood pressure effect in lead workers, although the picture is not consistent (Ehrlich *et al*., 1998; Maheswaran *et al*., 1993; Santos *et al*., 1994; Telisman *et al*., 2004).

There are clear indications of an effect of lead on blood pressure at lower exposures in general populations. Hence, associations between B-Pb and systolic and/or diastolic blood pressures have been seen in a long series of studies in many countries (Hense *et al*., 1993; Korrick *et al*., 1999; Kristensen, 1992; Møller and

Micciolo *et al*., 1994; Nash *et al*., 2003; Telisman *et al*., 2001; Vuppituri *et al*., 2003; Wolf *et al*., 1995), although not in all (Cheng *et al*., 2001; Nordberg *et al*., 2000; Staessen *et al*., 1996).

In a recent review, it was concluded that an effect of the blood pressure in lead workers might appear at B-Pb of 2.0–2.5 µmol/L (Table 3; Skerfving, 2005). Furthermore, it was concluded that in general populations, with mean B-Pb of 0.4 µmol/L or higher, there is an increase of systolic and/or diastolic blood pressure by approximately 1 mmHg for each doubling of B-Pb in the low range (although the picture is not consistent), less at higher. However, the variance of blood pressure explained by lead is minor.

The available information does not allow firm conclusions on whether the association between B-Pb and blood pressure is really causal. Thus, epidemiological studies of determinants of blood pressure are complicated, because there are many potential confounding and effect-modifying factors. For example, alcohol intake may cause confounding. However, in the latest studies, these problems seem to be reasonably well under control. Still, there is a risk of both residual confounding and overadjustment, which may result in overestimates or underestimates of the effect. The problems are well illustrated by the partly diverging results obtained in analyses of the same database (den Hond *et al*., 2002; Nash *et al*., 2003; Vuppituri *et al*., 2003). Also, reverse causality may exist: hypertension reduces glomerular filtration, which may decrease urinary lead excretion and cause a rise of B-Pb.

There are some indications that polymorphism in VDR might modify the relationship between lead and blood pressure (Lee *et al*., 2001a), although studies of *ALAD* polymorphism did not reveal such an association (Lee *et al*., 2001a; Smith *et al*., 1995a).

The mechanism(s) behind the likely association between lead exposure and blood pressure is not known. Several possibilities have been proposed: Direct effects on the excitability and contractibility of the heart, alteration of the compliance of the vascular smooth muscle tissue (perhaps through interaction with calcium signaling), altered vascular reactivity to α-adrenergic agents, interference with the renal synthesis of eicosanoids (resulting in depletion of prostaglandins with enhancement of sodium retention and the pressor response to angiotensin II and vasopressin), effects on the renal ion transport processes, and action on the parts of the CNS responsible for blood pressure regulation (Loghman-Adham, 1997). Furthermore, lead may cause a modification of the renin-angiotensin system.

Also, other effects mediated through kidney toxicity must be considered. Hence, it is possible that any effect on the blood pressure, at least at the high exposures in lead workers, is associated with lead-caused kidney damage.

2.7.4.2 Heart Disease and Stroke

The effect on blood pressure is not a health outcome *per se*, but is a risk factor for cardiovascular (coronary heart disease [CHD]) and cerebrovascular diseases. This risk is rather small from an individual subject risk point of view. However, in a population, it might be important. Indeed, a few studies showed increased risk of cerebrovascular diseases (perhaps also CHD) in lead workers (Gerhardsson *et al*., 1995b; Michaels *et al*., 1991), although most studies did not show such an effect (Cocco *et al*., 1997; Gerhardsson *et al*., 1995a; Gustavsson *et al*., 2001; Lundström *et al*., 1997; Steenland *et al*., 1992; Wingren and Axelson, 1993). In those studies, the referents were from the general population. This may be a problem, because there are indications of an effect already at such low B-Pb. Also, confounding by smoking and alcohol may be a problem.

In the general population, the information is limited. However, ECG changes were associated with B-Pb (Cheng *et al*., 1998a), as were CHD and stroke (Lustberg and Silbergeld, 2002; Møller and Kristensen, 1992). Also, children, who had been treated for lead poisoning, had higher cardiovascular and cerebrovascular risks as adults (McDonald and Potter, 1996).

In an attempt to calculate the global burden of disease (ishemic heart, cerebrovascular, hypertensive, and other cardiac) caused by lead, through an effect on blood pressure, it was estimated that 2% of the worldwide cardiovascular disease burden was due to lead exposure (annually 229,000 deaths and 3.1 million disability-adjusted life years [DALYs], i.e., the sum of years of life lost because of death and years of life with disability; Fewtrell *et al*., 2004).

As stated previously (Section 2.7.1.2), there were some indications of reduced HRV at B-Pb of 1.5 µmol/L and higher (Table 3; Skerfving, 2005).

2.7.5 Endocrine System

There is some indication of effects in lead workers on the hypothalamus-pituitary-thyroid/adrenal axes (Tiwari *et al*., 1985; Tuppurainen *et al*., 1988), although the picture is not consistent (Erfurth *et al*., 2001; Gennart *et al*., 1992a; Gustafsson *et al*., 1987), perhaps because of varying exposure levels. Furthermore, associations between serum prolactin and B-Pb have been reported (Lucchini *et al*., 2000; Manzo *et al*., 1996). In neither case is the level of action known.

Hence, there are indications that lead causes endocrine disruption. In a recent review, it was concluded that there were some indications of such effects at occupational lead exposure in the range of 1.5–2.0 µmol/L and higher (Table 3; Skerfving, 2005).

There are associations between lead and serum levels of vitamin D metabolites. In children, very high B-Pb was associated with low concentrations of 1,25-dihydroxycholecalciferol (1,25-$(OH)_3D_3$; Mahaffey *et al.*, 1982), possibly because of deficient hydroxylation in the kidney tubuli, no such relationship was seen in children with lower B-Pb (Koo *et al.*, 1991). However, the picture is complicated. Hence, in lead workers, high B-Pb was associated with *high* serum concentrations of 1,25-$(OH)_3D_3$ (Chalkley *et al.*, 1998; Kristal-Boneh *et al.*, 1998; Mason *et al.*, 1990). The latter may be due to compensatory increase of formation to compensate for lead-inhibited intestinal lead absorption (Fullmer, 1990). 1,25-$(OH)_3D_3$ may increase lead mobilization from the skeleton. Interactions between genetics of VDR and lead metabolism were discussed previously (Section 2.5.6), effects of lead on the skeleton will be treated later (Section 2.7.7).

Sex hormones will be discussed later (Section 2.11).

2.7.6 *Gastrointestinal Tract*

Lead is excreted in the saliva (Koh *et al.*, 2003), which may explain the gingival "lead line" (Burtonian line) sometimes seen in lead workers (alternately, the origin is lead in the gingival tissue). The greyish color is due to precipitated lead sulfide, which is formed by sulfur produced by bacteria. Hence, the sign is most prevalent in lead workers with bad oral hygiene and bacterial paradentosis.

Gastrointestinal symptoms (lead colic) are often the only clinical ones in lead poisoning. They appear in the beginning, often remain during the entire course of the disease and are often the reason why lead poisoning is diagnosed. They are often not characteristic. They most often start as protracted constipation, indigestion, and loss of appetite, but only occasionally diarrhea. The abdominal cramps are intermittent, often with pain-free intervals, most often localized in the hypogastrium (sometimes in the epigastrium), sometimes radiating to the urinary bladder, scrotum, and kidney. They may be very intensive. Vomiting is prevalent, as is tenesmus without defecation and miction without much urine. The clinical picture may be mistaken for obstructive ileus or appendicitis. The patients often compress their abdomen because of the pain, which excludes peritonitis as the cause. Probably, the gastrointestinal effects are due to interaction with calcium in the smooth muscle cells; calcium administration intravenously will give temporary relief.

In a recent review, it was concluded that gastrointestinal symptoms usually occur at B-Pb of 3 µmol/L and higher (Table 3; Skerfving, 2005).

2.7.7 *Other Organs*

Lead exposure is associated with a low skeletal growth, even at levels of exposure in the general population (Frisanch and Ryan, 1991; Schwartz *et al.*, 1986). The mechanism is not known. However, there are possibilities of interaction with both calcium metabolism and bone cell proteins (Sauk *et al.*, 1992).

2.8 Immunotoxicology

There is only limited information on immunotoxic effects of lead (McCabe, 1994), and the picture is not consistent. Some of the interstudy discrepancies may be due to the variations in exposure intensity and methodological differences. There is no evidence of a *marked* immunotoxic effect of lead at the exposure levels studied.

However, there are probably various effects on the humoral immunity—immunoglobulin levels in serum (Horiguchi *et al.*, 1992a; Pinkerton *et al.*, 1998; Queiroz *et al.*, 1993; 1994; Undeger *et al.*, 1996) and saliva (Pinkerton *et al.*, 1998; Queiroz *et al.*, 1994) in lead workers. Moreover, in lead-exposed workers, varying effects on leukocyte and lymphocyte subtypes and function have been described (Fishbein *et al.*, 1993; Pinkerton *et al.*, 1998; Queiroz *et al.*, 1994; Sata *et al.*, 1997; Undeger *et al.*, 1996; Valentino *et al.*, 1991). Their health implications are not clear, but there are some indications of increased sensitivity to infections in Japanese lead-refinery workers (Horiguchi *et al.*, 1992b).

The information is not easy to interpret in terms of exposure response. However, in a recent review it was concluded that various effects have been reported in groups of lead workers with mean B-Pb of approximately 2 µmol/L and higher (Table 3; Skerfving, 2005).

2.9 Mutagenicity

Lead has a clastogenic effect, inducing chromosome aberrations, micronuclei, and sister chromatid exchanges (SCEs) in lead workers (Dönmez *et al.*, 1998; Duydu *et al.*, 2001; Vaglenow *et al.*, 1998; 2001; Wu *et al.*, 2002). In a recent review, it was concluded that increased occurrences of such effects have been shown in peripheral lymphocytes of lead workers exposed at levels corresponding to average B-Pbs of approximately 1.5–2 µmol/L and higher (Table 3; Skerfving, 2005).

All these effects may increase the risk of cancer (Section 2.10). Indeed, it has now become clear that

structural chromosome aberrations—as had been assumed for a long time—are really associated with an increased risk of later cancer (Hagmar *et al*., 1998). For micronuclei and SCEs, the picture is less clear.

As to mechanism of the mutagenic effect, evidence shows that lead accumulates in the cell nucleus (Silbergeld *et al*., 2000). However, there is only limited evidence of direct genotoxic or DNA-damaging effects, except for lead chromate, where hexavalent chromium is probably the cause. Hence, lead has mostly been negative in *in vitro* gen-tox assays.

Rather, lead-induced nongenotoxic/epigenetic mechanisms seem to affect DNA (Silbergeld *et al*., 2000). Thus, lead exposure may increase the susceptibility to genotoxic agents. Hence, lead may bind to, and deplete, glutathione (a free-radical scavenger; Hunaiti *et al*., 2000), interfere with DNA repair (Hartwig, 1994), and bind to histones, thus decreasing their DNA protection (Quintanilla-Vega *et al*., 2000). In accordance with this, there was a multiplicative effect for coexposure in work environments to lead, cobalt, and cadmium, as regards induction of DNA single-strand breaks (Hengstler *et al*., 2003).

Lead-induced ALA accumulation (Section 2.6.2.1) can also generate reactive oxygen species, which may damage DNA (Silbergeld *et al*., 2000). Furthermore, experimental evidence shows that lead can substitute for zinc in several proteins that function as transcriptional regulators, including protamines. Lead also reduces the binding of these proteins to recognition elements in genomic DNA, which suggests an epigenetic involvement of lead in altered gene expression.

2.10 Cancer

Animal experiments have shown a tumorigenic effect of lead (Silbergeld *et al*., 2000). Hence, soluble lead salts, such as lead acetate and subacetate, have produced kidney and brain tumors, and lead phosphate kidney tumors, in rodents after oral or parenteral administration. Synergistic effects exist for the development of cancer between lead acetate and oxide, on the one hand, and some organic carcinogens, such as benzo(a)pyrene and nitrosamines, on the other.

In a series of epidemiological studies, lead workers had increased risks of total, kidney, lung, or stomach cancers (Anttila *et al*., 1995; Carta *et al*., 1994; Cocco *et al*., 1994a,b; Fu and Bofetta, 1995; Gerhardsson *et al*., 1995b; Wingren and Axelson, 1993; Wong and Harris, 2000). Also, there is limited support for a synergism with other carcinogens (e.g., diesel exhaust; Anttila *et al*., 1996). The studied cohorts have had a high exposure (mean B-Pb >3 μmol/L), at least in most of the studies (Skerfving, 2005).

There is some data indicating that lead exposure in the general population is associated with cancer risk (Lustberg and Silbergeld, 2002). Then, the exposure has been much lower.

However, the carcinogenic pattern in humans is not consistent (Cocco *et al*., 1997; Englyst *et al*., 2001; Ojajärvi *et al*., 2000; Steenland *et al*., 1992). Furthermore, there are major problems in terms of confounding (e.g., as regards coexposure to arsenic and cadmium in the occupational cohorts). Also, there may be selection bias. Hence, lead workers may differ in many ways besides the lead exposure. In particular, confounding by smoking is a problem, which has only occasionally been tackled. Also, the worker may be physically fit and may have different diets.

2.11 Reproduction

2.11.1 Females and Offspring

Lead is distributed to the ovary (Barry, 1975). There is some information indicating an effect of lead on female sexual maturation (Wu *et al*., 2003). Also, limited information may mean that lead causes a delay of time-to-pregnancy (longer time to become pregnant in women after a decision to try; Sallmén *et al*., 1995).

During pregnancy, the B-Pb changes in the woman. First, it decreases because of hemodilution (Lagerqvist *et al*., 1996; Rothenberg *et al*., 1994; 2000; Téllez-Rojo *et al*., 2004). Later on, lead is mobilized from the skeleton, causing an increase of B-Pb (Pires *et al*., 2001; Téllez-Rojo *et al*., 2004). In Australian women, B-Pb rose by 20%, of which 30% originated from the skeleton (Gulson *et al*., 1998b). Low calcium intake seems to cause an increase of blood and bone lead at a high lead exposure (Hernandez-Avila *et al*., 1996; Téllez-Rojo *et al*., 2002), but not at a low one (Berglund *et al*., 2000a; Téllez-Rojo *et al*., 2004; Vahter *et al*., 2002). The B-Pb during pregnancy is associated with a rise in blood pressure (Rothenberg *et al*., 1999a; 2002).

Lead is deposited in the placenta (Hubermont *et al*., 1978; Osman *et al*., 2000; Schramel *et al*., 1988). The levels in the placenta were higher in occupationally lead-exposed women than in nonexposed ones (Khera *et al*., 1988; Wang *et al*., 1989). Low lead levels are present in amniotic fluid (Klink *et al*., 1983). However, a large part of the lead mobilized from the skeleton and absorbed from the gastrointestinal tract is transferred through the placenta into the fetus (Ong *et al*., 1993). There is a close association between maternal and cord B-Pb; the cord blood concentration is approximately 85% of the maternal one.

Lead is embryotoxic and fetotoxic in experimental animals. Lead exposure in man has frequently been associated with spontaneous abortion (Hertz-Picciotto,

2000; Paul, 1860). However, most of the studies have been biased because of small sample size, poor ascertainment of outcome, lack of control of confounding, and/or deficiencies in exposure assessment. However, even when these factors were adequately accounted for, there was an association between risk and B-Pb (Borja-Aburto *et al*., 1999).

In a recent review, it was concluded that an increased risk of spontaneous abortion might occur at B-Pb approximately 0.5 µmol/L (Table 3; Skerfving, 2005), although there is a risk of confounding.

The mechanism for inducing pregnancy loss is not clear. Besides preconceptional chromosome damage (in the egg cell or in the testis, see later) or a direct teratogenic effect on the fetus, interference with the maternal/fetal hormone environment or developmental toxicity to the embryo/fetus is possible. Also, vascular effects on the placenta are plausible; elevated blood pressure during pregnancy means increased risks to both the mother and fetus (reduced birth weight, *abruptio placentae*, and perinatal mortality).

The available information does not firmly support a teratogenic effect (malformations) of lead in man, although an increased risk of neural tube defects has been reported (Bound *et al*., 1997; Irgens *et al*., 1998).

On the other hand, many studies have shown other effects on the fetus, including disturbance of heme synthesis (Lauwerys *et al*., 1978), stillbirth (Baghurst *et al*., 1987; Khera *et al*., 1988), and reduction of gestational age, birth weight, and head circumference (Hernandez-Avila *et al*., 2002; Rothenberg *et al*., 1999b). Effects on the size of the infant have been reported even at an average B-Pb of only 54 nmol/L (Osman *et al*., 2000), although the risk of unaccounted confounding is great. Reduced fetal growth is of particular interest because it may predict later cardiovascular, neurological, and metabolic disorders.

In a recent review, it was concluded that associations between lead exposure and size of the newborn have occasionally been reported at mean B-Pb in the mothers even at < 0.1 µmol/L (Table 3; Skerfving, 2005). However, again unadjusted confounding may be a problem.

During lactation, there is also a mobilization of lead from the skeleton of the woman (Berglund *et al*., 2000a; Téllez-Rojo *et al*., 2002). Lead is excreted into the milk (Oskarsson *et al*., 1995). Milk-lead levels in the range of 0.1–3 µmol/L have been reported from various countries (Ettinger *et al*., 2004a,b; Gulson *et al*., 2001; Hallén *et al*., 1995; Pires *et al*., 2001; Soweres *et al*., 2002; Tiran *et al*., 1994). Half of the milk lead originates from the mother's diet, half from her skeleton (Gulson *et al*., 2000). The milk-lead level is proportional to maternal bone (Ettinger *et al*., 2004a; Sowers *et al*., 2002) and blood (nonrectilinearly; Ettinger *et al*., 2004a; Namihira *et al*., 1993) lead. Possibly, there is some reduction of skeletal mobilization by calcium supplementation (Hernandez-Avila *et al*., 2003). There is a correlation between milk-lead concentrations in the lactating mother and the B-Pb in the breast-fed infant (Gulson *et al*., 1998b); the infant absorbs most of the lead content in milk (Gulson *et al*., 2003).

Experimental lead exposure of the dam (e.g., in primates) has shown toxic effects on the CNS of the offspring (Rice and Silbergeld, 1996). Moreover, many studies have shown effects by prenatal and postnatal lead exposure on the human fetus/infant/small child. This has been a major issue in lead toxicology.

Lead induces toxic effects on the CNS of the fetus, infant, and child. Mostly, it is not possible to conclude whether the effects seen in children are due to exposure prenatally, postnatally, or both. Hence, both will be discussed together here.

A long series of cross-sectional studies of general populations, with varying exposure intensities, have been reported in the last decade in Denmark, Germany, Croatia, the United States, Mexico, Saudi Arabia, China, Taiwan, Pakistan, and New Zealand (reviewed by Koller *et al*., 2004). The studies examined 80–4853 infants or children up to age 16. Mean B-Pb ranged between 29.4 and 973 µg/L. Generally, there were associations between B-Pb and cognitive functions. However, there are major methodological problems caused by the cross-sectional design of these studies.

Also, several prospective studies of pregnant women and their offspring have been published. Because the results are crucial for any conclusion on critical lead exposure, they deserve to be presented in some detail. Each study has, over the years, resulted in a series of publications. Here, only the latest one is quoted.

In the Cincinatti Lead Study, Ohio, there were, in 245 6-year-old children, significant negative associations between achievements in a comprehensive neuromotor assessment battery, on the one hand, and maternal first trimester (mean, 0.406 µmol/L), neonatal (0.233 µmol/L), and postnatal (maximum at 2 years, 0.824 µmol/L) B-Pb, on the other (Dietrich *et al*., 1993).

In a prospective study in Boston, Massachusetts, in 48 children whose B-Pb had never exceeded 100 µg/L at birth or at age 6, 12, 18, 24, 57, or 120 months, there was a negative association between adjusted IQ at age 10 and B-Pb, although the precise shape of the dose-effect relation remained uncertain (Bellinger and Needleman, 2003).

Around the smelter in Port Pirie, Australia, B-Pb (geometric mean, 83 µg/L at birth) in 375 children was negatively associated with adjusted cognitive performances at 2, 4, and 7 years of age (Tong *et al*., 2000).

However, in connection with a decrease of mean B-Pb from 1.02 µmol/L (212 µg/L) at age 2, to 0.38 µmol/L (79 µg/L) at ages 11–13, there was no significant relationship between the individual B-Pb decrease and the IQ change. The data may indicate a long-lasting effect of damage caused in the fetus and/or infant.

In the Yugoslavia Prospective Lead Study, IQ at 3, 4, 5, 7, or 10–12 years in 390 children to women living near a smelter (mean, B-Pb 309 µg/L at age 10–12) and a control (B-Pb, 61 µg/L) town displayed a negative association with prenatal and postnatal B-Pb (Wasserman *et al*., 2003). A doubling of the average lifetime B-Pb was associated with a decrease of full-scale IQ by 1.6 points. The association with tibia lead at age 10–12 was closer than with B-Pb.

In the Mexico City Prospective Lead Study, 112 children were followed to 3–5 years of age (Gomaa *et al*., 2002). B-Pb (geometric mean, approximately 100 µg/L) measured in cord blood was negatively associated with adjusted intellectual function, whereas postnatal B-Pb was not.

In an important study of 172 children in Rochester, New York, in which B-Pb was measured serially between the ages 6 months and 5 years, 101 did not have a recorded B-Pb >100 µg/L (Canfield *et al*., 2003; additional information in Koller *et al*., 2004). Strong and significant negative associations between B-Pb and adjusted IQ (0.74 points per 10 µg/L) were observed at 3 and 5 years. There were indications that the effect was larger at lower B-Pb than at higher ones.

In a thorough review of some of the recent prospective studies of children, it was concluded that even at B-Pb consistently <100 µg/L, there was intellectual impairment (Koller *et al*., 2004). Some of the reviewed studies indicate a supralinear exposure-response relationship (i.e., the loss of IQ at a B-Pb change from 0 to 100 µg/L would be larger than at a change from 100 to 200 µg/L) (Lanphear *et al*., 2005). Although this is under debate, it undermines the idea of a threshold. However, lead exposure was believed to account for only a small fraction (1–4%) of the total variation in cognitive ability while social and parenting factors account for 40% or more (Koller *et al*., 2004).

Critics have questioned the importance of such small decrements of IQs of individual children. However, the tests are blunt instruments for detecting subtle changes in brain function. Also, on a population basis, the effect is considerable. Furthermore, even small changes of IQ in large numbers of children will dramatically increase the proportion of children with low (e.g., <80) and decrease the proportions with high (>120) IQ.

It should be stressed that the confounding and effect-modification issues are complicated (Bellinger, 2000). All the aforementioned studies adjust for different covariates. However, still overadjustment and/or underadjustment, or incorrect inference, may easily occur. Also, the limits of the precision of analytical and psychometric measurements increase the uncertainty of any estimate of effect, especially at B-Pb <100–150 µg/L. Hence, if a threshold exists, it is unlikely to be detected (U.S. CDC, 2005). Likewise, if there is no threshold, that is also very difficult to prove.

There is limited information indicating a gene–environment interaction. Hence, children with the $ALAD^2$ genotype had low dentine lead and consistently better performance compared with children with $ALAD^1$, although the effects were small (Bellinger *et al*., 1994b).

Lead-associated declines in mental development in children living in contaminated housing were not reversed by chelation (Liu *et al*., 2002; Rogan *et al*., 2002). However, there are several caveats regarding these results.

In the U.S., Attention Deficit Hyperactivity Disorder (ADHD) in children (age 4-15) was associated with B-Pb (Braun *et al*., 2007). Further, in children (age 11) from Pittsburgh, Pennsylvania, there was a slight, but statistically significant, association between tibia-lead and delinquent behavior (Needleman *et al*., 1996). Furthermore, arrested and adjudicated youths had higher bone lead than controls (Needleman *et al*., 2002). It may be mentioned, that in the United States, there is an ecological relationship (at county level) between B-Pb and homicide rate (Stretesky and Lynch, 2001). Needless to say, such studies include large problems of residual confounding.

In summary, retardation of neurobehavioral development and growth, as well as electrophysiological and hearing changes, have in a long series of studies of infants and children been shown to be associated with B-Pb in the mother, newborn, infant, and child. It is not known which period is most critical. For example, the *in utero* exposure is often related to postnatal uptake of lead. Despite the many methodological problems in such studies, there were indications that effects may occur even at B-Pbs in the pregnant woman and infant of only 0.5 µmol/L, perhaps even lower (Table 3; Skerfving, 2005). WHO (2000b) estimated that there was a net decrease of 3.4 (95% confidence interval 1.1–5.0) IQ points at 150 µg/L. Although the disablement in the individual is small, as is the fraction of the total variance of the CNS function explained by lead, the effect is definitely adverse. The reversibility of the effects is not adequately known but they seem to be at least partly irreversible.

In an attempt to calculate the global burden of disease caused by lead-induced mild mental retardation, the estimate was 9.8 million disability-adjusted life years (DALYs), mostly in the West Pacific, Southeast Asia, and Central and South America regions (Fewtrell *et al*., 2004).

2.11.2 Males

In lead workers, slight, lead-associated effects on the hypothalamic-pituitary-testis axis (mainly luteinizing and follicle-stimulating hormones) have been reported (Braunstein *et al*., 1978; Erfurth *et al*., 2001; Gustafsson *et al*., 1989).

Lead is distributed to the testis (Barry, 1975). Also, lead accumulates in other parts of the male reproductive tract (epididymis, seminal vesicles, and prostate; Johansson and Wide, 1986). Furthermore, there is incorporation into seminal fluid.

Low levels of lead (a few μg/L) have been found in the seminal plasma in males without particular exposure (Chia *et al*., 1992; Dawson *et al*., 1998; Xu *et al*., 1994; 2003). It seems that a significant fraction of this originates from the prostate or the seminal vesicles (Butrimowitz *et al*., 1983). Furthermore, lead workers have increased lead levels in the seminal fluid; the levels were approximately one tenth of those in blood (Bonde *et al*., 2002; Kuo *et al*., 1997). The level in spermatozoa is similar to that in blood (Bonde *et al*., 2002).

In lead workers, there were associations between exposure and sperm count, motility, and morphology (Aribarg and Sukcharoen, 1996; Lerda, 1992; Telisman *et al*., 2000). In one study, the critical B-Pb seemed to be 440 μg/L (Bonde *et al*., 2002). Significant improvement has been reported after reduction of exposure (Viskum *et al*., 1999). Even in subjects without occupational lead exposure, there was an association between seminal fluid lead and sperm count (Xu *et al*., 2003).

Decreased libido, erectile dysfunction, and ejaculation problems have been reported in lead workers (Lancranjan *et al*., 1975), but there are methodological problems that preclude firm conclusions. In a study of Finnish lead workers, there was a blood-lead associated decrease of the standardized fertility ratio; an effect was seen at 0.5–0.9 μmol/L (Sallmén *et al*., 1992; 2000). Similar data have been reported from Belgium (Gennart *et al*., 1992b). However, other results have not been consistent (Apostoli *et al*., 2000; Lin *et al*., 1996).

There are indications of a risk of spontaneous abortion in wives of lead workers (Anttila and Sallmén, 1995). Such a risk was also suggested by a Finnish case-referent study of hospital-treated spontaneous abortion, although the data did not allow firm conclusions (Lindbohm *et al*., 1991).

Furthermore, preterm birth (Kristensen *et al*., 1993) and low birth weight in the offspring (Min *et al*., 1996) have been reported to be associated with occupational lead exposure of the father, but the risk of confounding by secondary exposure of the mother by lead dust brought home, as well as lifestyle and other occupational factors, is obvious. There is no conclusive evidence as to malformations (Irgens *et al*., 1998) or cancers (Kristensen and Andersen, 1992) in the offspring of male lead workers.

In a recent review, it was concluded that effects on male reproduction (endocrine function, sperm quality, and perhaps fertility) may occur at B-Pb of 1.5–2.0 μmol/L or higher (Table 3; Skerfving, 2005).

2.12 Overall Assessment of Risk

2.12.1 The Data Sets—Strengths and Limitations

In the last decades, the perspective of lead toxicity has changed in several aspects: Large lead-worker groups and general populations have been investigated. Some effects have been reported in general population strata with low exposures, in the range of the referent groups in many studies of lead workers. This is a problem in the interpretation of the occupational studies.

Also, explanations of the formerly intriguing differences in sensitivity have begun to appear in terms of gene–environment interactions, mainly as regards *ALAD* genotype. $ALAD^2$ subjects seem to have a higher B-Pb at the same exposure intensity (at least when it is not low) and perhaps less toxic effects. Thus, despite their higher B-Pb, they may be protected from at least some adverse effects and may tolerate higher exposure. These phenomena may induce selection at the workplace. Hence, if B-Pb is surveyed, $ALAD^2$ subjects may be more likely to be removed. On the other hand, if there is no such surveillance, $ALAD^1$ subjects may quit selectively because of early symptoms. Moreover, the prevalences of the genes differ between races, which may affect the risk patterns. Preemployment screening for *ALAD* genotype has been proposed. However, this seems premature.

One problem is that most studies do only allow conclusions about differences between groups with varying exposure; hence, it is not possible to define a no (NOAEL) or lowest (LOAEL) observed adverse effect levels. Also, the number of workers have often been low. On the other hand, when discrete exposure measures (B-Pb) are used in large populations, effects may be shown at low exposures, but then at a low rate.

At the same time, sensitive methods have been used. Then, the question whether the effects should really be considered adverse (i.e., indicating a health risk, and thus a basis for risk assessment) becomes more complicated.

There is still far too little information on the relationship between air lead and effects. Hence, biomarkers have to be used. However, the choice of biomarkers to define the exposure may seem less obvious than earlier. Hence, in the last decade, the use of *in vivo* determination of skeletal lead has exploded. Thus, bone lead has sometimes shown relationships to effects, when B-Pb has not.

Some information relating effects to plasma/serum lead has occurred. They may have advantages over B-Pb (Section 2.6.1.1.2), but data are still too limited to make it possible to use it in a risk assessment. As to urinary lead, there are several problems, in particular at low exposure; also, the information on exposure-response is limited and mostly obsolete. Furthermore, chelatable lead (after dosing of EDTA or DMSA, which differ), despite the fact that there is quite a lot of information as regards some effects, is not possible to use in practice, mainly because it is too complicated for risk surveillance.

Thus, because of the wealth of information, we still have to use B-Pb, keeping its limitations (mainly its nonlinearity) in mind. An important—and still mostly unsolved—problem is the time aspect of the toxic effects. Hence, it is possible that the current B-Pb is less interesting than the time-integrated/cumulated B-Pb (or bone lead, which is related to it) or the peak B-Pb. Furthermore, the reversibility of most of the critical effects is not known. It is perfectly clear that some effects (kidney damage by heavy exposure during childhood) stay for life despite reduction of exposure, but it is possible that others—at least partly—disappear.

2.12.2 Effects and Their Relation to Exposure

From the preceding review of effects on different organs, it is clear that the focus, from the point of view of the work environments, should be on two discrete effects: Toxicity in the fetus and/or breast-fed infant and on the blood pressure. In the general population, neurotoxicity in children must also be considered. These effects are adverse. It is not possible to define an overall NOAEL or LOAEL. It is not known whether there is a threshold (U.S. CDC, 2005).

It might well be that in some areas of the world, a considerable fraction of the general population suffers from effects. However, it is also clear that the fraction of the total variance of the effect explained by lead then is low. On an individual level, the effect of lead on disorders and disease processes may be subtle. However, on the population level, lead exposure may contribute an important fraction to the morbidity/mortality associated with theses disease processes.

A summary of the B-Pb associated with various adverse and nonadverse effects is given in Table 3 (Skerfving, 2005).

The CNS of the fetus and the infant/child seems to be affected at a mean B-Pb in the pregnant/lactating woman/infant/child of 0.5 μmol/L, probably even lower (Table 3; Skefving, 2005). In the last decade, the importance of the mobilization of lead from the skeleton of the pregnant and lactating women has become perfectly clear. This is a major source of exposure of the offspring. Considering the sensitivity of the fetus/infant, lead exposure in girls and fertile women should be as low as possible. Hence, before menopause, women should not be exposed in the workplace.

Effects on blood pressure in general populations have been shown at average B-Pb approximately 0.4 μmol/L (Table 3; Skerfving, 2005). Effects on the kidney may perhaps start at a mean B-Pb of 0.5 μmol/L, a level that is present in many general populations.

However, there is better evidence for slight effects on the kidney at a mean B-Pb of 1.5 μmol/L (Table 3; Skerfving, 2005), which is a level often present in lead workers and in the general population in some areas with high lead exposure. Adverse effects on the nervous and endocrine systems and male reproduction may occur at a mean B-Pb of 1.5–2.0 μmol/L.

There is some evidence of mutagenicity (clastogenicity) and effects on the immune system at a mean B-Pb of 1.5–2.0 μmol/L (Table 3; Skerfving, 2005). Adverse effects on blood formation seem to start at approximately 2.0–2.5 and on the gastrointestinal tract at approximately 3 μmol/L.

Because of the dramatic decrease of B-Pb in the general population in many areas (in some countries to approximately 0.1–0.2 μmol/L), it may be argued that the space for occupational exposure has increased somewhat.

It is not easy to translate B-Pbs into lead levels in air. Part of the uptake in work environments occurs through oral ingestion. Furthermore, it is clear that there is a wide difference between lead species with varying solubility and particle sizes. Also, the "background" exposure and the endogenous exposure from skeletal lead depots, caused by earlier exposure, must be considered. However, from the data just presented, it seems that the average worker would just not reach 1.5 μmol/L if exposed to 200 μg/m^3 of lead with low solubility or to approximately 30 μg/m^3 with high, if assuming small particle sizes (Figure 4). The metabolic models give the same picture.

Several human studies indicate limited evidence that lead is carcinogenic in man, but definite proof is lacking. Lead chromate and arsenate are carcinogenic in man, but that is not due to the lead moiety.

2.13 Exposure Standards and Classifications

2.13.1 Occupational Exposure Limits (OELs)

In the United States, permissible exposure limit is 50 μg/m^3 (action limit 30 μg/m^3) (U.S. OSHA, 1998). There is a requirement for monitoring of B-Pb,

medical surveillance, and reduction of exposure when the workers B-Pbs are >400 µg/L; at >600 µg/L, the worker should be removed from exposure. There are no additional restrictions for women. It may be mentioned, that in the U.S., the current goal of the Department of Health and Human Services is to eliminate all occupational exposures resulting in B-Pb >250 µg/L (1.2 µmol/L).

Also in the United States, ACGIH (2001; 2003) has a threshold limit value (TLV) in air for lead and its inorganic compounds of 0.05 mg/m^3 and consider them to be confirmed animal carcinogens with unknown relevance to humans. Lead chromate has the same TLV, but is listed as a suspected human carcinogen. Lead arsenate has a TLV of 0.15 mg/m^3 as the total compound to also protect from the toxic effects of arsenic. The recommended biological exposure index (BEI) for elemental and inorganic lead is 300 µg/L blood, with a special warning that women with a B-Pb >100 µg/L are at risk of delivering a child with a B-Pb over the U.S. CDC guideline of 100 µg/L, which should not be exceeded.

The OEL in the European Union is 150 µg/m^3 (EU, 1998b). Medical surveys shall be made if the air-Pb is >75 µg/m^3 (TWA during a 40-hour week) or if the B-Pb is >400 µg/L in an individual worker. However, the Scientific Committee on Occupational Exposure Limits (EU SCOEL, 2002) has recommended an OEL of 100 µg/m^3 (fumes and dust) and a biological limit value (BLV) for B-Pb of 300 µg/L (for both males and females). It is noted that it is not easy to set an OEL for airborne Pb, because a considerable fraction of the Pb is ingested orally. Furthermore, the B-Pb is not seen as entirely protective for the offspring of working women; no threshold for potential CNS effects in newborn and infants could be identified. Several within the EU apply lower OELs of 50 or 100 µg/m^3.

In Japan, the OEL is 0.1 mg/m^3 and the occupational exposure limit on the basis of biological monitoring is 400 µg/L (Japan Society for Occupational Health, 2002).

2.13.2 Other Assessments

2.13.2.1 Environmental Exposure

In the United States, the Centers for Disease Control and Prevention in 1991 identified a goal to reduce children's B-Pb below 100 µg/L (U.S. CDC, 2005). Interventions for individual children were recommended at levels of 150 µg/L and above.

The WHO B-Pb level of concern is 200 µg/L (WHO/ICPS, 1995). However, later a critical B-Pb of 100 µg/L was given (WHO, 2000a).

In Germany, as to "human biological monitoring values" for B-Pb for the general population, no risk was assumed at 100 µg/L in children < 12 years and women in the reproductive age, and 150 µg/L in men > 45 years, while there were an increased risk of adverse effects at 150 and 250 µg/L, respectively (Ewers *et al.*, 1999).

In the United States, the *ambient air* quality standard is 1.5 µg/m^3 (quarterly average; U.S. EPA, 2003). The EU (1999) ambient air quality guideline for Pb is 0.5 µg/m^3 on an annual basis (2005). In the immediate vicinity of specific industrial sources, the value is 1 µg/m^3 until 2010.

A provisional tolerable weekly intake (PTWI) of 25 µg/kg body weight through *food and drinking water* has been established for all age groups by the Joint FAO/WHO Expert Committee on Food Additives (JEFCA; WHO, 2000b).

The tolerable concentration of lead in drinking water in the EU is 10 µg/L (EU, 1998a). This is based on a health-based guideline value for bottle-fed infants (provisional tolerated weekly intake 25 µg/kg body weight = 3.5 µg/kg body weight/day; body weight 5 kg; 50% allocation to water; 0.75 L/day; rounded figure; WHO, 2003). In the United States, the EPA action level is 15 µg/L.

The maximal permissible level in foods within the European Union ranges from 0.02 mg/kg wet weight for milk and milk supplements for infants, over 0.1 mg/kg for most meat and vegetables up to 1 mg/kg for mussels, for example (EU, 2001).

2.13.2.2 Cancer and Reproduction

The International Agency for Research on Cancer (IARC, 2006) stated that there is limited evidence for carcinogenicity to humans of exposure to inorganic lead, but sufficient evidence in animals. Hence, IARC classified inorganic lead as probably carcinogenic to humans (Group 2A).

2.14 Diagnosis, Treatment, and Prognosis of Poisoning and Medical Surveillance

2.14.1 Diagnosis

The diagnosis of lead poisoning depends on a history of lead exposure, symptoms and signs compatible with lead toxicity, laboratory tests supporting the diagnosis, and exclusion of other, more reasonable explanations (differential diagnoses). The toxic effects have been discussed previously under the respective organ.

The clinical picture depends on the age of the subject and the duration and intensity of the exposure. In infants, encephalopathia with confusion and seizures is the most common presenting syndrome; lead poisoning is a differential diagnosis of infectious encephalopathia and brain tumor (Erickson and Thompson, 2005).

In adults, a short, intense exposure most often causes acute disease, dominated by symptoms and signs from the gastrointestinal tract, with constipation or diarrhea, epigastric pain, nausea, indigestion, loss of appetite, and colic. The clinical picture may be mistaken for obstructive ileus. Also, arthralgia and myalgia are common.

At long-term exposure of a lower intensity, there are sometimes gastrointestinal symptoms and signs, but other symptoms and signs usually dominate the clinical picture. There is often anemia ("lead pallor"), which is usually normocytic/slightly hypochromic and sideroblastic, with basophilic stippling (which may also be present in thalassemia). The anemia is usually moderate in adults (but is often the key to diagnosis), but may be severe in children. Also, there is peripheral neuropathy with motor (weakness in extensor muscles of forearms and legs with wrist and ankle drop; decreased pinch and grip strength) and sensory (tingling or numbness in arms or legs, muscle pain, affected sensory, and pain perceptions thresholds in fingers and decreased vibration thresholds in hands and/or toes) phenomena. In some cases, the clinical picture may be dominated by gout ("saturnine gouty arthritis").

Also, there is sometimes a gingival "lead line" (Burtonian line), in particular if the patient has paradentosis with bacterial infection.

The demonstration of increased lead absorption is crucial for the diagnosis. Of course, a history of lead exposure is important. Sources of exposure have been discussed previously (Section 2.4).

To verify the exposure, determination of high lead concentrations in blood is the key. In severe lead poisoning, the level is mostly 3 µmol/L or greater. If a possibility to determine lead in blood is not available, the diagnosis may be supported by determination of lead in urine, chelatable (EDTA or DMSA) lead, or ALA or porphyrins in serum or urine. Also, ZPP in blood is sometimes used.

2.14.2 Treatment

Lead-induced encephalopathia in *infants* is an acute, life-threatening emergency. Immediate treatment by chelating agent should be started, with intravenous EDTA infusion (1500 mg/m^2/day) for 5 days (Griesemer, 2001; Henretig, 1998). To prevent a transfer of chelated lead to the brain, intravenous administration of British Anti-Lewisite (BAL; 450 mg/m^2/day) should start 4 hours before and continue during the EDTA treatment. The treatment causes an increase of urinary lead and a decrease of B-Pb. However, only 1% of the body burden is removed. Hence, after the end of the chelation, there may be a rebound of B-Pb because of mobilization of lead from the skeleton. Thus, after 2 days, a new treatment should be given. Treatment for brain edema (hyperosmotic therapy, e.g. dexamethasone) and convulsions (e.g., benzodiazepam) may be needed.

Chelation should not be performed when lead colics are present. Furthermore, renal failure is a contraindication for EDTA, because the lead–EDTA complex is excreted by glomerular filtration and may cause aggravation of the renal disease after absorption. Because of the emetic effect of the BAL–iron complex, iron should not be dosed simultaneously with the EDTA. Even in absence of symptoms or signs, infants with B-Pb >450 µg/L may be chelated prophylactically.

An alternative to the BAL/EDTA regimen is DMSA, which may be given orally (30 mg/kg/day) in less severe cases and which has fewer side effects (Graziano and Howland, 1998). Glucose-6-phosphate dehydrogenase deficiency and allergy to sulfa drugs are contraindications. D-penicillamine orally was previously a choice. However, because of its toxicity, its tendency to increase lead absorption from the gastrointestinal tract, and the availability of DMSA nowadays, it should no longer be used.

Of course, the exposure source should be identified immediately and removed. The exposure at home should be assessed (US CDC, 2002). Also, the possibility of a lead object in the gastrointestinal tract should be considered. Furthermore, the nutritional status should be optimized, and especially iron deficiency should be avoided.

In *adults* with severe clinical lead poisoning, there is also indication for chelation therapy (Henretig, 1998). However, chelation should never be made in lead workers as a preventive measure in subjects with ongoing occupational exposure. Instead, heavily exposed subjects should be removed from exposure, and steps taken to reduce it.

2.14.3 Prognosis

As stated previously, the gastrointestinal symptoms and signs, anemia, and peripheral neuropathy in adults usually resolve during treatment and after reduction of exposure. Also, there are indications that tubular kidney effects are reversible.

However, after childhood encephalopathia, residual symptoms and signs from the CNS are common (Hu, 1991b; White *et al*., 1993). They also have an increased risk of cardiovascular and cerebrovascular disease (McDonald and Potter, 1996). In addition, kidney disease may appear later in life (Emmerson, 1973), although not always (McDonald and Potter, 1996; Moel and Sachs, 1992).

2.14.4 Medical Surveillance

2.14.4.1 Lead Workers

Subjects who are going to be hired as lead workers are often submitted to a preemployment medical surveillance. Individuals with medical conditions that may predispose for toxicity (i.e., nervous system disorders, anemia, hypertension, and kidney disorders) should be warned. Also, alcohol abuse and porphyrias/porphyrinurias are risk factors.

During employment, lead workers are often submitted to regular health examinations to detect predisposing disorders and early symptoms and signs of toxic effects. Also, B-Pb is determined (examples of biological limit values are given in Section 2.13.2.1). As a result, workers may be removed from exposure temporarily or permanently.

2.14.4.2 Children

In the United States, the Centers for Disease Control and Prevention in 1991 identified a goal to reduce children's B-Pb below 100 µg/L (US CDC, 2005). Interventions for individual children were recommended at levels of 150 µg/L and above. These levels have been criticized in light of the CNS effects recorded at lower B-Pbs.

3 ORGANIC LEAD

Organolead has attained much less interest lately than inorganic lead, probably because the use has decreased dramatically in many countries. Reviews have been published (Grandjean and Nielsen, 1979; IARC, 2006; Tsuchiya, 1979).

3.1 Physical and Chemical Properties

The only organolead compounds of practical industrial importance are tetramethyl lead (Me_4Pb; CAS 75-74-1) and tetraethyl lead (Et_4Pb; CAS 78-00-2). Both compounds are highly lipophilic with low water solubility.

For Me_4Pb, the following data apply: density, 1.99 g/cm^3; melting point, −28°C; boiling point, 110°C; vapor pressure, 3.2 kPa.

Correspondingly for Et_4Pb, the data are density, 1.66 g/cm^3; melting point, −137°C; boiling point, 198°C; vapor pressure, 0.03 kPa.

3.2 Methods and Problems of Analysis

The determination of organic lead requires in most cases speciation of the lead in the sample. A separate chapter of this book is dedicated to speciation (Chapter 2). The field of lead speciation has only attracted limited interest, probably because the organic lead compounds are metabolized into inorganic lead, and, therefore, organic lead is of little use in biological monitoring. However, to verify organolead exposure, the presence of high concentration of the metabolites, di-or trialkyl lead, in urine may prove useful (Section 3.6).

In brief, air sampling of organolead has been carried out by sampling on cold (typically −80°C or lower) gas chromatographic columns, or after removal of inorganic lead particles by a filter (Grandjean and Nielsen, 1979). Methods for speciation of ionic alkyl lead compounds in urine have been developed using gas chromatography with mass spectrometric detection (Pons *et al*., 1998) or high-performance liquid chromatography with ICP-MS detection (Shum *et al*., 1992). Also, the technique of chelating inorganic lead ions and then assuming that the remaining lead is organic has been used for urine (Vural and Duydy, 1995).

3.3 Production and Uses

The dominant use of Me_4Pb and Et_4Pb has been as an additive to gasoline (Section 2.3). That practice is now being discontinued, but leaded gasoline is still being used in Africa, for example (Section 2.4.1.6). At its peak during the early 1970s, approximately 250,000 tons/year of organic lead were added to gasoline in the United States only (US ATSDR, 1999).

3.4 Exposure

Several cases have been reported on organolead poisoning from sniffing of gasoline. Also, several poisonings, sometimes fatal, have occurred in the manufacturing and distribution of organoleads, especially in earlier years.

The exposure of the general population through foods seems to be insignificant.

Organolead is emitted to the air from leaded gasoline, especially at transfer of the gasoline, such as in filling stations. A review on emission of organolead to air is included in the review by Grandjean and Nielsen (1979). They conclude that at the time when leaded gasoline was still used in large quantities, although not at its highest peak, approximately 7000 tons of the annual world production of 300,000 tons of organolead were emitted to the atmosphere as organolead. Urban air 24-hour mean levels were often approximately 0.3 µg/m^3, but much higher concentrations had been detected at filling stations and in garages.

3.5 Toxicokinetics

The toxicokinetics of Me_4Pb and Et_4Pb show great similarities, although there are some differences (Grandjean and Nielsen, 1979).

Like other lipophilic compounds, Et_4Pb is rather readily absorbed through the skin; Me_4Pb seems to be somewhat more slowly absorbed (Grandjean and Nielsen, 1979). Et_4Pb is rapidly absorbed in the respiratory (Heard *et al*., 1979) and probably in the gastrointestinal tracts.

Et_4Pb is rapidly and widely distributed in the body (Grandjean and Nielsen, 1979). The tissue concentrations of Et_4Pb are low; the major part of lead is Et_3Pb. The highest levels are found in the liver, whereas the CNS contains several times less (Heard *et al*., 1979). In Danes without occupational exposure, and studied before the elimination of alkyl lead from petrol, R_3Pb made up approximately one fifth of the total lead content in the brain (Nielsen *et al*., 1978). Lead in blood is discussed later (Section 3.6.1).

High skeletal (probably in organic) lead levels have been found in subjects sniffing leaded petrol (Eastwell *et al*., 1983) and in organolead workers (exposed to both alkyl and inorganic lead; Glenn *et al*., 2003; Links *et al*., 2001; Schwartz *et al*., 2000b; 2001; 2002; Stewart *et al*., 1999; 2002; Tassler *et al*., 2001).

R_4Pb compounds are rapidly dealkylated by cytochrome P-450, into R_3Pb, R_2Pb, and inorganic lead (Grandjean and Nielsen, 1979). The degradation is most effective in the liver microsomes, but also occurs in other organs.

After exposure to R_4Pb compounds, there is excretion of lead in urine and feces (Grandjean and Nielsen, 1979). Because the urinary lead initially is nonprecipitable and nonextractable by organo-solvent, it is assumed that it is mainly present as R_2Pb, later as inorganic lead.

As stated previously, R_4Pb compounds are very rapidly dealkylated into R_3Pb, the turnover of which is fairly slow (Grandjean and Nielsen, 1979). In a case of human Et_4Pb poisoning, monitoring of Et_3Pb in blood from days 56 to 196 displayed at least two decay components, one with a half-time of 35, and one of approximately 100 days (Yamamura *et al*., 1975).

Increased (inorganic?) lead levels in bone were present long after end of exposure in former alkyl-lead workers (Glenn *et al*., 2003; Links *et al*., 2001; Schwartz *et al*., 2000b; 2001; 2002; Stewart *et al*., 1999; 2002; Tassler *et al*., 2001), which shows that the turnover is slow. Also, the B-Pb was persistently raised; hence, there is subsequent release of lead from bone, causing an endogenous exposure.

Among U.S. former organolead workers, the *VDR* genotype modified the kinetics of bone-lead (Schwartz *et al*., 2000a). Hence, there is a possible gene–environment interaction. However, it is not known whether this affects the organolead species or the inorganic lead after the biotransformation.

3.6 Biological Monitoring

3.6.1 Biomarkers of Exposure

In Et_4Pb poisoning, the lipid-associated lead in blood is increased (Grandjean and Nielsen, 1979). Presumably, the lead is mainly present as Et_3Pb. Also, some of the lead in blood should be inorganic lead. Hence, in organolead workers, there is an increase of B-Pb (Schwartz *et al*., 1993; 1995). Still, B-Pb is not the choice in biomonitoring of such workers. Instead, urinary lead should be used.

In subjects exposed to organolead, there is an increase of lead concentration in urine, which has been used as a mean to biomonitor exposure (Grandjean and Nielsen, 1979). In cases of poisoning, urinary lead is always $>200\,\mu g/L$, in fatal cases $>1000\,\mu g/L$. A disproportionally high concentration of lead in urine compared with those expected on the basis of the B-Pb is a sign of alkyl-lead exposure. It is quite uncommon that exposure to inorganic lead gives a lead concentration in urine that after density adjustment exceeds 10% of that in whole blood (Bergdahl *et al*., 1997c).

Alkyl-lead compounds are chelated to a much smaller extent than inorganic lead. There was an increase of chelatable lead in former organolead workers (Stewart *et al*., 1999), which should mainly reflect the body burden of inorganic lead.

Lead level in bone has been used as an index of alkyl-lead exposure in petrol sniffers (Eastewell *et al*., 1983) and workers (Glenn *et al*., 2003; Links *et al*., 2001; Schwartz *et al*., 2000b; 2001; 2002; Stewart *et al*., 1999; 2002; Tassler *et al*., 2001).

It can be speculated that plasma or serum could be useful in biomonitoring of alkyl-lead exposure, given the high levels in urine. There are, indeed, indications that alkyl-lead exposure leads to high lead concentrations in plasma (Winnik *et al*., 2004), but so far little data exist.

3.6.2 Biomarkers of Effects

Alkyl-lead compounds do not inhibit ALAD activity (Grandjean and Nielsen, 1979). Accordingly, organolead workers have a limited increase of ALA excretion in urine. Furthermore, they do not have a significant increase of FEP/ZPP in blood.

3.7 Organ Effects

3.7.1 Nervous System

In heavy exposure to Et_4Pb is, after a latency period of hours to days, the acute poisoning dominated by CNS symptoms: anorexia, nausea and vomiting, insomnia, hyperactivity, restlessness, irritability, concentration difficulties, aggression, headache, tremor, weakness and fatigue, depression, impairment of memory, confusion, body pain and sensations—later mania, convulsions, delirium, fever, coma, and death (Grandjean and Nielsen, 1979). Some 100 fatal cases have been reported. Me_4Pb is much less toxic; only one—nonfatal—case has been published.

The toxic mechanism is believed to be disturbance of the mitochondrial membrane with uncoupling of oxidative phosphorylation. Pathoanatomically, there is brain edema and neuron death in the cerebral and cerebellar cortex, reticular formation, and basal ganglia.

Cases of "chronic poisoning" have been reported after long-lasting sniffing of leaded gasoline (Grandjean and Nielsen, 1979). In children, disorientation, tremor, ataxic gait, and intermittent choreiform movements have been present. As stated previously, such exposure may cause a significant exposure to lead (Eastwell *et al*., 1984). However, it is not possible to judge whether these phenomena are due to the lead compounds or to the organic solvents of the gasoline. Also, CNS symptoms and EEG changes have been reported in workers exposed to leaded gasoline (Grandjean and Nielsen, 1979).

There are several studies of workers producing organolead compounds. In a study of *current* organolead workers (exposed to a mixture of organic and inorganic lead; "lifetime" mean B-Pb 261 µg/L), there were plenty of neurological findings (Mitchell *et al*., 1996); however, the group was selected. Among 222 workers (air-lead, inorganic 4–119; organic, 1–56 µg/m^3; weighted average B-Pb, 240 µg/L), manual dexterity, and verbal memory/learning were related to the exposure intensity (and urinary lead, although data were not given; Schwartz *et al*., 1993). No less than 42.6% had "subnormal" scores in a symptoms questionnaire versus 7.6% in a referent group of unexposed workers from the same factory (Bolla *et al*., 1995). The neurobehavioral and symptoms pictures were similar to those in workers exposed to organic solvents (Bolla *et al*., 1995; Schwartz *et al*., 1993).

In studies of *former* organolead workers, there were associations between tibia-lead, on the one hand, and performance in neuropsychological tests (Schwartz *et al*., 2000b; 2001; Stewart *et al*., 1999) and peripheral nervous function in hands (Tassler *et al*., 2001), on the other. Tibia-lead after end of exposure (i.e., endogenous exposure) may be of importance (Links *et al*., 2001). Apolipoprotein E genotype interacted with tibia-lead to explain CNS findings (Schwartz *et al*., 2002).

3.7.2 Other

Acute exposure to Et_4Pb through inhalation of leaded petrol caused renal and hepatic damage in adolescents (B-Pb 1200–1400 µg/L; Robinson, 1978).

In former U.S. organolead workers, there was an association between systolic and diastolic blood pressures and B-Pb (Glenn *et al*., 2003; Schwartz and Stewart, 2000). The gene for the alpha 2 subunit of Na(+)-K(+)ATPase seemed to modify the relationship (Glenn *et al*., 2001).

There is only limited evidence that alkyl-lead compounds are mutagenic in experimental systems. There was no clear increase of cancer or total deaths in organolead workers (Robinson 1976; Sweeney *et al*., 1986). According to IARC (2006), there is inadequate evidence for carcinogenicity. There are reports of disturbances of male reproduction, but the evidence is weak. The compounds pass the placenta, but effects on the embryo/fetus have not been established.

3.8 Diagnosis, Treatment, and Prognosis

There is no effective treatment of alkyl-lead poisoning (Grandjean and Nielsen, 1979). Chelating agents (BAL, EDTA, DMSA, penicillamine) do not work. Symptomatic treatment by barbiturates, for example, may be needed in some cases. In cases of acute poisoning, most of the symptoms disappear after end of exposure. After long-term occupational exposure, there may be residual symptoms and signs (Schwartz and Stewart, 2001; Schwartz *et al*., 2000b; Stewart *et al*., 1999; Tassler *et al*., 2001).

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CHAPTER

32

Manganese

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ABSTRACT

After the description of chemical properties, methods, and problems of analysis, this chapter discusses the occurrence of manganese inorganic and organic compounds, its production and uses, environmental levels and fate, and exposures. Manganese is ubiquitous in the environment. It occurs in soil, air, water, and food. Its concentration in food varies markedly, but on the whole, food is the major source of manganese intake for humans

Manganese absorption by inhalation, ingestion, and through the olfactory pathway, its distribution in the body, metabolism, and excretion are described using data from animal and available human studies. The absorption of ingested manganese is regulated by the homeostatic mechanism. However, manganese homeostasis is primarily achieved by excretion. Absorbed manganese is rapidly eliminated from the blood and at first concentrates in the liver where it conjugates with the bile and is excreted into the intestines. Reaching the intestines, most of the element is excreted in the feces. Urinary excretion is only approximately 0.01% per day of the body burden. Biological half-life in humans is 2–5 weeks, depending on body stores. Manganese crosses the blood–brain barrier and accumulates in the brain, where the half-life is much longer.

Manganese is an essential element for humans and animals. Although many people are likely below the estimated safe and adequate daily manganese intake, no large-scale deficiency has been reported.

Long-term exposure to increased concentrations of manganese, associated primarily with inhalation in occupational settings, may result in neurological and neurobehavioral effects. On the basis of the cumulative mechanism of neurotoxicity, concern is raised regarding the possible long-term effects on the central nervous system, represented by parkinsonian disturbances. Manganese-induced damage seems to occur postsynaptic to the nigrostrial system, predominantly in the globus pallidus. The principal mechanism of manganese neurotoxicity has not been cleared yet. Manganese may enhance the autooxidation or turnover of various intracellular catecholamines such as dopamine, leading to an increased production of free radicals, reactive oxygen species, and other cytotoxic metabolites. It may disturb also the γ-aminobutyric acid (GABA) regulation, as well as glutamatergic transmission. It also seems to impair the cellular antioxidant defense mechanism. Manganism, a manganese-induced brain disease, is a progressive irreversible impairment.

Lungs are the second target organs affected by exposure to manganese. Inhalation exposure to high concentrations of manganese dioxide (or tetroxide) can cause chemical pneumonitis, an inflammatory response in the lung. Moreover, increased incidence of acute respiratory diseases—bronchitis and pneumonia—have also been reported at rather low exposure levels, suggesting that inhaled manganese in a nonsoluble form can impair resistance to respiratory infections. This assumption has been supported by experimental and epidemiological studies.

The influence of manganese exposure on the reproductive, cardiovascular, hematological, endocrine, and immunological systems is also discussed in this chapter, including its genotoxic and carcinogenic effects. Although manganese causes mutations, it is not carcinogenic.

The last section deals with manganese concentrations in biological media and biomarkers of exposure and effects. On a group basis, average levels of manganese in blood seem to be related to manganese body burden, whereas average urinary excretion levels are believed to be the most indicative of recent exposure, although with a high degree of variability. Blood and urine levels are not a reliable predictor of exposure to manganese on an individual basis. No significant correlation has been found between fecal excretion of manganese and occupational exposure to the metal.

At this point, no biochemical indicator can detect early neurotoxic effects of manganese.

Reviews on the essentiality, toxicokinetics, and toxic effects of manganese are numerous, starting with Underwood (1971), NAS (1973), Mena (1974), and Utter (1976). In addition, a number of health assessment documents on manganese were prepared by WHO (1981; 1984a,b; 1986; 1987; 2000; 2004) and the US EPA (1984a,b; 1993; 1994). In 2000, the Agency for Toxic Substances and Disease Registry (ATSDR) of the U.S. Department of Health and Human Services, Public Health Services, made an extensive review of the toxicological profile of manganese.

1 PHYSICAL AND CHEMICAL PROPERTIES

Manganese (Mn): atomic weight, 54.94; atomic number, 25; density, 7.21–7.44, depending on the allotropic form; melting point, 1245°C; boiling point, 2097°C; whitish grey metal, harder than iron, very brittle. It belongs to group VIIB in the system of element and occurs in 11 oxidation states of which 2, 4, and 7 are the most important ones. Divalent manganese salts are most stable, four-valent manganese (MnO_2) is the most important compound of the tetravalent state, and permanganates of the +7 state. Manganese forms various organometallic compounds among which methylcyclopentadienyl manganese tricarbonyl (MMT) is of major practical interest.

Conversion factors: µg = 0.018 µmol; µg/L × 18.2 = nmol/L; µg/g cr. × 2.06 = nmol/mmol creatinine.

2 METHODS AND PROBLEMS OF ANALYSIS

Classical methods of analysis have been the periodate method for manganese in air and the permanganate method for manganese in water. However, these methods are not satisfactory for biological samples. Normally, the analysis is now performed by use of inductively coupled plasma atomic emission spectroscopy (ICP-AES). It is also possible to use flame atomic absorption spectroscopy (AAS) (Tsalev, 1983). In this method, a solution containing manganese is introduced into a flame, and the concentration of manganese is determined from the intensity of the color at 279.5 µm. Furnace atomic absorption analysis is often used for very low analyte levels (Baruthio *et al*., 1988). AAS has a lower sensitivity than ICP-AES and has generally been replaced by ICP-AES because of the greater suitability of ICP-AES for multielement analysis. High-resolution ICP mass spectrometry (ICP-MS) can also be used for analysis. This has a greater sensitivity than ICP-AES but is quite expensive.

Instrumental progress in ICP-MS was recently achieved by the introduction of the collision cell interface to dissociate many disturbing argon-based molecular ions, thermalize the ions, and neutralize the disturbing argon ions of plasma gas (Ar^+). The application of the collision cell in ICP-MS results in a higher ion transmission, improved sensitivity, and better precision of isotope ratio measurements compared with quadrupole ICP-MS without the collision cell (Becker *et al*., 2000).

Neutron activation analysis is also effective for determining manganese concentrations in different samples (Rose *et al*., 1999). This technique uses no reagents and a minimum of sample handling, thus potential contamination with exogenous sources of manganese can be avoided. It can be used both for environmental and biological samples. Other methods for measuring manganese include spectrophotometry, mass spectrometry, and X-ray fluorimetry.

Monitoring of manganese data is nearly always available as total manganese, because none of the listed methods distinguish between different oxidation states of manganese or between different manganese compounds.

Simple determination of total element manganese by atomic absorption and gas chromatography followed by flame-ionization detection can be used to quantify MMT concentrations in gasoline. By detecting the metallic portion of the compound, the detection of MMT is reported as manganese. In 1991, Walton *et al*. described high-performance liquid chromatography coupled with laser-excited atomic fluorescence spectrometry to detect various species of MMT.

To estimate the level of occupational exposure to manganese fungicide, mancozeb, Headley (1996) used an inductively coupled plasma-atomic emission spectrometry method by measuring the elemental manganese portion of fungicide.

As in other trace metal determinations, in the analysis of manganese in environmental media and in biological material where manganese is present, a high standard of cleanliness should be maintained to prevent contamination. The risk of obtaining falsely high values for manganese in serum exists as a result

of extraneous addition of the element (Versieck *et al*., 1987). Analysis of manganese in blood may be influenced by the anticoagulant used. High concentrations of manganese can be present in heparin.

Manganese in air exists as particulate matter, so sampling is done by drawing air through a filter to collect the suspended particles. A variety of filter types (e.g., glass fibers and cellulose acetate) and sampling devices (low volume, high volume, and dichotomous) are available, depending of the particle sizes of concern and the concentration range of interest. Material on the filter may be analyzed directly (e.g., by X-ray fluorescence), or the filter may be digested by ashing in acid before analysis. Sensitivity depends on the volume of air drawn through the filter before analysis, and typically detection limits are 1–2 μg/sample.

Standardization of the sampling criteria used for measurement of airborne dust concentrations in the workplace has led to the development of size-selective conventions for the inhalable fraction, the thoracic fraction, and the respirable fraction (CEN, 1993). Samplers are available for the collection of the inhalable fraction, such as the IOM inhalable sampler (Mark and Vincent, 1986), in addition to the Millipore 37-mm closed-face filter cassette (CFFC) sampler for the collection of "total dust." Correction factors between IOM and CFFC samples are generally between 1.0 and 2.5 (Tatum *et al*., 2001).

Water may be analyzed directly. If the concentration of manganese is low, a concentration step: evaporation, extraction, and binding to a resin, may be used. Detection limits range from 0.005–50 μg/L. In all cases, acid is added to the sample to prevent precipitation of manganese.

In a study performed by Beklemishev *et al*. (1997), a very low detection limit of 0.005 μg/L was obtained in measuring manganese in tap and river water. Their analytic method relied on an indicator reaction that is catalyzed by Mn(II) (The oxidation of 3,3′,5,5′-tetramethyl-benzidine [TMB] by potassium periodate [KIO_4] and is carried out on a surface of paper-based sorbent).

Determination of manganese levels in soils, sludge, or other wastes requires an acid extraction/digestion step before analysis. Treatment will usually involve heating in nitric acid, oxidation with hydrogen peroxide, and filtration and/or centrifugation to remove insoluble matter.

For measuring manganese levels in food, atomic absorption spectrometry has been the most widely used analytical technique. Tinggi *et al*. (1997) contributed a wet digestion technique using a 12:2(v/v) nitric/sulfuric acid mixture for their determination, and for food samples with low levels of manganese, they found that the more sensitive graphite furnace atomic absorption analysis is required.

3 OCCURRENCE, PRODUCTION, AND USES

3.1 Occurrence and Production

Manganese is widely distributed in the earth's crust making up approximately 0.1% of it (Graedel, 1978). Manganese does not occur naturally in its native state as a base metal, but it is found in more than 100 minerals. Oxides, carbonates, and silicates are most important among manganese-containing minerals. The most common manganese mineral is pyrolusite (manganese dioxide), with 60–63% of manganese. Commonly occurring are also rhodocrosite (manganese carbonate) and rhodonate (manganese silicate). Manganese occurs in nodules on the bottom of the ocean and in most iron ores. The manganese content in coal has been reported to be 6–100 μg/g (Ruch *et al*., 1973). Manganese also occurs over a wide range of concentrations as a natural trace element in crude oil.

Most manganese is mined in open pit or shallow underground mines. More than 80% of the high-grade manganese ore (>35% manganese) is mined in South Africa, and also Australia, Brazil, Gabon, and, India, and, more recently, China, and the Commonwealth of Independent States (CIS). The world production of all grades of manganese ore in 2002 was approximately 20 million tons according to the International Manganese Institute (IMnI, 2003), with China, South Africa, and CIS producing each 17% ot the total amount, followed by Brazil (12%), Australia (11%), Gabon (8.5%), India (7.8%), and Ghana (6%). Reserves of high-grade ore are approximately $500–600 \times 10^6$ tons, and those of low-grade ore are estimated at several billion tons (Gerber *et al*., 2002).

3.2 Uses

Manganese is mainly used in metallurgical processes. Approximately 90% of the total manganese is used in steel manufacture as a deoxidizing and desulfurizing additive and as an alloying constituent. Most manganese ore is smelted in electric furnaces to produce ferromanganese alloy widely used in the production of steel. Production of 97–98% pure manganese metal is achieved by aluminium reduction of low-iron–content manganese ore (HSDB, 1998). Manganese with <0.1% metallic impurities can be produced electrolytically from a manganese sulfate solution (EPA, 1984a; HSDB, 1998). The world production of manganese alloys in

2002, including ferro- and silico-manganese, was approximately 4 million tons, with China producing 31% of the total amount, followed by Ukraine (13%), South Africa (10%), Japan, Norway, and Brazil (5%), France (4%), and Spain (3%) (IMnI, 2003).

Metallic manganese (ferromanganese) is used principally in steel production to improve hardness, stiffness, and strength. It is used in carbon steel, stainless steel, high-temperature steel, and tool steel, along with cast iron and super alloys (EPA, 1984a; HSDB, 1998). Manganese is a minor, but indispensable, component of welding consumables. Most consumables contain <6% manganese. The forms in which it is used include ferromanganese, silicomanganese, and manganese carbonate. Manganese compounds are produced either from manganese ores or from manganese metal. Manganese compounds have a variety of uses. Manganese dioxide is commonly used in the manufacture of dry-cell batteries, matches, fireworks, porcelain and glass-bonding materials, amethyst glass, and as the starting material for production of other manganese compounds (EPA, 1984a; HSDB, 1998). Manganese sulfate is used primarily as a fertilizer and as livestock supplement where soils are deficient in manganese, then in some glazes, varnishes, ceramics, and fungicides (EPA, 1984a; HSDB, 1997; Windholz, 1983). Potassium permanganate, because of its oxidizing power, is used as a disinfectant, an antialgal agent, for metal cleaning, tanning and bleaching, and as a preservative for fresh flowers and fruits, as well as in water and waste-treatment plants for water purification purposes (HSDB, 1998). Other uses of manganese compounds are in textile bleaching, for linseed oil driers, in dyeing, in tanning of leather, and as an oxidizing agent for electrode coating in welding roads.

In 1985, Enzing reported about a common source of manganese in the street drug "Bazooka," which is a cocaine-based drug contaminated with manganese carbonate from free-base preparation methods.

There are two pesticides containing manganese: maneb and mancozeb. Maneb is ethylenebisdithiocarbonate with manganese sulfate or chloride. Mancozeb is a polymeric mixture of a zinc salt and maneb containing 20% manganese and 2.55% zinc (HSDB, 1999).

Among the organic manganese compounds, MMT was developed in the 1950s as a fuel additive to increase the octane level of gasoline and thus improve the antiknock properties of the fuel. It was introduced in Canada in 1976, and its use has increased so substantially that it completely replaced tetraethyl lead in gasoline in that country in 1990 (Zayed *et al.*, 1999). In 1977, MMT was banned as an additive in unleaded gasoline in the US by the EPA (EPA, 1978; 1979; 1981). In 1995, the ban was lifted, and a court decision ordered the EPA (EPA, 1995) to register the product for use as a fuel additive again, although testing for health effects continues. The Afton (former Ethyl) Corporation, the manufacturer of MMT, has been marketing its product to U.S. refineries since late 1995 (Davis 1999). Nevertheless, MMT is currently used only sparsely in the developed world. The major refiners in Canada have voluntarily stopped using MMT out of concern for the impact of MMT on advanced vehicles, and as a result, as much as 95% of Canadian gasoline is now MMT-free (Inside Fuels, 2004). MMT is not currently allowed in reformulated gasoline (RFG), a gasoline blended with oxygenates to reduce toxic pollutants, primarily from older vehicles, which is required in the United States in cities with the worst smog pollution, and is not used by the major oil companies in the United States. In Europe, MMT is used in Greece (Geivanidis *et al.*, 2003), in a couple of the Eastern countries, and perhaps by one small refiner in Belgium (Blumberg and Walsh, 2004). In much of the developing world, MMT use is being introduced with public relations and marketing campaign (Hill and Knowlton, 2003). MMT is used as an octane enhancer by approximately 50% of China's refineries (Walsh, 2003).

Another organic manganese compound, mangafodipir (manganese (II) dipyridoxyl diphosphate, MnDPDP), classified as a drug or therapeutic agent is used as both a liver and pancreas specific contrast agent for magnetic resonance imaging (MRI). It improves lesion detection in MRI of these organs by selectively enhancing the normal parenchyma, but not lesions, so that the contrast between tumorous and normal tissues is increased (Wang *et al.*, 1997).

4 LEVELS AND FATE IN THE ENVIRONMENT AND EXPOSURE

Manganese is ubiquitous in the environment. It occurs in soil, air, water, and food.

4.1 General Environment

4.1.1 Ambient Air

In ambient air, background concentrations of 0.05–5.4 ng/m^3 over the Atlantic Ocean (Duce *et al.*, 1975) and 0.01 ng/m^3 at the South Pole were reported (Zoller *et al.*, 1974).

Annual averages of manganese in urban areas without significant manganese pollution are in the range of 0.01–0.07 µg/m^3 (WHO, 2000). The daily intake of manganese in the air by the general population in areas without manganese-emitting industries was estimated to be <2 µg/day (WHO, 1981).

Above average exposures to manganese are likely to occur in or near a factory or a hazardous waste site that releases significant amounts of manganese dust into air. In areas with major foundry facilities, annual averages of manganese rise to 0.2–0.3 μg/m^3, and manganese intake may rise to 4–6 μg/day. In areas associated with ferroalloy industries, it may be as high as 0.5 μg/m^3 (intake 10 μg/day, with 24-hour peak exceeding 200 μg/day) (WHO, 1981). Additional releases of manganese into the environment occur from natural sources and from processes such as combustion of cement.

Manganese can be released into air by combustion of gasoline that contains MMT as an antiknock ingredient. It has been estimated that if MMT were used in all gasoline, urban air manganese levels would be increased by approximately 50 ng/m^3 (Cooper, 1984; Ter Haar *et al.*, 1975). The EPA (1994) estimated that a substantial number of people could be exposed to manganese particulate levels >0.1 μg/m^3 if there were 100% gasoline containing MMT. In Canada, where MMT in gasoline was already widely in use, the estimated 10% per year increase in manganese emission rates had been reported (Loranger and Zayed, 1997; Zayed *et al.*, 1999).

Because the releases of manganese into air are particulate in nature, the fate and transport of the particles are determined mainly by the wind and by the size and density of the particles. Elemental manganese and inorganic manganese have negligible vapor pressure but may exist in air as suspended particulate matter derived from industrial emissions or the erosion of soils. Manganese-containing particles are mainly removed from the atmosphere by gravitation settling, with large particles tending to fall out faster than small particles. The half-life of airborne particles is usually in the order of days, depending on the size of the particle and atmospheric conditions. Some removal by washout mechanisms such as rain may also occur, although it is of minor significance in comparison of dry deposition (EPA, 1984a; Turner *et al.*, 1985). Approximately 80% of the manganese in suspended particulate matter is associated with particles having a mass median equivalent of <5 μm (WHO, 1981). Approximately 50% have a mass median equivalent of <2 μm.

Very little information is available on atmospheric reactions of manganese (EPA, 1984a). Manganese can react with sulfur dioxide and nitrogen oxide, but the occurrence of such reactions in the atmosphere has not been demonstrated. MMT photolyses rapidly by sunlight in the atmosphere with a very short half-time, <2 minutes (Garrison *et al.*, 1995; Ter Haar *et al.*, 1975).

4.1.2 Water

Manganese may be released to water by discharge from industrial facilities or as leachate from landfills and soil.

Concentrations of manganese in surface water are usually reported as dissolved manganese. Some manganese compounds are readily soluble, so significant exposure can also occur by ingestion of contaminated drinking water. However, manganese in surface water may oxidize or adsorb to sediment particles and settle to the bottom. Manganese in soil can migrate as particulate matter in air, as mentioned previously, but also in water, or soluble compounds may be dissolved by water and leach from the soil. The extent of leaching is determined mainly by the characteristics of the soil and is highly variable.

The transport and partitioning of manganese in water is controlled by the solubility of the specific chemical form present, which in turn is determined by pH, oxidation–reduction potential, and the characteristics of the available anions. The metal may exist in water in any of four oxidation states. Manganese in water may be significantly bioconcentrated at lower trophic levels. A bioconcentration factor (BCF) relates the concentration of the chemical in the water in which they live. In general, the lower organisms such as algae have larger BCFs than higher organisms (Thompson *et al.*, 1972). To protect consumers from the risk of manganese bioconcentration in marine mollusks, the EPA has set a criterion for manganese at 0.1 mg/L for marine waters (EPA, 1993).

Manganese in water may undergo oxidation at high pH, and it is also subject to microbial activity (Johnston and Kipphut, 1988).

The rate of MMT degradation in natural aquifer and sediment systems is very slow under anaerobic conditions. Calculated half-lives range from approximately 0.2–1.5 years at 25°C (Garrison *et al.*, 1995). However, MMT photolyzes rapidly in purified, distilled water exposed to sunlight (calculated half-life of 0.93 min).

In a 1962–1967 survey of U.S. surface waters, dissolved manganese was detected in 51% of 1577 samples, at a mean concentration of 59 μg/L (EPA, 1984a). In a survey of U.S. river waters (1974–1981), values ranging from <11–>51 μg/L were reported with a medial dissolved manganese concentration of 24 μg/L (Smith *et al.*, 1987).

Natural concentrations of manganese in seawater reportedly range from 0.4–10 μg/L (EPA, 1984a), with an average of 2 μg/L (Barceloux, 1999). Concentrations in freshwater vary from <1 to several hundreds of micrograms per liter (Durum and Haffty, 1961; NAS, 1973). According to more recent data, levels of manganese in fresh water range from 1–200 μg/L. Higher levels in aerobic waters are associated with industrial pollution. In drinking waters, manganese concentrations range from 4–32 μg/L (EPA, 1984a; NAS, 1980; WHO, 1981).

Manganese intake from drinking water is normally substantially lower than intake from the median drinking water level of 10 µg/L. Daily water consumption is set at 2 L/day. The overall database supports the value of 0.4 mg/L as a health-based guideline value (WHO, 2004). Drinking mineral water regularly can add significantly to manganese intake (Dieter *et al*., 1992).

The highest level for manganese in fresh waters to prevent undesirable taste and discoloration is 50 µg/L (EPA, 1978; WHO, 1981). To avoid staining clothes or plumbing fixtures, the EPA recommends that the concentration of manganese in drinking water not be more than 0.05 mg/L. The FDA (1993) has set the same level for bottled water.

4.1.3 Soil

As a component of earth's crust, manganese occurs naturally in virtually all soils. Average natural (background) levels of manganese in soils range from approximately 40–900 mg/kg, with an estimated mean background concentration of 330 mg/kg (Cooper, 1984; Eckel and Langley, 1988; Rope *et al*., 1988). Accumulation of manganese in soil usually occurs in the subsoil and not in the soil surface; 60–70% of manganese is found in the sand fraction of the soil (WHO, 1981). Soil manganese concentrations arising from the use of methylcyclopentadienyl manganese tricarbonyl in unleaded gasoline are apparently connected with manganese oxides from Mn-containing motor vehicle exhaust (Lytle *et al*., 1995). Because they are correlated with distance from the roadway, it can be concluded that the environmental fate of manganese from MMT sources could be associated with increased total manganese in soil (Normandin *et al*., 1999).

The oxidation state of manganese in soils and sediments may be altered by microbial activity.

4.2 Food

Manganese is a natural component of most foods. Its concentration in foodstuffs varies markedly, but on the whole, food constitutes a major source of manganese intake for humans.

The highest manganese concentrations are found in some foods of plant origin, especially wheat and rice; here concentrations between 10 and 100 mg/kg have been reported (Guthrie, 1975; Schroeder *et al*., 1966). Polished rice and wheat flour contain less manganese because most of it is in the bran. High concentrations (up to 50 mg/kg) are found in nuts, tea, legumes, pineapples, and whole grains, and lower levels (up to 5 mg/kg) in milk products, meats, fish, and eggs (Davis *et al*., 1992; Pennington *et al*., 1986).

In connection with the levels of manganese in different foodstuffs, it is interesting to quote a study on bioaccumulation of manganese by plants. Lytle *et al*. (1994) reported increased concentrations of manganese in fruits and stems of oats grown in the organic and sandy soils at the station with the highest traffic density. In a study (Normandin *et al*., 1999) manganese was measured in the plants (flower, stem, leaves, and root). The authors tried to evaluate the potential of dandelion (*Taraxacum officinale*) as a bioindicator of manganese arising from the use of MMT in gasoline. Mn concentrations of the different parts of the plant were not correlated with the distance from the roadways.

Residues of maneb (< 0.1–4.0 mg/kg) were detected in various crop samples obtained from an area where maneb was used as a fungicide (Pease and Holt, 1977).

In a Canadian study (Méranger and Smith, 1972), it was estimated that of the total manganese intake, 54% comes from cereals. The second largest source was potatoes, which gave 14%, whereas meat, fish, and poultry provided only 2%. However, there is a difference in manganese concentrations for the same items in different countries and areas. Daily manganese intake between 5.8 and 12.4 mg has been reported from various regions in India (Soman *et al*., 1969), whereas in the United States (Schroeder *et al*., 1966) and Germany (Schelenz, 1977), average intake by adults has been estimated to range from 2.1–4.1 mg/day.

Variations in manganese intake can, to a large extent, be explained by differences in nutritional habits. In populations with cereals and rice as main food sources, the intake will be higher than in areas where meat and dairy products make up a larger part of the diet. Manganese intake can be higher in populations with a high tea consumption. A cup of tea may contain 0.4–1.3 mg manganese (ATSDR, 2000).

Summing up all the available data, it can be estimated that daily intake of manganese from food ranges from 2000–8800 µg (EPA, 1984a; WHO, 1981). Even though gastrointestinal absorption of manganese is low (3–5%), oral exposure is the primary source of absorbed manganese for general population. Adequate and safe intake of manganese for adults is considered to be 2–5 mg/day (NAS, 1980). According to some data from the United States, it is possible that a significant proportion of Americans, especially females, are not consuming sufficient manganese (Davis and Gregor, 1992; NAS, 1980; Pennington *et al*., 1986). However, infants may be ingesting more than the estimated safe and adequate dose of 0.3–1.0 mg/day for their age group (Pennington *et al*., 1986) because of relatively high manganese levels in prepared infant foods and formulas (Lönnerdal, 1997).

4.3 Working Environment

In the workplace, exposure to manganese occurs mostly by inhalation of manganese fumes or manganese-containing dusts. This is a concern mainly in the ferromanganese, iron, and steel, dry cell battery, and welding industries (WHO, 1986). Exposure also occurs during manganese mining and ore processing. In mining operations, manganese concentrations of 250 mg/m^3 or even higher have sometimes been found (Ansola *et al*., 1944, Rodier, 1955). High-speed drilling machines used to be the main cause of a large amount of manganese dioxide dust emitted in the working environment. In dry cell battery plants and ferromanganese plants, the concentrations of manganese in air are lower. Values up to 5.8 mg/m^3, but occasionally also higher, have been reported (Emara *et al*., 1971; Šarić *et al*., 1977; Suzuki *et al*., 1973). More recently in the United States in steel manufacturing facilities. a mean of 0.066 mg/m^3 median of 0.051 mg/m^3 as respirable dust, and 0.18 mg/m^3 in total dust have been reported (Gibbs *et al*., 1999). Among mineworkers, average exposure intensity across all jobs as Mn in total dust was 0.21 mg/m^3 in South African mineworkers (Myers *et al*., 2003a). In another study on miners in the Middle East, average concentrations were much higher, between 62 and 114 mg/m^3 of Mn in total dust, because of less effective control measures (Mashardi Abar Boojar and Goodarzi, 2002). For the ferroalloy industry, average inhalable manganese exposures, measured using IOM personal inhalable samplers, were 0.254 mg/m^3, and respirable exposures were 0.028 mg/m^3 in Norway (Ellingsen *et al*., 2000). Average exposure levels as Mn measured in total dust with the traditional CFFC samplers were 0.054 mg/m^3 among Italian ferroalloy workers (Lucchini *et al*., 1999). Among welders, manganese concentrations can vary from 0.1 mg/m^3 (Kucera *et al*., 2001) to 1.5 mg/m^3 (Lu *et al*., 2005) and 4 mg/m^3 (Korczynski, 2000), with peak exposure levels more frequently reached in the shipbuilding industry.

An important point is that in ferromanganese plants, but also in dry cell battery plants, the size distribution of manganese aerosols is such that small particles prevail absolutely compared with mine operations, where only a smaller proportion of respirable particles (<5 μm) are usually encountered. There is also some evidence that aerosols formed by condensation may be more harmful than those formed by disintegration (WHO, 1981). This is most probably caused by the differences in the particle size distribution.

Assuming a level of 1 mg/m^3 TWA (NIOSH, 2005) as recommended limit exposure (REL), maximum exposure would be 10 mg manganese/day. This low level compared with the permissive levels in occupational exposure used earlier still exceeds the average exposure from ambient air by a factor of more than 10^4 and is approximately 2.5 times the average exposure from the diet. The study by Roels *et al*. (1992) reported a lowest observable adverse effect level (LOAEL) of 0.150 mg/m^3 and suggested an 8-hour TWA of 0.09 mg/m^3 for total dust as a protective level to prevent the onset of neurotoxic effects. The LOAEL for total dust derived by Lucchini *et al*. (1999) was 0.10 mg/m^3 based on neurobehavioral changes. A LOAEL calculated by Mergler *et al*. (1994) was 0.035 mg/m^3 for the respirable fraction, based on neurotoxicity in alloy workers in a ferromanganese and silicomanganese alloy plant.

As far as occupational exposure to manganese from the combustion of MMT is concerned, in a study performed by Sierra *et al*. (1995) in Montreal, Canada, it was shown that 10% of the manganese exposure of the garage mechanics was due to MMT.

Occupational exposure to maneb and mancozeb can occur by inhalation or dermal routes during formulation and spray application of these pesticides (HSDB, 1999).

5 TOXICOKINETICS

5.1 Absorption

5.1.1 Inhalation

There are no quantitative animal data on absorption rates for inhaled manganese. Because manganese dioxide and other inhaled manganese compounds are practically insoluble in water, only manganese in particles small enough to reach the alveolar lining will be absorbed into the blood (WHO, 1981).

Particles that are deposited in the upper airways may be moved by mucociliary transport to the throat, where they are swallowed and enter the stomach. This process has been found to account for clearance of a significant fraction of manganese-containing particles initially deposited in the lung (Drown *et al*., 1986; Mena *et al*., 1969; Newland *et al*., 1987). Mena *et al*. found in 17 humans exposed to a nebulized solution of manganese chloride and in 4 subjects exposed to manganese oxide in a similar fashion that 40–70% of the deposited amount was recovered in feces. Both compounds were labeled with ^{54}Mn. In a study performed by Tjälve *et al*. (1996), the uptake of manganese in brain regions of weanling male Sprague–Dawley rats after intranasal administration of 4 μg/kg ^{54}Mn was investigated. Whole-body autoradiography of the rats at different time points revealed that the olfactory bulb contained the most measured manganese at 1, 3, and 7 days after

dosing (90, 69, and 47%, respectively), with values decreasing to a low of 16% at 12 weeks. Significant uptake of manganese by other brain regions was not observed until the third day, when the basal forebrain, cerebral cortex, hypothalamus, and striatum had 21, 2, 3, and 1% of the measured label, respectively. Maximum uptake of the label by these regions was observed 7 days after dosing, with percentage of label in the basal forebrain reaching 28%, and the other parts containing amounts slightly more than double their 3-day values. Liver and kidneys each contained approximately 1% of the measured manganese at 1, 3, and 7 days, with values decreasing consistently to 12 weeks.

Roels *et al*. (1997) measured blood manganese levels in 3-month-old rats after intratracheal administration of 1.22 mg manganese/kg as either $MnCl_2$ or MnO_2. Manganese levels in blood after $MnCl_2$ (water soluble compounds) reached a maximal of 30 minutes after dosing. After MnO_2 (nonsoluble compound) administration, the maximal blood manganese level was reached much slower at 168 hours after dosing. The authors also studied the relative uptake and distribution of manganese in the brain regions in the same rats after administration of the same manganese compounds. The results also indicated that absorption through the olfactory mucosa is a potential contributor to brain manganese deposition. Because such a route would bypass liver uptake and biliary excretion of manganese, its transport by the olfactory nerve directly to the brain might have toxicological importance (Fechter, 1999; Tjälve and Henriksson, 1999). Concerning the amount of manganese deposited in different brain regions, similar results were obtained for MnO_2 and $MnCl_2$, with the highest amount of manganese in the striatum. The transport of manganese across the olfactory pathway is further demonstrated for the ultrafine particles, and this may result in accumulation of manganese and inflammatory changes at the olfactory bulb in a greater manner than in the lung (Elder *et al*., 2006).

One study regards the absorption of primary combustion of MMT after inhalation exposure in animals. The primary combustion products of MMT are Mn phosphate, Mn sulfate, and an Mn phosphate/sulfate mixture. Salehi *et al*. (2003) carried out a study on 129 six-week-old male Sprague–Dawley rats divided in four groups—one as control, exposed in inhalation chambers to a manganese phosphate/sulfate mixture for a period of 13 weeks, 5 days a week, 6 hours a day. Exposure concentrations were 3000, 300, and 30 $\mu g/m^3$. At the end of the exposure period, locomotor activity and resting time tests were conducted for 36 hours by use of a computerized Optotrak system. Rats were then euthanized by exsanguinations, and Mn concentrations in different tissues and blood and brain were determined by neutron activation analysis. Increased manganese concentrations were found in blood, kidney, lung, testes, and in all brain sections in the highest exposure group. Manganese in the lung and in the olfactory bulb was dose dependent. Rats exposed to 300 and 3000 $\mu g/m^3$ exhibited significant body loss compared with the control group. Biochemical profiles also revealed significant differences in certain parameters, specifically alkaline phosphate, urea, and chlorate.

5.1.2 Ingestion

The amount of manganese absorbed across the gastrointestinal tract depends on the ingested amount. In humans, the absorption averages approximately 3–5% (Davidsson *et al*., 1988; 1989; Mena *et al*., 1969).

In animal studies, it was shown that manganese is absorbed equally well throughout the length of the small intestine (Thomson *et al*., 1971). On the other hand, Miller *et al*. (1972) found in calves that the upper sections of the small intestine absorb far more ^{54}Mn then the lower section. From studies in which the everted duodenal sac was used, it seems that manganese may be actively transported across the duodenal and ileal segment (Cikrt and Vostal, 1969). Grace (1975) found in sheep that absorption of manganese might also take place in the large intestine. It seems that there is not a marked difference between retention of manganese ingested in food or water (Davidsson *et al*., 1988; 1989; Ruoff, 1995), but absorption time depends of the water solubility of manganese compounds (EPA, 1995; Johnson *et al*., 1991).

Studies of oral absorption of manganese in animals have yielded results that are generally similar to those in humans. In mice and rats, absorption varies between 1 and 3.5% (Greenberg *et al*., 1943; Pollack *et al*., 1965). Manganese uptake in pigs, which have similar gastrointestinal tracts to humans, measured using labeled manganese administered orally, showed a mean absorption rate of 5% 1–6 hours after dosing, 7% 6–12 hours after dosing, and 3.8% 12–24 hours after dosing (Finley *et al*., 1997). Uptake of manganese is increased by iron deficiency (Pollack *et al*., 1965) and decreased by preexposure to high dietary levels of manganese (Abrams *et al*., 1976; Davis *et al*., 1992b).

Some studies performed in suckling rat pups (Lönnerdal *et al*., 1987; 1997) demonstrated differing absorptions of manganese from different milks and formulas. Infants fed cow's milk formula may retain 5 times more manganese, and infants fed soy formula may retain 25 times more manganese than breast-fed infants.

Because one of the key determinants of absorption of manganese seems to be dietary iron intake,

their relationship has been extensively investigated. The addition of manganese to diets depleted of iron resulted in depressed hemoglobin levels; the addition of iron to diets prevented the effect of manganese (Leach and Lilburn, 1978). An increase in the iron content of milk decreased the whole-body retention of orally administered ^{54}Mn in rats by a factor of 10 (Kostial *et al*., 1980). The interdependence between iron and manganese can be due to the fact that iron and manganese are absorbed by the same transport system in the gut (Chandra and Tandon, 1973; Diez-Ewald *et al*., 1968; Rehnberg *et al*., 1982). Both manganese and iron are bound by transferrin, and these elements compete for binding protein in the body. Therefore, diets that are low in iron allow transferrin to bind more manganese. Interaction between iron and manganese occurs only between non-heme iron and manganese (Davis *et al*., 1992b).

Manganese interactions with other elements (Cd, Ni, In, Rh, Se) at the level of gastrointestinal absorption were also observed (Burch *et al*., 1975; Doyle and Pfander, 1975; Jacobs *et al*., 1978). Ethanol was shown to influence the transport of manganese in the rat's small intestine (Schafer *et al*., 1974). High dietary intakes of phosphorus (Wedekind *et al*., 1991) and calcium (Wilgus and Paton, 1939) have been demonstrated to depress manganese uptake in chicks. More recent data also confirm that the absorption of manganese is related inversely to the level of calcium in the diet (Lutz *et al*., 1993).

Manganese absorption is age dependent. Dorner *et al*. (1989) have shown that infants, especially premature infants, retain a higher proportion of manganese than adults. Animal studies also support these findings (Kostial *et al*., 1978; Rehnberg *et al*., 1980; 1982; 1985). Such age-dependent differences in retention of manganese could also be due to differences in excretory ability (Miller *et al*. 1975) or to age-related changes in dietary intake level of iron and manganese (Ballatory *et al*., 1987). Animal studies show that absorption and/or retention of manganese is higher in neonates but returns to the level of older animals at approximately postgestational day 17–18 (Lönnerdal *et al*., 1987; Rehnberg *et al*., 1982). Dorner *et al*. (1989) do not provide adequate data to determine when this transition takes place in human infants. In a recent study published by Kostial *et al*. (2004) on regulation of manganese in perinatally exposed rat pups, it was shown that the concentration of manganese in perinatally exposed pups (whose mothers were exposed orally to Mn in drink—as manganese chloride, dose of 2000 mg/L Mn, throughout pregnancy and 11 days of lactation) was 6–8 times higher than in controls, irrespective of the period and duration of exposure. After cessation of exposure, the Mn concentration decreased almost to control levels. These results indicate the existence of an accurate regulation of Mn accumulation in pups exposed to Mn during the perinatal period.

There are no studies regarding absorption of manganese after oral exposure to MMT in either humans or animals.

5.1.3 Dermal Exposure

There is only a case report of a man burned with a hot acid solution containing 6% manganese. The authors (Laitung and Mercer, 1983) speculated that manganese absorption had occurred across the burn area because the man had slightly elevated urinary manganese levels. Studies reporting systemic effects in animals after dermal exposure to organic manganese compounds indicate absorption have occurred (ATSDR, 2000).

5.2 Distribution

Manganese is a normal component of human and animal tissues and fluids with highest levels in the liver, pancreas, and kidney and the lowest levels in bone and fat. Most tissue concentrations range between 0.1 and 1 µg manganese/g wet weight (Tipton and Cook, 1963; Sumino *et al*., 1975).

Absorbed manganese is rapidly eliminated from the blood and at first concentrates in the liver. Excess metal may be distributed to other tissues. Manganese is distributed in the body in constant concentrations, which are characteristic of the individual tissues and almost independent of the species (Cotzias, 1958; Underwood, 1971). Generally, organs and tissues do not accumulate large concentrations of manganese. Minor amounts go to the brain and bone, as shown in experiments on mice (Kato, 1963), rats (Dastur *et al*., 1969), and monkeys (Dastur *et al*., 1971). In contrast to many other trace metals, manganese does not accumulate significantly in the lungs with age (Newberne, 1973). Manganese preferentially accumulates in tissues rich in mitochondria (Maynard and Cotzias, 1955). The brain has a small amount of manganese, but retention time is long. Experiments on rats have shown that manganese elimination from the brain is slower than from the whole body (Dastur *et al*., 1969). In monkeys, it was also shown that elimination from the cerebrum was very slow; whereas the half-time in whole body was estimated to be 95 days for the slow component, it was not possible to obtain an estimate for the cerebrum even after 278 days, indicating an extremely long biological half-time (Dastur *et al*., 1971). In 1999, Aschner wrote a review on manganese homeostasis in the CNS, focusing on mechanisms of the transport across the blood–brain

barrier, on transport mechanisms associated with regulation of manganese uptake and release by astrocytes and its requirement for glutamine synthetase function and regulation. The transport across blood–brain barrier was recently attributed to an active carrier-mediated mechanism for the influx into the brain and to a passive diffusive mechanism for the efflux from the brain (Crossgrove and Yokel, 2005; Yokel and Crossgrove, 2004). This would be an important mechanism to explain the occurrence of a brain overload after excessive absorption of manganese but also after prolonged exposure to low doses. In a review on the transport of manganese across the blood–brain barrier, putative carriers for manganese into and out of the brain were examined, with an alternative explanation for the efflux of manganese from the brain (Aschner, 2006).

Vitarella *et al*. (2000) exposed adult rats to airborne doses of particulate manganese, as Mn phosphate, at 0, 0.03, 0.3, 3 mg/manganese/m^3. The particles had a mean diameter of 1.5 µm. Exposure lasted for 6 hours/day for either 5 days/week (10 exposures) or 7 days/week (14 exposures). The following tissues were analyzed for manganese content by use of neutron activation analysis: plasma, erythrocytes, olfactory bulb, striatum, cerebellum, lung, liver, femur, and skeletal muscle after exposure to 3 mg/m^3 (after either dosing regiment). A lower dose of 0.3 mg/m^3 resulted in increased manganese concentrations in olfactory bulb and lung (14-dose regimen only). Striatal manganese levels were increased at the two highest doses only after 14 days of exposure. Concentrations in the cerebellum were similarly elevated, which was interpreted by the authors to indicate that accumulation of manganese was not selective for the striatum. Red blood cell and plasma manganese levels were increased only in rats exposed to the highest dose for the 10-day exposure period. These data indicate that even at lower doses, manganese can accumulate in the olfactory bulb. However, it must be noted that tissues were collected and analyzed at the cessation of exposure. It is not clear whether the distribution of manganese would be similar if tissues had been analyzed at a later time (ATSDR, 2000).

Studies in humans with chronic liver disease or some other liver dysfunction (e.g., cirrhosis, portacaval shunt) after oral exposure, presumably through the diet, with impaired manganese excretion showed that manganese preferentially accumulates in the basal ganglia, especially the globus pallidus and the substantia nigra. For determining the accumulation of manganese Tl-weighted MRI or neutron activation analysis was used (Devenyi *et al*., 1994; Fell *et al*., 1996; Hauzer *et al*., 1996; Lucchini *et al*., 2000; Pomier-Layrargues *et al*., 1998; Rose *et al*., 1999).

The distribution of manganese in the brain was investigated in *Cebus* (Newland and Weiss, 1992; Newland *et al*., 1989) and *Macaque* (Newland *et al*., 1989) monkeys given intravenous injections of $MnCl_2$ that reached a cumulative dose of 10–40 mg manganese/kg. Magnetic resonance images indicated a symmetrical hyperintensity in the globus pallidus and substantia nigra consistent with an accumulation of manganese in these areas. Substantial accumulation of manganese was also noted in the pituitary at low cumulative doses. London *et al*. (1989) reported a rapid localization of manganese in the choroid plexus observed on MRI.

Some of the experimental animal studies showed that large increases in tissue levels of manganese compared with the controls occurred in rats over the first 24 days of exposure to 214 mg manganese/kg (body weight)/day (as Mn_3O_4), for up to 224 days. But levels tended to decrease toward the control levels as exposure was continued (Kristensson *et al*., 1986; Rehnberg *et al*., 1980). This pattern is thought to be due to a homeostatic mechanism that leads to decreased absorption and/or increased excretion of manganese when manganese intake levels are high (Abrams *et al*., 1976; Ballatory *et al*., 1987; Mena *et al*., 1967). Davis *et al*. (1992b) and Malecki *et al*. (1996) demonstrated that rats fed elevated levels of manganese for several weeks had increased tissue manganese concentrations, despite increased gut endogenous losses of manganese, as biliary manganese.

Manganese penetrates the placental barrier in all species. Children are exposed *in utero* because manganese in maternal blood crosses the placenta to satisfy the fetus's need for manganese. Manganese has been measured in cord blood plasma of premature and full-term infants and their mothers (Wilson *et al*., 1991).

In a human study on manganese levels during pregnancy and at birth performed in a southwest Quebec population (Takser *et al*., 2004), it was shown that mothers' manganese blood levels increased significantly during pregnancy, and cord blood manganese levels were significantly higher than those for mothers' blood. This study also indicated that lifestyle (mothers' smoking habit) and environmental factors might interfere with the balance and homeostatic mechanisms required to maintain manganese at optimal levels for physiological changes during pregnancy. In a previous study, levels of Mn in the umbilical cord and in maternal blood were compared between 160 pairs of mothers-neonates in Montreal and 206 pairs in Paris. The prevalence of high Mn levels in umbilical cord blood was found significantly higher in Montreal, where MMT was in use in gasoline in 1977 (Smargiassi *et al*., 2002).

Manganese is secreted into milk. Its concentrations in breast milk vary between 3.4–10 μg/L (Arnaud and Favier, 1995; Collip *et al*., 1983).

Manganese is more uniformly distributed in fetal than in adult tissue. A study on age-related retention and distribution of ingested Mn_3O_4 in rats (Cahill *et al*., 1980) demonstrated that 24 hours after ingestion, infant rats retained up to 20 times more Mn than the adolescent or the adult. The retention of manganese was also a function of age at exposure within infancy. The liver was the principal site of deposition at all ages. Brain concentrations of manganese from a single dose were easily detectable in infants but not adults. A study measuring the retention of a single oral dose of radiolabeled manganese in adult and neonatal rats indicated that retention of the label 6 days after exposure was much greater in pups. The addition of manganese to the animals' drinking water decreased radiolabel retention in pups and adults (Kostial *et al*., 1989).

A high rate of manganese absorption and distribution in selected tissues, especially the cerebrum, hypothalamus, and pituitary, was found in preweanling rats dosed daily with particulate Mn_3O_4 for 12 or 27 days postpartum (Rehnberg *et al*., 1982).

In the blood, manganese is bound to proteins. In the trivalent state, it can be bound to transferrin, and in the bivalent state to an α-macroglobulin (Gibbons *et al*., 1976). The plasmatic content of manganese is lower than the manganese content in red blood cells (ratio 1:5) (Weissman, 1981). After absorption, manganese is transported in blood bound to proteins. The quote of erythrocytary manganese is approximately 85% (Weissman, 1981) or 66% according to Milne *et al*. (1990).

The organic manganese compound MMT is rapidly metabolized in rats. After oral exposure and injection, Moore *et al*. (1975) found that the liver, kidney, and lungs contained the highest concentrations. The distribution in general was similar to that seen after exposure to inorganic manganese. Similar results were obtained in some of the studies performed later. In 1985, Gianutsos *et al*. administered 0, 11, or 22 mg manganese/kg as MMT (dissolved in propylene glycol) to male adult mice by subcutaneous injection. The experiment was divided into an acute study (one dose) or a "chronic" study (10 doses), and the accumulation of manganese in brain was followed. The brain manganese level 24 hours after the single dose of MMT was significantly higher than the control values: after 10 doses of 11 mg/kg and 22 mg/kg, the brain manganese levels in both cases were significantly higher than the control values and were significantly different than the levels reported after the acute exposure. Manganese levels in the brains of mice given a single dose of MMT at 22 mg manganese/kg were also compared with those after injection of the same manganese dose of $MnCl_2$. The manganese level in brain after $MnCl_2$ exposure followed the same increasing trend over 24-hour analysis as in the case of MMT application but was somewhat higher at each time point with a maximum value of >2.0 μg/g wet weight compared with ~1.4 μg/g wet weight after MMT exposure.

Another study included young adult rats, which were administered MMT dissolved in propylene glycol by subcutaneous injection at a dose of 1 mg manganese/kg (McGinley *et al*., 1987). The rats were sacrificed 1.5, 3, 6, 12, 24, 48, or 96 hours after injection. Maximum accumulation of MMT-derived manganese measured 3 hours after dosing was found primarily in lung (~ 9 mg/kg), kidney (3.9 mg/kg), liver (2.75 mg/kg), and blood (~0.75 mg/kg). Concentrations of manganese in these four tissues were still elevated (~1 mg/kg) 96 hours after dosing. Brain manganese concentrations were not significantly elevated over control levels in MMT-treated animals.

In connection with the clinical use of mangafodipir as a contrast agent, several studies have shown the qualitative presence of manganese in liver because of increased signal in that organ after mangafodipir administration of 0.17–0.83 mg manganese/kg on Tl-weighted MRI (Bernardino *et al*., 1992; Lim *et al*., 1991; Padovani *et al*., 1996; Wang *et al*., 1997). Several studies (Gallez *et al*., 1997; Grant *et al*., 1997; Hustvedt *et al*., 1997) have determined the distribution of manganese in tissues of animals after intravenous administration of mangafodipir. The results of these studies indicate that single, clinical doses of mangafodipir are not likely to cause persistent accumulation in the brain.

5.3 Metabolism

Manganese is capable of existing in a number of oxidation states, and limited data suggest that it may undergo changes in oxidation state in the body. The oxidation state of the manganese ion in several enzymes seems to be Mn(III) (Leach and Lilburn, 1978; Utter, 1976), whereas most manganese intake from the environments is either Mn(II) or Mn(IV). However, Gibbons *et al*. (1976) suggested that the oxidation of manganese occurs in the body. It was observed that human ceruloplasmin led to the oxidation of Mn(II) to Mn(III) *in vitro*, and although the process was not studied *in vivo*, it is a likely mechanism for manganese oxidation in the blood. The same authors also noted that manganese oxidation led to a shift in manganese binding *in vitro* from α_2-macroglobulin to transferrin and that *in vivo* clearance on Mn(II)–α_2–macroglobulin from cows was much more rapid

than the clearance of Mn(III)–transferrin. Another line of evidence that manganese may undergo changes in oxidation state in the body is based on measurements of manganese in tissues and fluids by use of electron spin resonance (ESR), which detects the unpaired electrons in Mn(II), Mn(III), and Mn(IV). When animals were injected with $MnCl_2$, levels of manganese increased in bile and tissues, but only a small portion of this was in a form that gave an ESR signal (Sakurai *et al*., 1985; Tichy and Cikrt, 1972). This suggests that Mn(II) is converted to another oxidation state (probably Mn(III), but it is also possible that formation of complexes between Mn(II) and biological molecules (bile salts, proteins, nucleotides, etc.) results in loss of the ESR signal without formal oxidation of the manganese ion (ATSDR, 2000).

The extent of manganese reduction–oxidation reactions may be important determinants of manganese retention and toxicity in the body. In a study by Komura and Sakamoto (1991), it was shown that the form in which the manganese was administered in the diet affected tissue levels of manganese in rats. In animals fed manganese acetate or $MnCO_3$, levels of manganese were significantly higher than in animals fed $MnCl_2$ or MnO_2. Differences in manganese concentrations in blood and brain regions depending on the oxidation state of manganese were also noted in another study (Roels *et al*., 1997).

MMT metabolizes to hydroxymethylcyclopentadienyl manganese tricarbonyl (CMT-CH_2OH) and carboxycyclopentadienyl manganese tricarbonyl (CMT-COOH), both present in urine after intravenous administration of MMT in male rats, as shown in a study by Hanzlik *et al*. (1980). Metabolites are also present in the bile as indicated by the fecal recovery of ^3H from the ring structure in MMT after intravenous or intraperitoneal administration of radiolabeled compounds to rats (Hanzlik *et al*., 1980).

Maneb and mancozeb are metabolized to several compounds, including ethylenethiourea (ETU), ethylene urea, and lenediamine in plants and animals (Jordan and Neal, 1979). ETU has been identified in the urine of occupationally exposed sprayers of these fungicides (Kurttio and Savolainen, 1990; Kurttio *et al*., 1990).

Mangafodipir, after an infusion of the clinical dose of 5 µmol/kg or 0.25 mg/kg, is rapidly dephosphorylated to manganese dipyridoxyl monophosphate (MnDPMP). The monophosphate then is fully dephosphorylated to manganese dipyridoxyl ethylenediamine (MnPLED). This compound has been isolated in blood from 18 minutes after the start of an infusion of mangafodipir until 40 minutes after the start (Toft *et al*., 1997). Clinical doses of mangafodipir are not likely to cause persistent accumulation of manganese in the brain.

5.4 Excretion

Manganese homeostasis is primarily achieved by excretion. Absorbed manganese is almost totally excreted through the intestinal wall by several routes (Newberne, 1973). The main excretion route is through the bile. Manganese is removed from the blood by the liver, where it conjugates with bile and is excreted into the intestines. Reaching the intestines, most of the element is excreted in the feces (Cikrt, 1973; Davis *et al*., 1993; Malecki *et al*., 1996; Papavasiliou *et al*., 1966). Klaassen (1974) found in rats, 5 days after intravenous dosing, that 99% of the manganese administered had been eliminated through feces. Liver lysosomes play an important role in intrahepatic movement and affect biliary excretion of absorbed manganese (Suzuki and Wada, 1981). At excessive exposure, other gastrointestinal routes may participate (Bertinshamps *et al*., 1965). Some of the manganese in the intestine is reabsorbed through enterohepatic circulation (Schroeder *et al*., 1966) Manganese retention is prolonged after ligation of the bile duct (Papavisiliou *et al*., 1966). Small amounts of manganese can also be found in urine, sweets, and milk. In humans, the urinary excretion of manganese is less than 1 µg/L. Because the body burden of an adult is approximately 10 mg manganese, only approximately 0.01% of the body burden is excreted daily by this route.

In humans who inhaled $MnCl_2$, approximately 60% of manganese originally deposited in the lung was excreted in the feces within 4 days (Mena *et al*., 1969). Rats exposed to either $MnCl_2$ or Mn_3O_4 by intratracheal instillation excreted approximately 50% of the dose in the feces within 3–7 days (Drown *et al*., 1986). Monkeys exposed to an aerosol of $^{54}MnCl_2$, excreted most of the manganese, with a half-time of 0.2–0.36 days (Newland *et al*., 1987). However, a portion of the compound was retained in the lung and brain. Clearance of this label was slower, occurring with half-times of 12–250 days.

Occupationally exposed male workers were reported to have urine manganese levels that were significantly higher than unexposed persons (Alessio *et al*., 1989; Lucchini *et al*., 1995; Roels *et al*., 1987). Mergler *et al*. (1994) did not report a significant difference in urinary manganese levels between the exposed and control workers. The differences in urinary excretion may be due to differences in duration and extent of exposure.

Humans who ingested tracer levels of radioactive manganese, usually as $MnCl_2$, excreted manganese with whole-body retention half times of 13–37 days (Davidson *et al*., 1989; Mena *et al*., 1969; Sandstrom *et al*., 1986). In studies by Greger *et al*. (1990) and Davis

and Greger (1992), urinary excretion of manganese by men was 7.0 µmole/g creatinine and by women 9.3 µmole/day. Urinary excretion was not responsive to oral intake of manganese.

Animals on low-manganese diets still show a substantial excretion of manganese. This indicates that the ability to conserve manganese by decreasing excretion is limited (Britton and Cotzias, 1966) and that increase of dietary manganese is important for the homeostasis (Abrams *et al*., 1976).

After exposure to the organic compound MMT, manganese excretion takes place to a large extent through urine and is of the same magnitude as the gastrointestinal excretion. This has been explained as a result of biotransformation of the organic compound in the kidney (Moore *et al*., 1975). Manganese originating from clinical application of mangafodipir is primarily excreted in the feces through the bile and is incompletely cleared from the body 24 hours after administration, and approximately 7–8% of a dose is still retained in the body after 1 week (Hustvedt *et al*., 1997).

A qualitative physiologically based pharmacokinetic model (PBPK) for manganese disposition in humans and animals was developed some years ago (1999) by Andersen *et al*. Because several data gaps exist concerning manganese pharmacokinetics, this model is anticipated to change with time and might also include a separate compartment for the brain regions versus a general CNS compartment (Andersen *et al*., 1999).

6 HEALTH EFFECTS

6.1 Manganese Deficiency

Manganese, like zinc and copper, is essential for normal prenatal and neonatal development. It is essential for humans and animals and plays a role in bone mineralization, protein and energy metabolism, metabolic regulation, cellular protection from damaging free radical species, and the formation of glycosaminoglycans (Welder, 1994). Mitochondrial superoxide-dismutase, pyruvate carboxylase, and liver arginase are known manganese metalloenzymes (NAS/NRC, 1989; Welder, 1994). Several enzyme systems, transferases, decarboxylases, hydrolases, dehydrogenases, synthetases, and lyases, have been reported to interact with or depend on manganese for their catalytic or regulatory function (Welder, 1994). Manganese has been shown to stimulate the synthesis of chondroitin sulfate, an important constituent of the cartilage and connective tissue (Utter, 1976). There is limited evidence that some disorders or diseases in humans such as amyotrophic lateral sclerosis, acromegaly, catabolic disease, and epilepsy may be associated with an imbalance in tissue levels of manganese (Aihara *et al*., 1985; Carl *et al*., 1993; Tulikoura and Vuori, 1986). However, no large-scale deficiency has been reported, although manganese intakes of many people are believed to be less than the estimated safe and adequate daily dietary intake. This may reflect, in part, the lack of adequate methods to monitor nutritional status in regard to manganese (ATSDR, 2000).

In animals, it has been shown experimentally that manganese deficiency will cause skeletal abnormalities and impaired growth (Underwood, 1971). Also the inner ear can be affected, because maternal manganese deficiency will cause ataxia and defective otoliths (Hurley and Theriault-Bell, 1974).

Doisy (1973) described the case of a male participant in a vitamin K study, who was on a diet low in manganese (0.35 mg manganese/day) and vitamin K during 16 weeks that resulted in a decreased level of clotting proteins, decreased serum cholesterol, reddening of black hair and beard, slow growth of hair and nails, and scaly dermatitis. After vitamin K was returned to the diet, the subject continued to exhibit these symptoms, but when manganese was returned to the diet the symptoms were eliminated.

In a study by Friedman *et al*. (1987), five of seven men fed a manganese-deficient diet for 39 days exhibited dermatitis (*Miliaria cristallina*). The dermatitis cleared rapidly when manganese was returned to the normal diet. Considering the fact supported by studies in humans that manganese is an essential element, the US Food and Nutrition Board of the National Research Council established ESADDI (estimated safe and adequate daily dietary intake) levels as follows: 0.3–0.6 mg/day for infants from birth to 6 months; 0.6–1.0 mg/day for infants from 6 months to 1 year; 1.0–1.5 mg/day for children from 1–3 years; 1.0–2.0 mg/day for children from 4–6 years and 7–10 years; and 2.0–5.0 mg/day for adolescents (>11 years) and adults (NRC, 1989). However, the Food and Nutrition Board has cautioned that upper levels of these provisional dietary intake of ESADDIs should not be exceeded on a routine basis, because toxic levels may be only several times the usual intake levels (NRC, 1989).

6.2 Acute Effects

Manganese is less toxic than most of the metals (Bienvenu *et al*., 1963). The average LD_{50} values range from 400–830 mg/Mn/kg (guinea pig, mouse, rat) for oral administration of soluble manganese compounds (Sigan and Vitvickaja, 1971) and from 38–64 mg Mn/kg (rat, mouse) for parenteral injections (Bienvenu *et al*., 1963; Holbrook *et al*., 1975). It has been suggested that

manganese cations are more toxic than the anionic forms and that bivalent cation is approximately three times more toxic than a trivalent cation (EPA, 1975). The inhalation LD_{50} for MMT was determined to be 62 mg Mn/m^3 (247 mg MMT/m^3) for a 1-hour exposure and 19 mg Mn/m^3 for a 4-hour exposure.

Because manganese is regarded as a metal with relatively low toxicity, acute poisoning by manganese in humans is rare. There is a case report on methemoglobinemia induced by ingestion of potassium permanganate (Mahomedy *et al*., 1975). Hobbesland *et al*. (1997) reported a significantly increased incidence in sudden death mortality for workers in ferromanganese/silicomanganese plants during their employment period (standardized mortality ratio, SMR = 2.47). However, the authors caution that the association of increased death and manganese exposure is speculative, and the incidence in sudden death could also be caused by common furnace work conditions (heat, stress, noise, carbon monoxide, etc.).

There is only one study (Kawamura *et al*., 1941) in which death in humans may have been associated with ingestion of manganese. In this report, death from "emaciation" occurred in two adults who ingested drinking water contaminated with high levels of manganese (14 ml/L). Although many of the symptoms in the group of six Japanese families (approximately 25 persons) could be connected with the exposure to manganese, there is considerable doubt that all of the features of this outbreak (particularly the deaths) were due to manganese alone (ATSDR, 2000). Namely, symptoms seemed to have developed very quickly; the course of the disease was very rapid, in one case progressing from initial symptoms to death in 3 days; all survivors recovered from the symptoms even before the manganese content of the well had decreased significantly after removal of the batteries buried near the well from where manganese leached to the drinking water.

Freshly formed manganese oxide fumes of respirable particles size, along with a number of other metals, may cause metal fume fever.

6.3 Adverse Effects of Prolonged Exposure

6.3.1 Neurotoxic Effect

Central nervous system (CNS) is the primary target of manganese toxicity. Although it is known that manganese is a cellular toxicant that can impair transport systems, enzyme activities, and receptor functions, the principal manner in which manganese neurotoxicity occurs has not been yet clearly established (Aschner and Aschner, 1991).

6.3.1.1 Mode of Action

Studies of the neuropathological bases for manganese neurotoxicity have pointed to the involvement of the corpus striatum and the extrapyramidal motor system (Archibald and Tyree, 1987, Eriksson *et al*., 1987; 1992). The specific area of injury in humans seems to be primarily in the globus pallidus. The substantia nigra is sometimes affected but generally to a lesser extent (Katsuragi *et al*., 1996; Yamada *et al*., 1986). Studies in nonhuman primates have produced similar findings (Newland *et al*., 1989; 1992.). The likelihood that the CNS effects of manganese are mediated downstream of the substantia nigra, predominantly in the globus pallidus, was also supported in the discussions at the International Conference on Manganese, 1997 (Aschner, 2002).

Limited evidence suggests that dopamine levels in caudate nucleus and putamen are decreased in affected patients (Bernheimer *et al*., 1973). In terms of the neurochemistry of manganese toxicity, some other studies have shown that dopamine levels are affected by manganese exposure in humans, monkeys, and rodents, with various indications of an initial increase in dopamine followed by a longer-term decrease (Barbeau, 1984; Donaldson, 1984). The loss of dopamine in the brain and the concomitant neuronal cell damage could be expressed as an increase in motor activity (Bonilla, 1984; Nachtman *et al*., 1986). Calabresi *et al*. (2001) also reported that Mn-treated rats exhibited a complex behavior syndrome with increased activity in the absence of significant striatal neuronal loss and gliosis.

The precise biochemical mechanism by which manganese leads to selective destruction of dopaminergic neurons is not known, but many researchers believe that manganese ion enhances the autooxidation or turnover of various intracellular catecholamines, leading to increased production of free radicals (Donaldson *et al*., 1982), reactive oxygen species, and other cytotoxic metabolites, along with a depletion of cellular antioxidant defense mechanism (Barbeau, 1984; Donaldson, 1987; Graham *et al*., 1984; Liccione and Maines, 1988; Nachtman, 1986; Verity, 1999). Desole *et al*. (1994) in an experimental study performed on 6-month-old rats provided supporting evidence for the hypothesis that high levels of manganese exert neurotoxicity through oxidation. However, a study by Sziraki *et al*. (1999) demonstrated atypical antioxidative properties of manganese in iron-induced brain lipid peroxidation and copper-dependent low-density lipoprotein conjugation, but the underlying mechanisms of the antioxidant effects are not clear. In a study by Brenneman *et al*. (1999), in which reaction oxygen species in the brains of neonatal rats administered up to 22 mg/manganese/kg/day for up to 49 days were followed, also did not

support the hypothesis that oxidative damage is a mechanism of action in manganese-induced neurotoxicity in rat.

The role of mitochondrial energy metabolism in manganese toxicity was indicated by two studies (Aschner and Aschner, 1991; Gavin *et al*., 1990). A study by Brouillet *et al*. (1993) suggested that the mitochondrial dysfunctional effects of manganese result in various oxidative stress to cellular defense mechanisms and secondary free radical damage to mitochondrial DNA. At the previously mentioned Conference on Manganese, the mitochondria were singled out as critical organelles in the cell, and the role they might play in manganese-induced cellular damage was targeted as an important subject for further investigation (Aschner, 2002). Oxidative stress generated through mitochondrial perturbation may be a key event in the demise of the affected central nervous system cells. Studies with primary astrocyte cultures have revealed that they are a critical component in the defenses against manganese-induced neurotoxicity (Dobson *et al*., 2004). Enhanced oxidative stress may take place particularly in catecholaminergic (i.e., dopamine) cells (Erikson *et al*., 2004).

Transcriptional patterns of genes related to oxidative stress of inflammation were examined in the brains of rats exposed to inhaled manganese during either gestation or early adulthood (HaMai *et al*., 2006). The expression of genes encoding for proteins critical to an inflammatory response and/or possessing prooxidant properties, including TGFß and nNOS, were slightly depressed by prenatal exposure, whereas inhalation exposure to manganese during adulthood markedly down-regulated their transcription. When exposure to manganese occurred during gestation, the extent of altered gene expression induced by subsequent exposure to manganese in adulthood was reduced. The obtained results suggest that prior exposure to manganese may have attenuated the effects of inhalation exposure to manganese in adulthood, in which the expression of inflammation-related genes were suppressed.

Some experimental evidence suggests that the mechanisms of manganese toxicity may depend on the oxidation state of manganese. However, both the trivalent (MnIII) and divalent (MnII) forms have been demonstrated to be neurotoxic (Aschner and Aschner, 1991), but it is important to note that the oxidation of catechols is more efficient with Mn(III) than with Mn(II) or Mn(IV) (Achibald and Tyree, 1987). Formation of Mn(III) may occur by oxidation of Mn(II) by superoxide (Hussain *et al*., 1997). Both Mn(III) and Mn(II) can cross the blood–brain barrier, although it is suggested that Mn(III) is predominantly transported bound to the protein transferrin, whereas Mn(II) may enter the brain independently of such a transport mechanism (Murphy *et al*., 1991). A large portion of manganese is bound to manganese metalloproteins, especially glutamine synthetase in astrocytes. A portion of manganese probably exists in the synaptic vesicles in glutamatergic neurons, and manganese is dynamically coupled to the electrophysiological activity of the neurons. Manganese released into the synaptic cleft may influence synaptic transmission. So, as pointed out by Takeda (2002), understanding the movement and action of manganese in synapses may be important to clarify the function and toxicity of manganese in the brain.

6.3.1.2 Health Impairment

Syndrome (disease) of neurological effects of manganese is referred to as "manganism." As early as 1837, John Couper reported five cases of poisoning, with a picture similar to paralysis agitans (Parkinson's disease), among men employed in grinding manganese dioxide in the manufacture of chlorine for bleaching powder. Couper's description of the disease remained forgotten for almost a century. In 1901, Von Jaksch in Prague and Embden in Hamburg rediscovered the disease and for three and four cases, respectively, they provided an accurate clinical description of manganism. After that time, a large number of cases of chronic manganese poisoning have been reported. Up to 1935, 152 cases of manganism had been described in the literature (Voss, 1939). According to the EPA (1993), 7550 cases of manganism had been recorded since the first report in 1837. A notable increase during the past 50 years may be presumably explained by use of different diagnostic criteria for manganism. Although earlier clinically fully developed cases only had been considered as manganism, later cases with initial symptoms and signs might be registered as well.

Neurological effects, as the hallmark of excessive exposure to manganese in humans, are associated primarily with inhalation in occupational settings. Exposure to manganese dioxide in mines or in ore processing plants had been the most common cause of the disease. There have been reports of manganism in Huelva miners in Spain (Dantin Galego, 1935), Sinai miners (Nazif, 1936), German miners (Büttner and Lenz, 1937), Morrocan miners (Rodier and Rodier, 1949), Chilean miners (Ansola *et al*., 1944), Cuban miners (Garcia Avila and Peñalver, 1953), Romanian miners (Wassermann *et al*., 1954), miners in Mexico (Roldan, 1955), miners in the former U.S.S.R. (Khazan, *et al*. 1956), Japanese miners (Suzuki *et al*., 1960), and Indian miners (Chandra *et al*., 1974). Manganism occurring in ferromanganese plants have been described by Dogan and Beritic (1953), Jonderko *et al*. (1971), Rosenstock

et al. (1971), Smyth *et al*. (1973), Suzuki *et al*. (1973a,b), Šarić *et al*. (1977), and in the manufacture of dry cell batteries, as well as in some other processes by Tanaka and Lieben (1969), Horriuchi *et al*. (1970), and Emara *et al*. (1971).

However, some of the studies performed more recently in children, despite numerous limitations that preclude firm conclusions, indicate that ingestion of water and/or foodstuffs containing increased concentrations of manganese may result in adverse neurological effects. Infants have increased absorption of manganese related to adults (Dorner *et al*., 1989). Iron deficiency leads to increased brain manganese concentrations. Children who have cholestatic liver disease or gastrointestinal disorders that mandate they be given total parenteral nutrition may be at increased risk from overexposure to manganese (Devenyi *et al*., 1994; Fell *et al*., 1996; Ono *et al*., 1995). Interruption of bile secretion and flow can impair the body's ability to clear manganese. In some studies carried out in such circumstances, signs of manganism were observed (Fell *et al*., 1996; Kafritsa *et al*., 1998; Nagatomo *et al*., 1999; Ono *et al*., 1995).

The clinical picture of manganism shows both psychiatric and neurological manifestations mainly related to the extrapyramidal system. The disease typically begins with feeling of weakness and lethargy. As the disease progresses, a number of other neurological signs may become manifest. Although not all individuals develop identical signs, the most common are a slow and clumsy gait, speech disturbances, a masklike face, and tremors. At further exposure, more pronounced and objective signs appear. The speech disorder may progress to muteness. Patients will develop a fixed facial expression. Increasing clumsiness and inability to make certain movements have also been observed. A neurological examination at this stage, however, does not reveal anything specific except increased reflexes in the lower limbs. In the third stage, the disease is fully developed. Difficulties in walking caused by muscular hypertonia, muscle rigidity, and tremor in the upper limbs, as well as difficulties writing, are among the symptoms and signs. Patients may become completely and permanently disabled (Rodier, 1955). In addition, a syndrome of psychological and psychic disturbances (aggressiveness, hallucination, psychosis) frequently emerges, although such symptoms are sometimes absent (Cook *et al*., 1974).

Although, there is evidence that indicates that the neurological symptoms may be improved in some cases (Shuqin *et al*., 1992; Smyth *et al*., 1973); in most cases, however, the symptoms and signs were found to be irreversible, persisting for many years after exposure ceases (Cotzias *et al*., 1968). More recently, Huang *et al*. (1998) documented the progression of clinical symptoms of manganism by a follow-up study of a group of workers in a ferromanganese plant.

Despite the similarities, significant differences between parkinsonism and manganism exist. Barbeau (1984) reported that the hypokinesia and tremor, which is mainly of intentional type, present in patients with manganism differs from those seen in Parkinson's disease. Psychiatric disturbances early in the disease (although not always), a cock walk, a propensity to fall backward when displaced, less frequent resting tremor, more frequent dystonia, and failure to respond to dopaminomimetics in manganism, are other characteristics that distinguish manganism from Parkinson's disease (Calne *et al*., 1994). In Parkinson's disease, patients' pathological lesions are found in the substantia nigra and other pigmented areas of the brain (Barbeau, 1984). Parkinsonism is believed to be due to the selective loss of subcortical neurons, whose cell bodies lie in the substantia nigra and whose axons terminate in the basal ganglia, which includes the caudate nucleus, the putamen, the globus pallidus, and other structures. These nigral neurons use dopamine as their neurotransmitter. Manganese seems to affect pathways that are postsynaptic to the nigrostrial system, most likely the globus pallidus (Chu *et al*., 1995). Magnetic resonance imaging (MRI) of the brain reveals accumulation of manganese in cases of manganism but few or no changes in subjects with Parkinson's disease. Fluorodopa positron emission tomography (PET) scans are generally normal in cases of manganism but abnormal in subjects with Parkinson's disease (Calne *et al*., 1994). Nevertheless, it cannot be excluded that the damage induced by manganese can extend also onto the dopaminergic pathways that are typically damaged in Parkinson's disease (Baek *et al*., 2004; Kim *et al*., 2002). In fact, typical signs of manganese deposition at MRI scan, with elevated MnB and abnormal Fluoro-Dopa PET scan, have been shown in welders (Kim *et al*., 1999). More recently, a patient with parkinsonism associated with elevated serum manganese from hepatic dysfunction demonstrated relatively symmetrical and severely reduced ^{18}F-dopa levels in the posterior putamen. MRI revealed selective abnormality within the internal segment of the pallidum (Racette *et al*., 2005a). This case suggests that the clinical and pathophysiological features of manganese-associated parkinsonism may overlap with that of Parkinson's disease. It has also been suggested that manganese exposure may play a role in the development of idiopathic Parkinson's disease by acting as an environmental trigger able to accelerate the onset of the neurovegetative damage (Martin, 2006). The relationship between these two manifestations need further research, also in view

of the close interconnection between the target area of manganese toxicity, which is represented by the globus pallidus and the substantia nigra, where the degeneration of dopaminergic neurons takes place (Weiss, 2006).

In the development of manganism, individual susceptibility has been discussed, because poisoning was found in young people after only a few months of exposure, whereas in many workers with more than 20 years of exposure, no adverse neurological effect was observed (Mena *et al*., 1969; Tanaka and Lieben, 1969). Typically, the clinical effects of high-level inhalation exposure to manganese do not become apparent until exposure has occurred for several years. Studies have been initiated with multigene arrays encoding for >1000 genes to detect and categorize changes in gene expression caused by manganese (Aschner, 2002). Such toxicogenomic studies could help to explain the observed differences in susceptibility to neurotoxic effects of manganese.

More recent studies to estimate the impact of occupational exposure to manganese on neurological health have used a number of sensitive tests designed to detect early signs of neuropsychological and neuromotor deficit in the absence of symptoms (Iregren, 1999).

In descriptions of neurotoxic effects of manganese, data on manganese levels in air were not regularly reported in the older literature. Time-weighted averages of current exposure are reported more frequently, without clear dose-response relationships that were shown on the contrary when exposure was expressed with cumulative exposure indices (Lucchini *et al*., 1995; 1999; Roels *et al*., 1987; 1992). Manganese levels reported to lead to early signs of nervous system toxicity after inhalation exposure range from 0.027–1 mg Mn/m^3 (Chia *et al*., 1993; 1995; Iregren, 1990; Lucchini *et al*., 1995; 1997; 1999; Mergler *et al*., 1994; Roels *et al*., 1987; 1992; Wennberg *et al*., 1991). On the other hand, overt manganism has been observed at exposure levels ranging from 2–22 mg Mn/m^3 (Rodier, 1955; Šarić *et al*., 1977; Schuler *et al*., 1957; Tanaka and Lieben, 1969). According to WHO (1981), clinical manifestation of manganese intoxication can also occur at 1 mg/m^3 in susceptible individuals.

Roels *et al*. (1987) detected early preclinical neurological effects (alterations in simple reaction time, audio verbal short-term memory capacity, and hand tremor) in workers exposed to 0.97 mg manganese (median concentration in total dust)/m^3, for a group average of 7.1 years. Similarly, Iregren (1990) used neurobehavioral tests (simple reaction time, digit span, finger tapping, verbal ability, hand dexterity, and finger dexterity tests from Swedish Performance evaluation system [SPES]) to study adverse effects in 30 male workers from two different manganese foundries exposed to an estimated median concentration of 0.14 mg manganese (in total dust)/m^3 as MnO_2 for 1–35 years. The exposed workers had below-average scores on a number of the tests, such as reaction time and finger tapping compared with matched controls. Similar results were reported in a more recent study by Roels *et al*. (1992), as well as in a study by Mergler *et al*. (1994). In Mergler *et al*.'s study, the exposed workers also differed significantly from the controls in cognitive flexibility and emotional state. They also exhibited significantly greater levels of tension, fatigue, and confusion, as well as a significantly lower olfactory threshold than controls. Environmental levels of manganese in this study were measured at 0.014–11.48 mg/m^3, whereas manganese levels in respirable fraction were 0.001–1.273 mg/m^3, and mean duration of exposure was 16.7 years.

The epidemiological studies by Lucchini *et al*. (1995; 1997; 1999) that used similar neurobehavioral tests have shown similar findings on neurobehavioral and motor function. These studies have also shown dose-effect relationships with cumulative exposure indices. An 8-year follow-up study by Roels *et al*. (1999) showed a normalization of performance at the "Eye hand coordination test" after a decrease of exposure levels, only for the workers with average annual exposure of 0.4 mg/m^3 of Mn in total dust. No improvement was observed for hand stability and simple visual reaction time for the workers with average annual exposure of 0.6 and 2 mg/m^3. In a study by Gibbs *et al*. (1999), hand steadiness was also analyzed using Movemap steady, Movemap square, and tremor meter. Although technically sophisticated Movemap test has not been observed to discriminate between exposure groups any better than the other neurobehavioral methods (Iregren, 1999).

Mergler and Baldwin (1997) pointed out that although outcomes from individual studies using neurophysiologic and neurobehavioral tests in the absence of clinical manifestation can vary, they collectively show a pattern of slowing motor functions, increased tremor, reduced response speed, enhanced olfactory sense, possible memory and intellectual deficits, and mood changes. Several studies suggest the existence of dose-effect relationships.

An increased frequency of parkinsonian disturbances has been recently shown in case-control studies on large groups of welders (Racette *et al*., 2005b). The estimated prevalence of parkinsonism among active male welders age 40–69 was 977–1336 cases/100,000 population and was significantly higher than age-standardized data for the general population (prevalence ratio, 10.19, 95% confidence interval [CI], 4.43–23.43). The parkinsonian features in welders seem

not to be different from idiopathic Parkinson's disease, except for a younger age of onset and a tendency to familiarity (Racette *et al*., 2001). Two studies in Korea (Park *et al*., 2006) and Sweden (Fored *et al*., 2006) did not observe an increased prevalence of disturbances in welders' cohorts.

Manganese neurotoxicity can also result from prolonged exposure to much lower levels occurring in the general population. This has been studied in a population of residents in the proximity of industries emitting manganese in the environment. In 1999, a dose-effect association was found between manganese in blood and several neurobehavioral tests examining motor and cognitive functions and mood in residents in the vicinities of a former ferroalloy plant in south Quebec. These effects were more pronounced in men and individuals older than 50 years (Mergler *et al*., 1999).

Santos-Burgoa *et al*. (2001) published a cross-sectional (pilot) study on the health effects of manganese in a group of adult subjects from a mining district in Central Mexico, with different levels of airborne manganese. By using a multivariate logistic regression analysis, the authors identified a significant relationship between deficient cognitive performance (Mini-Mental score of less than 17) and manganese blood levels. The same group has further confirmed the observation of impaired motor functions in residents in the vicinities of manganese mines, as associated to the airborne concentration of manganese that averaged 0.13 μg/m^3 as a geometric mean (Rodriguez-Agudelo *et al*., 2006).

The frequency of parkinsonian disturbances in the surroundings of ferroalloy plants in an Italian Pre-Alps valley (crude prevalence = 358/100.000 population, standardized for age and gender = 438) was significantly higher (s.m.r. = 1.58; CI = 1.41–1.76) compared with the other counties of the same province (crude prevalence, 246/100,000) (Lucchini *et al*., 2003). This observation could indicate the interaction of prolonged environmental exposure to manganese and genetic factors potentially relevant in this mountain population.

Manganese can also cause neurotoxic effects in children, who may represent a more sensitive subgroup of the general population. A cross-sectional investigation of children exposed to manganese through drinking water in Bangladesh showed a dose-response association between manganese and reduced full-scale, performance, and verbal raw scores of intellectual functions (Wasserman *et al*., 2006). The average concentration of manganese in water was 793 μg/L. In the United States, approximately 6% of domestic household wells have Mn concentrations that exceed 300 μg Mn/L, the U.S. Environmental Protection Agency lifetime health advisory level.

A study conducted to explore the potential associations between hair metal levels and the neuropsychological function and behavior of school-aged children showed children's general intelligence scores, particularly verbal IQ scores, as inversely related to manganese and arsenic levels. Significant interaction between these two metals was observed. The mean hair metal levels were 471.5 ng/g for manganese and 17.8 ng/g for arsenic (Wright *et al*., 2006).

The findings on welders and environmentally exposed populations suggest the hypothesis that manganese neurotoxicity may differ according to exposure levels. Exposure levels >1 μg/m^3 can cause the clinical picture of classical manganism, whereas at lower levels, manganese may represent an "environmental trigger" able to accelerate the physiological aging of the brain, possibly interacting with genetical factors (Le Coteur *et al*., 2002; Zatta *et al*., 2003).

6.3.1.3 Treatment of Manganism

The similarity of manganism to Parkinson's disease prompted Mena *et al*. (1970) to try the amino acids L-dopa(3,4-dihydroxyphylamine) and 5-hydroxytryptophane-precursors of dopamine and serotonin, respectively, in the treatment of chronic manganese poisoning. Treatment of Parkinson's patients with L-dopa often relieves some of the symptoms of Parkinson's disease (Bernheimer *et al*., 1973). Oral doses of up to 8 g/day of L-dopa were given to patients with manganism. Improvements were noticed in five of eight patients. One patient got worse but responded to treatment with 5-hyroxytryptophane. Barbeau (1984) also reported that oral L-dopa could temporarily improve symptoms of manganese-induced neurotoxicity. In an experimental study on rats, Shukla and Chandra (1981) found that L-dopa treatment might markedly increase the striatal dopamine level. Therefore, they advised that L-dopa therapy should not be tried in early manganese intoxication, because it may aggravate manic symptoms caused by the increase in brain dopamine.

However, most studies show that manganism patients typically do not respond to L-dopa treatment (Calne *et al*., 1994; Chu *et al*., 1995; Huang *et al*., 1989), indicating that they likely had degeneration of the receptors and neurons that normally respond to this neurochemical (Chu *et al*., 1995).

Chelating agents have been also tried in the therapy, mainly without success. Nevertheless, recent experiences have reported that chelation treatment may improve the clinical features in overexposed welders when administered in the early phases of the clinical onset (Discalzi *et al*., 2000). In a follow-up of seven patients affected by manganese-induced parkinsonism

after treatment with $CaNa_2$ EDTA, good clinical, biological, and neurological results were obtained by timely removal from exposure and chelating therapy (Herrero Hernandez *et al*., 2006).

Sodium paraaminosalicylic acid (PAS) has also been observed as effective in treating manganese-induced clinical manifestations in severe cases (Jiang *et al*., 2006).

6.4 Effect on the Lungs

6.4.1 Mode of Action

Acute or intermediate exposure to excess manganese affects the respiratory system. Inhalation exposure to high concentrations of manganese dusts (manganese dioxide and manganese tetroxide) can cause an inflammatory response in the lung (chemical pneumonitis). But exposure to manganese in nonsoluble form, such as MnO_2, may also be harmful for the lung at low exposure levels. As quoted in the WHO Regional Office for Europe document (2001), an increase in pneumonia incidence has been registered in rats exposed to 43–139 µg manganese/m^3 as MnO_2 (mean MMAD = 0.76 µg; mean standard geometric deviation (Sg) = 2.28) for 2 weeks (Shiotsuka 1984), pulmonary congestion in monkeys exposed to 0.7 or 3.0 manganese/m^3 as MnO_2 (80% < 1 µm) for 5 months (Nishiyama *et al*. 1975), pulmonary emphysema in monkeys exposed to 0.7–3.0 mg manganese/m^3 as MnO_2 (80% < 1 µm) for 10 months (Suzuki *et al*. 1978), bronchiolar lesions in rats and hamsters exposed to 0.117 mg manganese/m^3 as Mn_3O_4 (0.29 µm) for 56 days (Moore *et al*. 1975). Nevertheless, no significant pulmonary effects were detected in some other studies of rats and monkeys exposed to as much as 1.15 mg manganese/m^3 as Mn_3O_4 for 9 months (Urlich *et al*., 1979a,b) and rabbits exposed to as much as 3.9 mg manganese/m^3 as $MnCl_2$ for 4–6 weeks (Camner *et al*., 1985).

Laboratory animal studies have also shown that inhaled manganese may increase susceptibility to infectious agents such as *Streptococcus pyogenes* in mice (Adkins *et al*., 1980), *Enterobacter cloacae* in guinea pigs (Bergström, 1977), *Klebssiella pneumonia* in mice (Maigetter *et al*., 1976), and *Streptococcus haemolyticus* in mice (Lloyd Davies, 1946). Sufficient evidence indicates that exposure to manganese has cytotoxic effects, including a depressive effect on the number and phagocytic capacity of alveolar macrophages (Fisher and Škreb, 1980; Graham *et al*., 1975; Shanker *et al*., 1976; Škreb *et al*., 1980; Waters *et al*., 1975). Thus, toxicological findings in experimental animals and *in vitro* studies have supported human studies and corroborated the assumption about the mechanism of manganese action on the lungs. However, it has to be taken into consideration that an inflammatory response is not unique to manganese-containing particles but is characteristic of nearly all inhalable particulate matter (EPA, 1984).

6.4.2 Human Studies of Lung Impairment

An increased morbidity and mortality rate from pneumonia has been found among workers exposed to manganese dusts (Baader, 1937; Dervillée *et al*., 1966; Heine, 1943; Lloyd Davies, 1946; Lloyd Davies and Harding, 1949; Rodier, 1955; Van Beukering, 1966; Wassermann and Mihail, 1961). The association between exposure to manganese and a higher rate of pneumonia was first suspected by Brezina (1921), who reported that 5 of 10 workers in an Italian pyrolusite mill died of croupous pneumonia within 27 months.

Clinically, manganese pneumonia has been characterized as an acute alveolar inflammation with marked dyspnea. A typical finding was that antibiotics were often without effect (Rodier, 1955). Manganese concentrations in air had not been reported in most of the earlier studies, but Rodier (1955) found that concentrations reached 800 mg/m^3 or more in some parts of a mine. An increased incidence of pneumonia, as well as bronchitis, was found in workers exposed to manganese concentrations of 0.39–16.35 mg/m^3 in a factory producing manganese alloys (Šarić *et al*., 1977). In this study, smoking habits were taken into account.

However, in a number of published articles describing manganism in exposed workers, there was no report of lung disease (Flinn *et al*., 1940; Schuler *et al*., 1957; Smith *et al*., 1973; Suzuki *et al*., 1960). Some of the quoted reports had quantitative environmental data, and examinations or records were such that one would predict that the presence of excess pulmonary disease would have been detected.

Studies on populations living in the vicinity of manganese-emitting plants have also indicated that manganese exposure might affect the respiratory system. Elstad (1939) noted an eightfold increase in the mortality from pneumonia and a fourfold increase in pneumonia morbidity rate in the general population living near a ferromanganese plant in Norway. Production had started in 1923, but in 1931, air concentrations of manganese were only measured once and reported to be 46 µg/m^3 3 km from the plant. The time of sampling was not stated. It was also reported that the incidence of pneumonia followed the production of manganese alloys in the plant. Analysis of lung tissues from 11 persons who died from pneumonia showed manganese concentrations of 0.35–1.63 µg/kg wet weight.

In a study by Nogawa *et al*. (1973), it was found that children living in the vicinity of a ferromanganese plant in Japan and attending a school situated only 100 m away from the factory showed a higher prevalence of nose and throat symptoms than children in a school 5 km away. Manganese concentrations were reported in a 5-day study to average 6.7 μg/m^3 at 300 m from the factory. On another occasion, a maximum value of 260 μg/m^3 was recorded (time not stated). After installation of a dust collector, the concentration at the same site was 3.7 g/m^3 (Kagamimori *et al*., 1973). Objective tests of lung function, such as forced expiratory volumes and maximum expiratory flow, showed lower values among exposed children than controls. Tests were repeated after reduction of manganese emission. The frequency of effects had been shown to decrease when concentrations of manganese in dust declined.

The effects at still lower levels of airborne manganese have been claimed (Šarić *et al*., 1978) to occur in a population living near a ferromanganese plant (Šibenik, Croatia). Over a period of 4 years, acute bronchitis and peribronchitis were consistently more common among people living near the plant. A less consistent pattern for pneumonia could be seen. Average annual manganese concentrations were in two zones of the town area near the plant 0.236–0.39 and 0.164–0.243 μg/m^3, whereas in a third zone at a distance of 4–5 km from the plant, the annual means for 4 years varied from 0.042–0.099 μg/m^3. The highest weekly means for the zones were 1.24, 1.08, and 0.27 μg/m^3, respectively. In addition, in a study of school children carried out in the same area and repeated 1 year later, it was shown that, during the study period of 6 months, the recorded incidence of acute respiratory diseases, as well as the values of forced expiratory volumes, correlated in both studies with the levels of manganese measured at the locations of the schools attended by the studied children (two schools were in zones with higher manganese in air levels, and one in a zone with levels of manganese approximately 10 times lower). Acute bronchitis was significantly more frequent, and forced expiratory volumes were lower, in children from the schools with higher manganese levels (Hrustic and Šarić, 1980).

Bergström (1977) summarized data from epidemiological and experimental studies concerning the effect of manganese on the lung. He supported the concept that a primary inflammatory reaction can occur in the lung after exposure to MnO_2 without the presence of pathogenic bacteria. In his opinion, such a reaction pattern also explains the ineffectiveness of the antibiotics commonly used for treatment of pneumonia caused by manganese. On the other hand, experimental studies suggest that exposure to MnO_2 causes a decrease in the resistance toward respiratory infections. Consequently, some of the observed cases of manganese-induced pneumonia might have had a bacterial genesis.

It has been suggested (Šarić and Lucic-Palaic, 1977) that long-term exposure to manganese combined with smoking habit may contribute to the development of chronic nonspecific lung disease.

In a recent longitudinal follow-up study on pulmonary function and respiratory symptoms in 145 miners exposed to manganese and 65 matched controls (Mashhadi Abar Boojar and Goodarzi, 2002), the obtained results indicated a synergistic effect of manganese and smoking in the development of chronic respiratory impairment.

6.5 Effects on Other Organs and Systems

6.5.1 Reproductive Effects

Impotence and loss of libido are common complaints in workers occupationally exposed to manganese who have been afflicted with clinical signs of manganism (Emara *et al*., 1971; Mena *et al*., 1967; Penalver, 1955; Rodier, 1955; Schuler, 1957). Impaired fertility (measured as a decreased number of children/married couple) has been observed in male workers exposed for 1–19 years at levels that did not produce frank manganism (Lauwerys *et al*., 1985). On the other hand, in a study performed by Jiang *et al*. (1996a) on 314 men in a manganese plant exposed up to 35 years to manganese at levels of 0.145 mg/m^3 as MnO_2 (geometric mean), no significant differences were found in reproductive outcomes between exposed and control workers. But impotence and lack of sexual desire were higher in manganese-exposed workers. Gennart *et al*. (1992), in a reproductive study on 70 male workers in a dry alkaline battery plant exposed to manganese dioxide at a median concentration of 0.71 mg/m^3 in total dust for an average of 6.2 year, did not register any difference compared with the controls. Wu *et al*. (1996), in a study carried out in three groups of workers occupationally exposed to manganese (63 miners or ore processing workers, 38 electric welders in mechanical fields, and 110 electric welders in shipbuilding) found increased semen liquefaction time and decreased sperm count and viability. Although this study indicates that manganese exposure can cause sperm toxicity, the presence of other metals in welders, copper, nickel, chromium and iron, which were also elevated in semen, had to be taken into consideration as well. In animal experiments (on rats) performed by Chandra (1971), and on rats and rabbits (Chandra *et al*., 1973), it was shown that an excessive manganese exposure might produce marked degenerative changes in the seminiferous tubules. This

effect did not occur immediately but developed slowly over the course of 4–8 months after the exposure.

Direct damage to the testes has not been reported in humans occupationally exposed for longer periods.

Reported data reveal conflicting evidence for whether occupational exposure to manganese causes adverse reproductive effects. Noted effects may occur as a secondary result of neurotoxicity to manganese (ATSDR, 2000).

6.5.2 Cardiovascular Effects

During an epidemiological study performed in ferroalloy workers, a decrease in systolic blood pressure was found (Šarić and Hrustic, 1975). Arterial blood pressure was measured and compared in three groups of male workers aged 20–59 years at different exposure levels to the airborne manganese. The lowest mean values of the systolic blood pressure were found in workers with the highest occupational exposure (0.39–20.44 mg/m^3), although this group was comparatively the oldest. The lowest mean diastolic pressure values were found in control workers. Age, body bulk, and smoking habits, as factors, which might have an impact on the obtained results, were considered. Jiang *et al*. (1996b) studied the potential cardiotoxicity of MnO_2 exposure in 656 workers (547 males and 109 females) engaged in manganese milling, smelting, and sintering. The geometric mean of manganese in air was 0.13 mg/m^3. Length of exposure varied from 0–35 years. There was no increase of abnormal electrocardiograms between manganese workers and their matched controls. Arterial blood pressure values showed a greater frequency of low diastolic pressure, but this effect was highest in young workers with the lowest tenure in the plant.

A manganese-induced decrease of blood pressure was also noted in animal experiments by Schroeder and Perry (1955) and Kostial *et al*. (1974).

In 1974, Kamiyama and Sacki carried out a study on myocardial action potentials in the canine ventricle and effects of manganese ions. The results showed that Mn^{2+} decreased the contractile tension of the muscle. In 1978, Kimura *et al*. reported that dietary exposure to 564 mg/kg of manganese in rats produced a significant increase in the serotonin concentrations and a decrease in blood pressure. The authors attributed the final marked decrease of blood pressure to the elevated blood serotonin concentrations, most probably released from different tissues.

There are three case reports concerning possible cardiac effects after acute exposure to fungicide maneb without taking protective measures. In two cases (De Carvalho *et al*., 1989; Israeli *et al*., 1983) no adverse cardiac effects were observed. In a case report by Koizumi *et al*. (1979), ECG analysis of heart function suggested myocardial ischemia. The affected man underwent hemodialysis after developing renal failure from exposure to the fungicide. ECG analysis made 2 months later showed no abnormalities.

6.5.3 Hematological Effects

In subjects chronically exposed to high levels of manganese in the workplace, as a rule, no significant hematological effects had been observed. In a study by Flinn *et al*. (1941), a low white cell count was found in a number of workers affected by manganese. Paterni (1954) claimed that small amounts of manganese had a stimulatory effect on erythropoiesis. Similar findings were also reported by Kesic and Häusler (1954). From some findings referring to chronic manganese poisoning, it was presumed that large amounts of manganese caused depression of both erythropoiesis and granulocyte formation (Cotzias, 1958; Rodier, 1955). Yiin *et al*. (1996) observed increased erythrocyte superdioxide dismutase and plasma malondialdehyde in men who worked in manganese smelters.

6.5.4 Endocrine Effects

In a study by Alessio *et al*. (1989) performed in foundry workers exposed to manganese levels of 0.04–1.1 mg/m^3 (particulate matter) and 0.5–0.9 mg/m^3 as fumes for approximately 10 years, elevated prolactin (PRL) levels and cortisol levels were reported. No changes in the levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were noted. Smargiassi and Mutti (1999) also reported an increase of serum prolactin levels in a group of workers from a ferroalloy plant. Measurements were made twice in the same workers (in 1992 and 1997). Serum PRL levels that were significantly elevated in the earlier analysis (1992) had also increased significantly over the earlier measurement in 1997. During the 5-year period between studies, exposure levels were consistent.

6.5.5 Immunological Effects

In a study performed in male welders exposed to manganese (0.29-0.64 mg/m^3) for an unspecified duration, suppression of T and B lymphocytes characterized by reductions in serum immunoglobulin G (IgG) and total E-rosette–forming cells was observed (Boshnakova *et al*., 1989). However, the welders in this study were exposed to other compounds, including cobalt, carbon dioxide, and nitric acid, as well as to noise and vibration. Also, it is not known whether any of

the registered changes are associated with significant impairment of immunological system function.

6.5.6 Genotoxic and Carcinogenic Effects

Relatively high doses of manganese affect DNA replication and repair in bacteria and cause mutations in microorganism and mammalian cells, although the Ames test does not seem to be particularly responsive to manganese. In mammalian cells, manganese causes DNA damage and chromosome aberrations (Gerber *et al*., 2002). Large amounts of manganese affect fertility in mammals and are toxic to the embryo and fetus (Colomina *et al*., 1996). The fungicide maneb and the contrasting agent MnDPDP also can be embryotoxic, but MnDPDP only at doses much higher than those clinically used (Maci and Arias, 1987).

There is only one study in humans regarding genotoxic effects of exposure to manganese. In this study, an increased incidence of chromosomal aberrations was registered in three groups of welders with occupational exposures lasting 10–26 years (Elias *et al*., 1989). Because workers, in addition to manganese with median concentrations of 0.18 mg/m^3 for respirable dust and 0.71 mg/m^3 for total dust, were also exposed to nickel and chromium, the observed increase in chromosomal aberrations could not be attributed to any one metal exposure (nickel is known to cause chromosomal aberrations by inhalation route).

In a study on the genotoxic effects of occupational exposure to mancozeb (in 14 women and 30 men, all of reproductive age), Jablonická *et al*. (1989) showed a significantly increased incidence of chromatid breaks in exposed women compared with controls and the combined exposed cohort compared with the combined control group; isochromatid breaks were significantly increased in exposed men and the combined group compared with their respective controls; gaps were significantly increased in both exposed groups. Chromatid exchanges (SCEs) were not significantly increased in any group. Mancozeb-exposed smokers had a significantly increased incidence of SCEs compared with exposed nonsmokers.

Information on cancer caused by manganese is scanty, but the available data do not indicate that inorganic manganese is carcinogenic. The US EPA (1988) has concluded that manganese is not classifiable as a human carcinogen. IARC has not evaluated manganese (Boffetta, 1993).

7 GUIDELINES/REGULATIONS

When describing the levels of exposure to manganese in different environmental medias, some established guidelines and regulations have been already mentioned.

In a WHO Regional Office for Europe document from 2000, a guideline value for manganese of 0.15 µg/m^3 air was declared. On the basis of neurotoxic effects observed in occupationally exposed workers and using the benchmark approach, an estimated NOAEL (the lower 95% confidence limit of the $BMDL_5$) of 30 µg/m^3 was obtained. A guideline value was derived by dividing by a factor of 4.2 to adjust for continuous exposure and an uncertainty factor of 50 (40 for inter-individual variation and 5 for developmental effects in younger children, supported by evidence from studies of experimental animals). As pointed out in the explanation, the adjustment for continuous exposure was considered sufficient to account for long-term exposure based on knowledge of the half-time of manganese in the brain. The guideline value should be applied as an annual average.

The upper range of the estimated safe and adequate daily dietary intake of 5 mg/day (NCR, 1989) has been adapted as a provisional guidance value (0.07 mg/kg/day) for oral exposure to manganese. The US EPA has derived a chronic oral reference dose (RfD) of 0.14 mg/kg/day for manganese. This value corresponds to the average daily intake of manganese in the diet (10 mg/day) that is considered adequate and safe. The RfD was derived assuming an average body weight of 70 kg and was based on a composite of data provided by WHO and NRC. An uncertainty factor was not used for reasons that were listed as follows: the information used to determine the RfD was taken from many large populations; humans exert an efficient homeostatic control over manganese such that body burdens are kept constant with variations in diet; there are no subpopulations that are believed to be more sensitive to manganese at this level; manganese is an essential element that is required for normal human growth and maintenance of health (ATSDR, 2000). However, the EPA has recommended that a "modifying factor" of 3 be applied when assessing risk from manganese in drinking water or soil, because the study by Kondakis *et al*. (1989) raises significant concerns about possible adverse neurological effects at doses not far from the range of essentiality (ATSDR, 2000).

Manganese is regulated by a 0.05 mg/L secondary maximum contaminant level (MCL), a standard established to address aesthetic issues (discoloration) of drinking water, not health concerns. As stated earlier, the WHO health-based guideline value for drinking water is 0.4 mg/L. Considering the potential health concerns of high levels of manganese in drinking water as appropriate, given the possibility of neurological effects, a health-based action level for manganese in addressing high manganese levels in drinking water sources has been introduced. DHSs' drinking water

action level for manganese is 0.5 mg/L. With the establishment of the 0.5 mg/L action level, DHS recommends follow-up monitoring for those water supply systems that historically have shown manganese higher than the 0.5 mg/L concentration, but that lack recent data (USDHS, 2003).

In 1996, the WHO recommended 0.3 mg/m^3 as the exposure limit for respirable manganese particles in workplace air. Threshold limit value as time-weighted average (TLV-TWA) set by American Conference of Governmental Industrial Hygienists (ACGIH) in 1998 for manganese tetroxide compound and manganese fume was 1 mg/m^3. Currently, ACGIH for manganese is 0.2 mg/m^3 TWA (TLV listed as manganese and inorganic compounds, as Mn). The Occupational Safety and Health Administration (OSHA) in 1998 proposed 0.2 mg/m^3 as PEL TWA for manganese, elemental and inorganic compounds. Earlier OSHA's permissible exposure limit value of 5 mg/m^3 is still valid for general and construction industries and maritime, but as ceiling value. Manganese cyclopentadienyl tricarbonyl (MMT) as manganese (skin exposure) set by OSHA in 1998 was 0.1 mg/m^3. The ACGIH value for MMT set in 1998 was 0.2 mg/m^3, as manganese and, also 0.1 mg/m^3 (skin).

8 MANGANESE CONCENTRATIONS IN BIOLOGICAL MEDIA AND BIOMARKERS OF EXPOSURE AND EFFECTS

Manganese can be measured with good sensitivity in biological fluids and tissues, and levels in blood, urine, feces, and hair have been investigated as possible biomarkers of exposure.

The reported normal concentrations of manganese in blood, serum, and urine show a wide range. Data by Muzzarelli and Rocketti (1975), Buchet *et al*. (1976), Tsalev *et al*. (1977), and Zielhuis *et al*. (1978), determined by electrothermal atomic absorption, indicate that the average normal level in whole blood is 7–12 µg/L. These data agreed with data by Olehy *et al*. (1966), who used neutron activation and found concentrations of 16 µg/L and 4.3 µg/L in erythrocytes and plasma, respectively. Assuming a hematocrit value of 45%, this would correspond to 9.5 µg/L in whole blood. Cotzias *et al*. (1966) found by neutron activation a mean normal level of 8.4 µg/L. Approximately 85% of blood manganese is in the erythrocytes. In serum and plasma, average manganese concentrations are reported to be 0.6–4.3 µg/L as determined by neutron activation analysis (Cotzias *et al*., 1966; Hagenfeldt *et al*., 1973; Olehy *et al*., 1966) and 0.6–1.0 µg/L as determined by electrothermal atomic absorption (Halls and Fell, 1981). Greger *et al*. (1990) and Davis and Greger (1992) reported serum manganese concentrations in a group of healthy men and women in Wisconsin of 1.06 µg/L and 0.86 µg/L, respectively. In healthy, unexposed people living in the Lombardi region in northern Italy, manganese in blood was 8.8 ± 0.2 µg/L and in serum 0.6 ± 0.014 µg/L (Minoia *et al*., 1990). In woman, a hormonal influence may cause variations in plasma manganese (Hagenfeldt *et al*., 1973).

The concentration of manganese in blood and serum seems to be fairly stable over long periods of time (Cotzias *et al*., 1966; Mahoney *et al*., 1969). A slight variation of blood manganese concentrations was observed with somewhat lower levels during summer and autumn months (Horiushi *et al*., 1967). Sullivan *et al*. (1979) found elevated manganese serum levels in patients with congestive heart failure, infection, and psychoses. Elevated manganese levels in blood to approximately twice-normal values were found after acute coronary inclusion (Newberne, 1973). Elevated manganese concentrations in red cells but not in the serum occur in patients with rheumatoid arthritis (Cotzias *et al*., 1968).

The reported normal concentrations of manganese in urine vary to a large extent. Data presented by Buchet *et al*. (1976), Halls and Fell (1981), and Minoia *et al*. (1990) indicate that normally less than 1 µg/L is found.

As a group, workers exposed to a mean concentration of 1 mg manganese/m^3 had higher levels of manganese in the blood and urine than unexposed controls (Roels *et al*., 1987). The group average levels in blood seem to be related to manganese body burden, whereas average urinary excretion levels are judged to be most indicative of recent exposures. However, the individual measurements do not correspond to individual exposure.

There is only one study by Lucchini *et al*. (1995) suggesting that blood and urine levels were correlated with exposure levels on an individual basis. In this study, a correlation was observed between a cumulative exposure index (CEI) and manganese in blood (MnB), and both MnB and CEI were associated with neurobehavioral outcomes. Workers had been tested during a month of layoff, and all the correlations increased proportionally to the latency from the exposure cessation. Mergler and Baldwin (1997) suggested that in this study the correlation between CEI and MnB and the dose-effect relationships were evident because MnB levels were not influenced by current exposure and, therefore, they were better related to the manganese levels accumulated in the body storage deposits. This unique situation has not been replied in the literature.

In a follow-up study on currently exposed workers, Lucchini and coauthors (1999) did not observe any correlation between CEI and MnB, although CEI was still associated with the impairment of neurobehavioral functions.

Other studies by Järvisalo *et al*. (1992), Roels *et al*. (1987; 1992), and Smyth *et al*. (1973) on currently exposed workers have indicated that, on an individual basis, the correlation between the level of workplace exposure and the levels of manganese in blood and urine is not a reliable predictor of exposure. Järvisalo *et al*., as well as Roels *et al*., suggested that blood and urinary manganese levels may be useful to monitor group exposure. A factor that limits the usefulness of measuring manganese in blood and urine as a measure of excess manganese exposure is the relatively rapid rate of manganese clearance from the body with very little excretion in urine.

There is not a significant correlation between fecal excretion of manganese and occupational exposure to the metal (Valentin and Schiele, 1983).

In a Canadian study by Baldwin *et al*. (1999) on the relationship between the levels of manganese in air and blood manganese, significantly higher levels of blood manganese were correlated with high levels of airborne manganese. Data obtained in this study combined with the occupational studies indicated that there might be a plateau level of homeostatic control of manganese. At low levels, blood manganese concentrations would be related to food, water, and air levels of manganese, and large differences in individual blood levels would be observed. At high exposure levels, such as in occupational environments, this plateau may be reached or exceeded.

Lymphocyte manganese-dependent superoxide dismutase activity increases with increased manganese uptake (Yiin *et al*., 1996). As suggested by the results of some studies (Davis and Greger, 1992; Greger, 1999), the analysis of this enzyme, in conjunction with serum manganese levels, may be used in assessing low and moderate levels of exposure to manganese.

Magnetic resonance imaging (MRI) can be useful to track manganese distribution in the brain. Brain MRI scans may be useful in assessing excessive manganese exposure even among industrial workers exposed to airborne manganese (Nelson *et al*., 1993). These scans also have been successfully used to identify accumulation of manganese in children exposed to excess manganese (Devenyi *et al*., 1994; Fell *et al*., 1996; Ono *et al*., 1995).

The use of hair as a possible biomarker of manganese exposure is problematic. In hair, manganese concentrations are reported to be normally <4 mg/kg (Eads and Lambdin, 1973). Although some studies have found a correlation between exposure levels and manganese concentration in hair (Collip *et al*., 1983), in most studies performed, different limits concerning the validity of the scalp hair analysis as a biomarker of exposure have been pointed out (Lyden *et al*., 1984; Stauber *et al*., 1987; Sturaro *et al*., 1994).

Ethylene thiourea is readily detected in the urine of humans (and animals) exposed to maneb and mancozeb, but there is not a clear relationship between dose and urinary levels (Kurttio and Savolainen, 1990; Kurttio *et al*., 1990).

Clara cell protein CC16 is a potential biomarker for pulmonary effects from exposure to MMT (Halatek *et al*., 1998). Clara cells are unciliated cells occurring at the boundary where alveolar ducts branch from the bronchioles. Damage of CC16 causes a significant change in the levels of this protein. The protein can be quantified in serum and urine. Until now the protein has only been studied experimentally after intraperitoneal administration of MMT.

No biochemical indicator is currently available for the detection of the early neurotoxic effects of manganese.

Serum PRL has been shown to be a possible biomarker of manganese effects on dopamine neurotransmission. Namely, tuberoinfundibular dopaminergic system that exerts tonic inhibition of PRL secretion is affected by manganese action (Smargiassi and Mutti, 1999). Still, it is not clear whether PRL levels indicate recent or cumulative exposure. It has to be mentioned, also, that in a study by Roels *et al*. (1992) and more recently in a large South African study on ferroalloy workers (Myers *et al*., 2003b), serum PRL levels were not increased in workers chronically exposed to airborne manganese.

Position emission tomography (PET) with ^{18}F-dopa or single-photon emission tomography (SPET) afford a differentiation between manganism and parkinsonism, which is marked by neurodegradation in the dopaminergic nigrostriatal pathway, whereas manganism-induced damage occurs postsynaptic to the nigrostriatal system. Further work with the PET scan is needed to clarify whether manganese damage can also extend to the nigrostriatal pathway as suggested by the most recent findings (Racette *et al*., 2005a). Neuroimaging research should also aim at developing specific and sensitive parameters for manganism in Mn-exposed individuals (Kim, 2006).

Brain MRI scans with a battery of specific neurobehavioral tests may also be useful in assessing adverse effects of manganese (Greger, 1998).

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CHAPTER

33

Mercury

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ABSTRACT

Mercury occurs as elemental mercury and as inorganic and organic compounds (mercury vapor, mercury liquid, mercury salts, short-chain alkylmercury compounds, alkoxyalkylmercury compounds, and phenyl mercury compounds), all having different toxicological properties. Total mercury can be analyzed in water, air, and biological material by cold vapor atomic absorption methods and by neutron activation analysis and can be detected down to concentrations of a tenth of a nanogram per gram in biological material. Methylmercury can be detected in biological material at levels of a few nanograms by extraction with benzene after strong acidification with hydrochloric acid, followed by gas chromatographic analysis of methylmercury chloride. More recently, analytical methods for speciating inorganic mercury and several of the organo mercurial forms have been published. These methods include isotope dilution mass spectrometry, time of flight mass spectrometry, HPLC-ICP-MS, capillary electrophoresis-ICP, gas chromatography-ICP, and X-ray absorption fine structure spectroscopy.

Mercury is circulated naturally in the biosphere, with 30,000–50,000 tons being released into the atmosphere by degassing from the earth's crust and the oceans. In addition, 20,000 tons of mercury is released into the environment each year by human activities, such as combustion of fossil fuels and other industrial releases. Yearly, approximately 2000 tons of mercury is produced for industrial use, a small part of which is used for synthesizing organic mercury compounds. The world production of mercury for commercial uses has been slowly declining over the past 20 years.

In nature, methylmercury is produced from inorganic mercury as a consequence of microbial activity. In mammals, oxidative demethylation occurs *in vivo* to produce inorganic mercury. In fish, the major amount of mercury is methylmercury. Factors determining the methylmercury concentration in fish are mercury content in water and bottom sediments, pH, and redox potential of water, species, age, and size of the fish.

The toxic properties of mercury vapor are due to mercury accumulation in the brain causing neurological signs, involving an unspecific psychoasthenic and vegetative syndrome (micromercurialism). At high exposure levels, mercurial tremor is seen accompanied by severe behavioral and personality changes, increased excitability, loss of memory, and insomnia. Mercurials may also affect other organ systems, such as the kidney. On a group basis, exposure levels are likely to be reflected in mercury concentrations in blood and urine. Occupational exposure to mercury concentrations in air >0.1 mg/m^3 may produce mercurialism. Micromercurialism has not been reported at concentrations <0.01 mg/m^3. Exposure to mercury vapor inhibits fetal brain development in primates. The exact dose-response relationship in humans is not known. Inorganic but not methylmercury has been found to induce and bind to the low molecular weight metal-binding protein, metallothionein.

The acute and long-term actions of mercuric salts, phenylmercury compounds, and alkoxyalkylmercury compounds are likely to be gastrointestinal disturbance

and renal damage appearing as a tubular dysfunction with tubular necrosis in severe cases. The lethal dose in man is approximately 1 g of mercuric salt. The mercury load on the kidney is best determined by analysis of renal biopsy. Mercury concentrations in the kidney between 10 and 70 mg/kg have been reported in poison cases with renal injury. Levels <3 mg/kg may be found in normal cases. Occasionally, mercuric compounds may cause idiosyncratic skin symptoms, which may develop into severe exfoliative dermatitis or may cause glomerular nephritis. Animal and clinical observations have shown that mercuric mercury stimulates and methylmercury inhibits the immune system A specific form of idiosyncrasy, called acrodynia or pink disease, is seen in children. Most cases are associated with mercury exposure where increased levels of mercury in urine are observed.

The hazards involved in long-term intake of food containing methylmercury and in occupational exposure to methylmercury are due to the efficient absorption (90%) of methylmercury in man and the long retention time (half-life of 70 days) with an accumulation of methylmercury in the brain. Chronic poisoning results in degeneration and atrophy of the sensory cerebral cortex, paresthesia, ataxia, hearing, and visual impairment. Prenatal exposure causes cerebral palsy and, in less severe cases, psychomotor retardation. Methylmercury concentration in blood and hair reflects the body burden and the concentration in brain of methylmercury. Intake resulting in body burdens of <0.5 mg/kg body weight is not likely to give rise to detectable neurological signs in adults. This intake corresponds to blood values of <200 μg/L and mercury levels in hair of <50 mg/kg. However, this level of methylmercury exposure in pregnant women may result in inhibited brain development of the fetus with psychomotor retardation of the child. The highest level of methylmercury load in pregnant women, not associated with inhibition of fetal brain development, is not known.

The history of mercury has been reviewed by Goldwater (1972). The pharmacology and toxicology of mercury has been previously reviewed by Clarkson *et al*. (1972), the chemistry of mercury in biological systems by Carty and Malone (1979), the toxicology of methylmercury by a Swedish Expert Group (1971), and the toxicology and epidemiology by Friberg and Vostal (1972), by the Task Group on Metal Accumulation (1973), by the Task Group on Metal Toxicity (1976), and by the WHO (1976; 1980; 1990; 1991). More recently, the toxicology of mercury has been reviewed in the USEPA Report to Congress (1998), ATSDR (1999), NAS/NRC (2000), and IOM Report on Mercury in Vaccines (2004).

1 INTRODUCTION

Mercury (Hg) is a metal that is in liquid state at room temperature. In addition to its metallic state, mercury occurs in compounds as monovalent mercurous mercury and divalent mercuric mercury. Mercury also exists in nature as organometallic compounds in which mercury is covalently bound to carbon, in compounds of the type RHg+ and RHgR′, where R and R′ represent the organic moiety. The carbon–mercury bond is chemically stable because of the low affinity of mercury for oxygen.

The affinity of mercury for sulfur and sulfhydryl groups is a major factor underlying the biochemical properties of mercury and mercury compounds. The mercury-containing moiety binds to sulfhydryl groups of proteins in membranes and enzymes, thereby interfering with membrane structure and function and with enzyme activity. The toxicological properties of the different forms of mercury (Hg) in mammalian organisms are largely a function of the complex binding interactions that occur between mercuric ions and various protein and nonprotein thiols in the extracellular and intracellular compartments of target cells. Because of the predilection of mercurous or mercuric ions binding to sulfhydryl groups, as well as other nucleophiles, there is an extremely low probability of these ions existing in an unbound, "free" ionic state in extracellular and intracellular compartments of the body (Hughes, 1957). Instead, ionic species of Hg have a particular propensity to bind to the sulfhydryl groups on glutathione (GSH), cysteine (Cys), homocysteine (Hcy), *N*-acetylcysteine (NAC), metallothionein (MT), and albumin. In the presence of an excess of low molecular weight, thiol-containing molecules, mercuric ions will bind to these molecules in a linear I or II coordinate covalent manner, depending on the species of mercuric ion (Canty *et al*., 1994; Fuhr and Rabenstein, 1973; Rubino *et al*., 2004). Mercuric–thiol conjugates formed in an aqueous environment have been shown to be thermodynamically stable at pH 1–14 (Fuhr and Rabenstein, 1973). It should also be stressed that the formation constant for the thiol–Hg bond is as much as 10 orders of magnitude greater than the constant for the bonds formed between mercuric ions and other nucleophiles present in the same environment (Zalups, 2000a).

All forms of Hg induce toxic effects in mammalian species, including humans. The extent of the adverse effects induced by Hg depends on the form of Hg at the time of exposure, the duration of exposure, and the route of exposure. Toxic effects of Hg have been demonstrated in the cardiovascular system (Carmignani *et al*., 1992; Soni *et al*., 1992; Wakita, 1987; Warkany

and Hubbard, 1953), gastrointestinal system (Afonso and deAlvarez, 1960; Bluhm *et al*., 1992; Lundgren and Swensson, 1949; Murphy *et al*., 1979), liver (Jaffe *et al*., 1983; Murphy *et al*., 1979; Samuels *et al*., 1982), kidneys (Murphy *et al*., 1979; Rowens, *et al*., 1991; Samuels *et al*., 1982; Yasutake *et al*., 1991), and nervous system (Jaffe *et al*., 1983; Lin and Lim, 1993; Tsubaki and Takahashi, 1986). More recent studies have described a variety of effects on cells of the immune system including apoptosis (Shenker *et al*., 1998; 2000; Ziemba *et al*., 2005), alterations in signal transduction pathways (Ziemba *et al*., 2006), and autoimmunity responses (Abedi-Valugerdi *et al*., 2005; Haggqvist *et al*., 2005; Havarinasab and Hultman, 2006; Havarinasab *et al*., 2004; Martinsson and Hultman, 2006; Roether *et al*., 2002; Zheng *et al*., 2005). For a more detailed discussion of mercury and the immune system, see Chapter 11.

From a toxicological point of view, mercury compounds should be divided into inorganic compounds and organic compounds. Among the inorganic compounds, elemental mercury and the divalent mercury salt are the compounds of toxicological interest. It is doubtful whether mercurous mercury survives in the organism. However, it is possible that mercurous mercury is an intermediate step in the redox transformation of elemental and mercuric mercury or vice versa in the body.

The organic mercury compounds can be divided into mercurials, which are relatively stable, such as methylmercury, and those that rapidly split in the mammalian body. The pharmaceutically used short-chain alkylmercury compounds, such as ethylmercury, undergo dealkylation more rapidly than methylmercury *in vivo* to release inorganic mercury. Dimethylmercury is transformed to methylmercury when absorbed. Mercurials, previously used as diuretics, are mainly excreted conjugated or unchanged by the kidneys.

Phenylmercury compounds and methoxyalkylmercury compounds are among the organic compounds that are cleaved rapidly in the body and are of toxicological importance. Both are used extensively in pesticides and preservatives. In the following discussion, the toxicology of each chemical form mentioned is treated separately.

2 PHYSICAL AND CHEMICAL PROPERTIES

Mercury (Hg): atomic weight, 200.6; atomic number, 80; density, 13.6; melting point, –38.9°C; boiling point, 356.6°C; crystalline form silver-white metallic liquid; oxidation states +1, +2.

Metallic mercury is rather volatile. A saturated atmosphere of mercury vapor contains approximately 18 mg Hg/m^3 at 24°C. Mercury vapor is regarded as insoluble in water. However, at room temperature its solubility is approximately 60 mg Hg/L. Its solubility in lipids is on the order of 5–50 mg/L. In the presence of oxygen, metallic mercury is rapidly oxidized to ionic form. Mercuric salts, like halides, sulfates, and nitrates, are water soluble. In aqueous solutions, an equilibrium is established between Hg^0, Hg^{+1} (mercurous), and Hg^{2+} (mercuric). The representation of the three oxidation stages is determined by the redox potential of the solution and the presence of compounds that can form complexes with the mercuric ions. Mercuric ion Hg^{2+} is able to form many stable complexes with biologically important molecules or moieties, such as sulfhydryl groups. Mercuric mercury forms four different complexes with the chloride anion in water, $HgCl^+$, $HgCl_2$, $HgCl^{-3}$, and $HgCl_4^{-2}$. Mercurous mercury is rather unstable in the presence of biological molecules. In the presence of sulfhydryl groups, it undergoes disproportionation to one atom of metallic mercury and to one ion of mercuric mercury.

The short-chain alkylmercuric compounds form salts with the halogens, which are highly volatile at room temperature, resulting in highly poisonous air concentrations when saturation occurs. The saturation concentration of methylmercury chloride at 20°C is 90,000 mg Hg/m^3 (Swensson and Ulfvarson, 1963). Other salts, such as hydroxide and nitrate of methylmercury, are less volatile and thus less hazardous. Methylmercury dicyandiamide has been used in commercial pesticides because of its rather low vapor pressure. Methylmercury and ethylmercury chloride are highly soluble in solvents and lipids. Sulfhydryl groups on proteins have a high affinity for the methylmercuric group. Therefore, methylmercury occurs in the organism bound mainly to sulfhydryl groups of large molecules.

Phenylmercury salts and methoxyalkylmercury compounds decompose readily and release mercury vapor.

3 METHODS AND PROBLEMS OF ANALYSIS

Mercury analysis is complicated, difficult, and requires considerable skill and experience, because concentrations found in the environment and in biological material are often low (1–200 µg/kg). Colorimetric, cold vapor atomic absorption (CVAA), neutron activation analysis (NAA), and various types of plasma atomic emission spectrometry (AES) are the principal analytical methods that are used. More recently, other

methods such as capillary electrophoresis and ICP-MS have been applied for speciation of mercury compounds.

The colorimetric method is based on the conversion of mercury in the sample to a dithizone complex that is extracted with organic solvents, and the amount of dithizonate is then determined colorimetrically. This method was formerly used extensively, but its two disadvantages are that the limit of detection—around a few micrograms, 0.05 mg/kg in a sample of 0.01 kg (Analytic Methods Committee, 1965) is not impressive, and it is time-consuming.

CVAA and NAA have replaced the colorimetric methods. The detection limit for atomic absorption is 1–5 ng of Hg. The reported precision, expressed as a variation coefficient, is generally better than 20% (Burrows, 1973). The detection limit for NAA is 0.1–0.3 mg Hg in a 0.3 g sample; it has high specificity, and the precision in terms of the variation coefficient is better than 10%, as is the accuracy (Westermark and Ljunggren, 1972). The atomic absorption method developed by Magos and coworkers (Magos, 1971; Magos and Clarkson, 1972) for separating inorganic and organomercurials such as methylmercury in blood and other matrices has been recently improved by Berglund *et al*. (2005) by means of an atomic fluorescence method.

Even lower detection limits can be obtained with the AES methods. Brosset (1983) reported the detection limit for plasma AES to be better than 0.005 ng.

Several interlaboratory comparisons of the errors encountered in analysis of biological material under controlled practical conditions have been published (Lindstedt and Skerfving, 1972). Because mercury is volatile and easily lost, the greatest errors are probably connected with the collection, storage, transport, and handling of the samples. Mercury vapor diffuses through plastic materials, adsorbs to surfaces, and absorbs into material such as polyethylene, silicone, and rubber. In biological material, bacterial activity can reduce mercuric mercury, resulting in the loss of mercury vapor. In aqueous solutions containing low concentrations of mercury, mercury tends to adhere to the surface of the collecting vessels.

For the determination of mercury in air, mercury can be collected by oxidative solution in an impinger flask with permanganate or by amalgamation with gold in a gold trap. For a more detailed discussion on sampling and analysis of mercury in air and water, see Lindqvist *et al*. (1984).

These instruments measure the absorption of light emitted from a mercury vapor lamp. The units are, however, subject to interference from substances such as hydrocarbon solvents, often present in the working environment. Strong electromagnetic fields have also been reported to influence the result. Reactive tubes (Dreger tubes) have been devised for simple screening. Measurement of very low levels of mercury in water requires preconcentration by, for example, dithizone extraction or electrodeposition.

Analysis of mercury in biological material may require oxidative digestion or combustion in oxygen or some kind of extraction. The method must be selected and adapted to the type of material analyzed. More specific nondestructive physical methods of mercury analysis have been applied successfully to human hair strands by X-ray fluorescence (Torabara *et al*., 1982) and proton-induced X-ray emission (PIXE) (Li, 1983), with a detection limit of 1–5 μg/g hair.

Alkylmercury compounds can be identified by thin-layer chromatography or gas-liquid chromatography. Quantitative analysis of methylmercury is carried out by use of gas-liquid chromatographic techniques after extraction of alkylmercury from the sample with benzene (Westöö, 1966; 1967). Because all techniques used involve nondestructive extraction of the alkylmercury from the sample, the recovery has to be checked for every different type of sample matrix, because deficiency of extraction of mercury will be determined by both the nature of the sample matrix and the extraction procedures themselves. The detection limit of the method, according to Westöö, is approximately 1–5 μg/kg with a sample of 0.01 kg. The precision is 3% at the 0.005 μg level for fish-muscle samples. The recovery is generally >90% but varies with the type of sample. Samples such as liver and kidney are more difficult to extract than fish meat.

More recently, a number of new analytical methods have been developed that are capable of speciating the various forms of mercury by combining a number of recently developed analytical methods in tandem. Examples of these methods include cold vapor AAS (Ertas and Tezel, 2004), which gave a detection limit of 1.84 ug/L and a quantitation limit of 4.03 ug/L and a detection limit of 0.2 ug/g of hair (Gill *et al*., 2004) and gas chromatography-cold vapor AAS Diez and Bayonna (2002), which gave detection limits of 50 and 80 ug Hg/g of hair for methyl mercury and Hg2+, respectively. Lee *et al*. (2003) reported a detection limit of 0.2 pg/m^3 for air samples from Gothenburg, Sweden. Montuori *et al*. (2004) reported a detection limit of 0.08 pg. Gas chromatography-atomic emission spectroscopy (Rodil *et al*., 2002) gave detection limits for methylmercury and mercuric mercury of 0.12 ng/mL and 0.86 ng/mL, respectively, from certified reference materials after derivatization with sodium tetraphenylborate.

For gas chromatography-ICPMS, Bettinelli *et al*. (2002) reported a detection limit of 0.03 ug/L; Bouyssiere *et al*. (2002) and Yang *et al*. (2003) reported 0.037 μg/g in dogfish liver standard reference materials. Centineo *et al*. (2004) and Monperrus *et al*. (2005) reported 10–60 pg/L; whereas Gomez-Ariza *et al*. (2005) reported 12 ng/mL and 8 ng/mL for Hg^{2+} and methyl mercury, respectively. For gas chromatography-IC-TOF spectroscopy (Jitaru and Adams, 2004; Jitaru *et al*., 2005) or gas chromatography coupled to ICPMS and atomic fluorescence spectroscopy (Krystek and Ritsema, 2005), methylmercury concentrations in shark filets between 0.9–3.6 μg Hg/g were reported. In addition, capillary electrophoresis coupled with GFAAS (Li *et al*., 2005a) gave a detection limit of 3.0 ± 0.15 pg for Hg^{2+} for phenyl mercury and methylmercury, respectively. Capillary gas chromatography coupled on line with atomic fluorescence spectrometry has been reported to give detection limits of 0.005 ng for both methylmercury and ethylmercury (Shi *et al*., 2005). Capillary electrophoresis coupled with atomic fluorescence spectrometry (Li *et al*., 2005b; Yan *et al*., 2003) have been reported to give detection limits over the range of 6.8–16.5 μg/L for Hg^{2+} for methylmercury, ethylmercury, and phenylmercury. NS: Capillary electrophoresis-ICPMS (Michalke, 2005) have also been reported as methods for mercurial speciation. X-ray absorption fine structure spectroscopy (XAFS) is another technique for conducting mercury speciation (Kim *et al*., 2003).

4 PRODUCTION AND USES

4.1 Production

Mercury occurs in the earth's crust mainly in the form of sulfides. The red sulfide, cinnabar, is the main component of the mercury-rich ores that are mined and may contain up to 70% mercury. The world production around 1975 was near 9000 metric tons (U.S. Bureau of Mines, Minerals Commodity Studies, 1976) but started falling quickly around 1990. The world production in 2003 was less than 2000 metric tons (USGS, 2005). The world production data for mercury over the past 25 years from the USGS are shown in Figure 1. In addition to the pure mercury production, mercury is released into the environment by human activities like combustion of fossil fuels, waste disposal, and industrial activities. These sources amount to approximately another 7250 short tons per year (U.S. Bureau of Mines, Minerals Commodity Studies, 1979). These figures should be compared with the release of mercury by degassing from the earth's crust and the oceans, which is estimated to be in the range of 30,000–150,000 tons per year, 30,000 tons being a minimum figure (Korringa and Hagel, 1974). Organic mercury compounds released into the environment are often broken down to elemental mercury or mercuric compounds.

Methylmercury is formed naturally in the aquatic and terrestrial environment from elemental mercury and mercuric mercury. The methylation is likely to occur in upper sedimentary layers of sea or lake beds

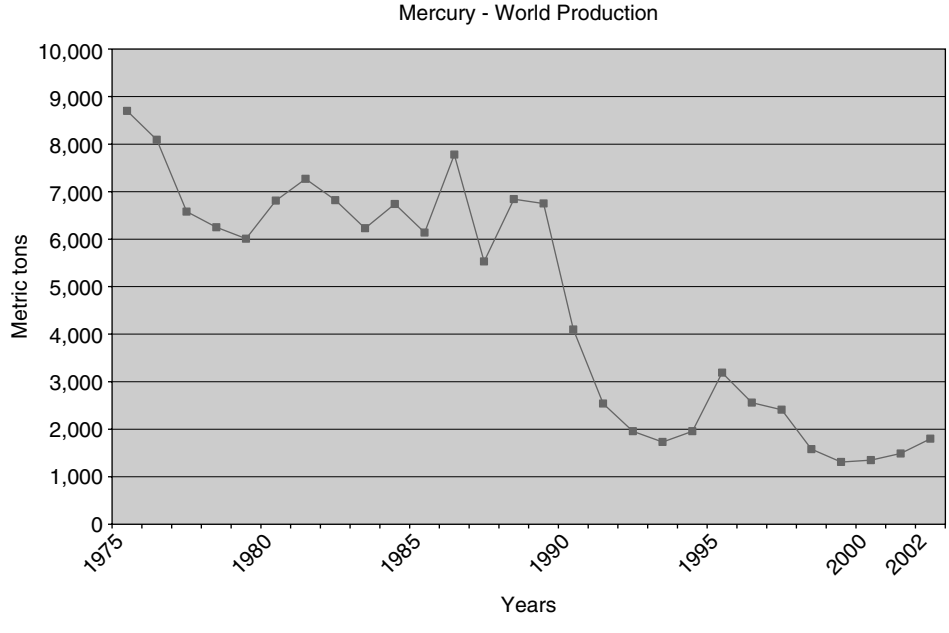

FIGURE 1 World production of mercury for the period from 1975–2003. (Source: USGS, 2005).

(Jensen and Jernelöv, 1967; 1969; Jernelöv, 1968). The methylmercury formed is rapidly taken up by living organisms in the aquatic environment, and, by degradation, gases of $(CH_3)_2Hg$ are formed and released into the air (Jensen and Jernelöv, 1968). Dimethylmercury may be decomposed in the atmosphere by acidic rainwater to monomethylmercury compounds and thereby reenter the aquatic environment (Jensen and Jernelöv, 1972). It may eventually be demethylated, thereby completing the cycle. Little is known about the quantitative aspects of these cycles. The local load of MeHg may be considerably increased by industrial release of mercuric compounds. This has been shown for mercury release from chemical factories—paper-pulp factories and alkaline-chlorine factories (Swedish Expert Group, 1971) and the Almaden mercury-mining region in Spain (Gray *et al*., 2004). These investigators found that mine waste contained total mercury in the range of 160–34,000 μg/g and that methylmercury varied from <0.2–3100 ng/g. They also reported variations in the methylation rates at various sites and that stream water near the Alamaden mine contained total mercury in concentrations up to 13,000 ng/L and methylmercury up to 30 ng/L. Stream sediments also contained total mercury concentrations up to 2300 μg/g and methylmercury concentrations of up to 82 μg/g. These data suggest a dynamic relationship between the stream sediments and the water column.

More recent studies (Mehrotra and Sedlak, 2005) have shown decreased mercury methylation rates by factors of 2.1–6.6 after iron amendment of anoxic wetlands sediment slurries with 30 mmol/L Fe(II). Hence the chemical nature of local environments may have a marked effect on the environmental mercury methylation cycle. In addition, the transalkylation of mercury (Hg^{2+}) from tetraethyl lead to form ethylmercury at a tetraethyl lead–contaminated site in Germany (Hempel *et al*., 2000) was reported, indicating that other processes and forms of alkyl mercurials should be considered in evaluating the environmental cycling of mercury.

4.2 Uses

According to the USGS (2005), only approximately 20 metric tons of mercury are now consumed domestically in the United States. Recent estimates indicate that of this amount approximately 50% is used for chlorine-caustic soda manufacture and the remaining 50% for dental amalgams, mercury electronic switches, and fluorescent lamps and other electronic devices. This represents a marked decline in consumption, because USGS (2005) has estimated that the chlorine–caustic soda industry alone used approximately 100 metric tons per year for the period 1996–2005. The uses have changed dramatically over time. In the mid-70s, of the mercury produced yearly for commercial purposes, 25% was consumed by the chloralkali industry, 20% used in electrical equipment, 15% in paints, 10% in measurement and control systems (e.g., thermometers and sphygmomanometers), 5% in agriculture, 3% in dentistry, and 2% in laboratories. The remaining 20% was divided among military uses, such as detonators, mercury-containing catalysts, preservatives in the paper-pulp industry, pharmaceuticals, cosmetic preparations, and others (Korringa and Hagel, 1974; Nriagu, 1979). The cytotoxic properties of mercury compounds have resulted in their widespread use as germicides and fungicides in pharmaceuticals, plastics, paints, and other products. Methylmercury and ethylmercury have been extensively used in seed treatment. Most industrial countries have banned this use, and the production of alkylmercury compounds has decreased. Dimethylmercury is used by chemists as a reagent. Phenylmercury acetate has been extensively used as a fungicide and algaecide in paints, plastics, and other products. Methoxyethylmercury compounds have replaced the short-chain alkylmercury compounds in seed treatment. Under the conditions in which they are used, both types of compounds are unstable and slowly release inorganic mercury.

5 ENVIRONMENTAL LEVELS AND EXPOSURES

5.1 General Environment

5.1.1 Food and Daily Intake

Alkylmercury, formed in the bottom sediment of the ocean and in freshwater systems, is enriched to a high degree in the aquatic food chain, with the highest levels occurring in predatory fish. From the aquatic environment, methylmercury becomes incorporated in the terrestrial environment by species feeding on aquatic organisms. However, enrichment has not been seen to the same extent in the terrestrial food chain. Large tuna fish, exceeding 60 kg, have levels up to 1 mg/kg of methylmercury in their muscles (Peterson *et al*., 1973), whereas terrestrial animals rarely have levels in muscles exceeding 50 μg/kg and generally average 20 μg/kg (Swedish Expert Group, 1971). In areas with polluted water, levels of methylmercury in fish flesh may exceed 10 mg/kg, with a tendency to increasing levels with increasing size and age of fish. Another determining factor for the methylmercury content in fish is pH. A positive association

between acidification of lakes and mercury content in fish has been observed (Lindqvist *et al*., 1984). For a detailed discussion of the mechanism behind this association, see Lindqvist *et al*. (1984). After agricultural use of alkylmercury, levels of methylmercury in game birds may reach toxic levels (Swedish Expert Group, 1971). Methylmercury, but not ethylmercury, has been recently reported in rice samples from 15 provinces of China (Shi *et al*., 2005). Methylmercury concentrations in the rice samples ranged from 1.9–10.5 ng MeHg/g and accounted for 7–44% of the total mercury measured.

A large part of the mercury in food—at least in animal products—is likely to be in the form of methylmercury. The major proportion of the mercury in fish is methylmercury. The mercury concentration in food products, excluding fish, varies from a few micrograms to 50 μg/kg (Bouquiaux, 1974). Thus, the daily intake of methylmercury mainly depends on fish consumption and the concentrations of MeHg in consumed fish. Fish consumption varies among countries, with some individuals and ethnic groups having virtually no consumption to others having 500 g or more per day. The average daily intake of fish flesh for the Swedish population was estimated at 30 g/day (Swedish Expert Group, 1971). This level will result in a daily intake of MeHg of between 1 and 20 μg/day with consumption of uncontaminated fish. In epidemiological studies of the consumption of tuna fish in the American Samoan population, blood levels have been found indicating a daily intake of between 200 and 300 μg/day of MeHg (Clarkson *et al*., 1975). Should the water be polluted, the daily intake from fish consumption can rise to toxic levels, as occurred in Minamata and Niigata in Japan in the period 1953–1966 (Swedish Expert Group, 1971). Concentrations of methylmercury in fish of 1–20 mg/kg have been reported to result in a maximum daily intake in persons with high fish consumption (200–500 g/day) of approximately 5 mg/day (Swedish Expert Group, 1971). More recently the USEPA and NAS/NRC (EPA, 1997; NAS, 2001) have revised the acceptable daily intake of fish species containing methylmercury to lower consumption rates for pregnant or nursing women. The current reference dose (RfD) for methylmercury is now 0.1 μg/kg/day.

It should be noted that, in the absence of occupational exposure, the daily intake of inorganic mercury would probably not exceed 10 μg/day from mercury inhalation, drinking water, and food. However, a major contribution may result from the release of mercury vapor from dental amalgam fillings, if present (Aronsson *et al*, 1989; ATSDR, 1999; Brune and Evje, 1985; USEPA, 1998).

Mercury from dental amalgam fillings is the dominant source of mercury exposure in terms of uptake and retention in the organism for the general population of industrialized countries (WHO, 1991). It is also the overwhelmingly dominant source of mercury in the central nervous system of the general population. Dental amalgam primarily contributes to the daily absorption of mercury in two ways. Mercury is released in vapor form, inhaled, and reabsorbed to 80% in the airways. Abraded amalgam particles are swallowed and to a smaller extent oxidized in the intestinal tract. Less than 10% of such ingested mercury is reabsorbed as Hg^{+2}. Mercury can also be taken up in the nerve endings and transported in a retrograde direction to ganglia and central nerve cells (Arvidson, 1990; 1994). The average daily retention in the population from amalgam is estimated at 3–17μg with the note that substantial individual variations exist (WHO, 1991). This dose interval has since been confirmed in several studies (Halbach, 1995; Sandborgh-Englund, 1998; Weiner and Nylander, 1995). The size of the variation is illustrated by Barregård *et al*. (1995), who described three patients experiencing symptoms of mercury toxicity who all eliminated large quantities of mercury in their urine (54, 53, and 25 μg/g creatinine, respectively) and had no source of exposure other than their amalgam fillings; all of them used nicotine chewing gum. When the amalgam fillings were removed in the first two cases with the highest elimination levels, the mercury elimination fell to expected values and the symptoms disappeared. In the third case, the patient refused to have the amalgam removed, but the elimination seemed to decrease with reduced chewing gum consumption. A similar case was published by Langworth and Strömberg (1996). These cases demonstrate a mercury uptake of approximately 100 μg/day, approximately 10 times higher than the average uptake from amalgam according to WHO (1991).

Mercury uptake from amalgam increases tissue concentrations in the brain, plasma, and kidneys in proportion to the number of amalgam fillings. Mercury concentration in plasma and urine in amalgam-free subjects amounts to 0.2 μg/L and 2 μg/L, respectively (Sandborgh-Englund *et al*., 1998). Mercury content in the brains (occipital cortex) of nonamalgam bearers was found in autopsies to be approximately 7 ng/g (mean, 6.7; range, 1.9–22.1). The brains of amalgam bearers contained approximately 15 ng/g (mean, 15.2; range, 3.8–121.4) (WHO 1991). In the fetus, an increase also occurs in the brain and kidneys, with an average of twice the mercury concentration in those with mothers who have amalgams compared with those whose mothers do not (Drasch *et al*., 1994; Lutz *et al*., 1996).

5.1.2 Water

Because of the low levels of mercury in seawater and freshwater and the limitations of analytical methods used in the past (Olafsson, 1982), variations in mercury concentrations in surface water are generally well within the limits of analytical errors. Analysis with more sensitive methods indicates that dissolved mercury in uncontaminated marine water and freshwater amounts to 1–10 ng/L (Lindqvist *et al*., 1984). However, bottom sediment of lakes and oceans may contain 20–250 μg/kg of mercury (Lindqvist *et al*., 1984). More recent studies (Boudou *et al*., 2005) reported higher concentrations of mercury in a gold mining river in French Guiana. Fish collected from below a hydroelectric dam on the river had 8-fold higher concentrations of methyl mercury than fish collected upstream. A downstream reservoir seemed to play a central role in these differences by providing a deep anoxic sink that facilitated the environmental formation of methylmercury.

5.1.3 Ambient Air

The mercury concentration in the general atmosphere varies from a few ng/m^3 in remote uncontaminated areas up to approximately 20 ng/m^3 in urbanized areas (Lindqvist *et al*., 1984; Williston, 1968). High levels of mercury have been reported in the atmosphere close to active volcanoes, up to 18 μg/m^3 (Siegel and Siegel, 1979). From a community close to the mercury mines in Spain, values of 0.8 mg/m^3 have been reported (Fernandez *et al*., 1966). More recently, attention has been focused on mercury emissions from coal-fired power plants in the United States (Boylan *et al*., 2003; Gustin and Ladwig, 2004; Heebink and Hassett, 2002; Laudal *et al*., 2000), Spain (Otero-Rey *et al*., 2003), The Netherlands (Meij *et al*., 2002), Finland (Mukherjee *et al*., 2000), Canada (Goodarzi, 2004), Japan (Yokoyama *et al*., 2000), Korea (Jun *et al*., 2004), and China (Horvat *et al*., 2003; Zhang *et al*., 2002). Laudal *et al*. (2000) reported total gaseous mercury emissions from a coal-fired power plant in North Dakota of 6.95–15.66 g/hour, which was primarily in the form of mercury vapor (Hg^0) (6.59–12.62 g/hour), whereas Hg^{2+} was emitted at a rate of 0.34–3.68 g/hour. Heebink and Hassett (2002) studied release of Hg^0 from coal combustion by-products (CCBs), such as coal flyash, and found relatively little release from this source. The atmospheric emissions of mercury from coal-fired power plants is, hence, a matter of local, regional, and international concern. Release of mercury vapor or mercury-containing particles into the atmosphere will eventually result in deposition of a fraction of the total mercury released into bodies of water by rain, snow, or dry particle deposition with subsequent uptake by aquatic species (e.g., fish) consumed as food by man.

5.1.4 Soils and Sediments

Horvat *et al*. (2003) studied total mercury and methylmercury in soil and rice in Guizhou Province of China and reported high levels near the Wanshan mining and ore-processing facilities and near a chemical plant in Quingzhen. They reported total mercury concentrations in rice of up to 569 μg/kg, with 145 μg/kg in the form of methylmercury as a result of mercury methylation in soil, water, and sediments used to grow the rice. Tomiyasu *et al*. (2003) reported vertical variations in the mercury concentrations in soils around the Sakurajima volcano in Japan and found that the concentration in soils increased with distance from the volcano at three sampling sites in the order of 6.5 ng/g, 29 ng/g, and 229 ng/g for Sakurajima, Takatoge, and Suzuyama. The mercury concentrations correlated with organic matter but not with soil grain size. Neculita *et al*. (2005) studied mercury speciation in soils near chloralkali plants that use chemical extraction. They reported that the soils contained mean concentrations of 295–11,500 mg Hg/kg in coarse-grained, alkaline soils with high chloride content and low organic matter. Methylmercury concentrations were low, but a large percentage of the mercury present was in a volatile form in the residual soil fraction. Grigal (2003) reviewed mercury sequestration in forests and peat lands. This investigator noted the strong binding of Hg by reduced sulfur in these areas and that, in general, wetlands sequestered more mercury than adjacent upland areas. Wetland areas are important sites for the production of methylmercury. Gabriel and Williamson (2004) noted that in terrestrial environments, OH–, Cl–, and S– have the greatest impact on formation of complexes with Hg. In reduced soils, sulfur and chlorine complexes predominate. Clay and soils with high organic content have the greatest mercury-binding capacity. Soil permeability and temperature are major factors influencing mercury volatilization from soils. As a result of these various factors, the movement of mercury through soil environments will vary across regions. Examples of this variability are shown in the studies of Almeida *et al*. (2005) in Brazil and Tack *et al*. (2005) in Belgium. Almeida *et al*. (2005) reported that the loss of mercury from soils increased after conversion of Amazon forested areas to pastureland. Soil in forested areas was found to have concentrations on the order of 128 ng Hg/g at the surface versus 69 ng Hg/g for pasturelands at the soil surface. The rate of mercury loss was most marked for surface areas of the soils, with mercury losses estimated to be between 8.5 and 18.5 mg/m^2 for the first 20 cm of soil. The loss rates were directly related to the mercury concentrations present in the soil. Tack *et al*. (2005) reported differences in

Flemish soils with higher values in the eastern regions (0.09–0.43 mg/kg) versus the western region (0.7–0.13) and Antwerpen (0.7–0.15 mg/kg).

Sediments are important sites for mercury deposition and microbial conversion to methylmercury. Oliverso and Solano (1998) reported mercury in river sediments of a gold mining region of Colombia, South America, to range between 140 and 355 μg/kg. Hylander *et al*. (2000) reported data from studies on sediments from nine flood plain lakes in the Pantanal region of Brazil, where gold mining with the use of mercury is a common practice. The average mercury content of sediments in the Bento Gomes River was 88.9 ng/g on a dry weight basis with lower concentrations in related flood plain lakes (average, 33.2 ng/g Hg on a dry weight basis). Heaven *et al*. (2000) reported sediment concentrations in the river Nura in Central Kazakhstan in the range of 150–240 mg/kg downstream from an acetaldehyde plant and from power station flyash. Wasserman *et al*. (2002) studied sediments in the Sepetiba Bay, Brazil, and reported inorganic mercury concentrations between 22.65 and 134.61 ng/g with methylmercury concentrations between <2.0 and 4.4 ng/g. Arnason and Fletcher (2003) evaluated mercury concentrations in the Patroon Reservoir in New York and found mercury concentrations from <1.0 mg/kg in sediments at 1.68 m to 6.2 mg/kg at 0.8 m on a dry weight basis and found lower levels in surface sediments. Ram *et al*. (2003) reported mercury concentrations in the Ulhas estuary in India that receives discharges from various industrial sources including chloralkali plants. Sediment concentrations were generally elevated in the upper estuary with the highest concentrations found to be 38.45 ug Hg/g. Higueras *et al*. (2005) found mercury concentrations in stream sediments downstream from the Almaden mercury mining area in Spain to range up to 16,000 μg Hg/g. Extensive mercury contamination of crayfish and plants in the river were also noted.

5.2 Working Environment

Exposure to mercury vapor is the most common form of occupational exposure to mercury. Such exposure occurs in a variety of industries (e.g., in mercury mining, chloralkali factories, and instrument and fluorescent lamp manufacturing, as well as in physics and medical laboratories). Most reports of hazardous exposure come from mining and chloralkali industries. Air levels of mercury as high as 5 mg/m^3 have been reported in connection with mining operations. Human exposure is often related to specific working conditions or spillage of mercury compounds on the work clothes from which it evaporates and is inhaled.

Although the most common exposure to inorganic mercury is by mercury vapor, mixed exposures to organic mercury compounds in aerosols, or mercuric inorganic mercury aerosols also occur. In chloralkali industries, the presence of chlorine in combination with mercury vapor gives rise to the formation of mercuric chloride aerosols. The handling of mercuric salts in the chemical industry may also cause exposure to aerosols of inorganic mercury. Exposure to methylmercury and ethylmercury has been described in connection with manufacturing and use of these salts in chemical industrial workers and in personnel carrying out seed treatment. Because of the restrictions in agricultural application of alkylmercury compounds, it is likely that occupational exposure is rare at present in most industrialized countries.

Dental and medical health professionals may also be exposed to mercury (Hg^0) vapor as a result of releases from carpets or flooring contaminated with mercury metal spills during preparation of amalgam fillings or breakage of mercury-containing thermometers.

Gold miners in the Amazon Basin (Aks *et al*., 1995; Branches *et al*., 1993; Drake *et al*., 2001), and Slovenia (Franko *et al*, 2005; Grum *et al*., 2006) have been reported to show a variety of neurological and renal effects associated with occupational mercury vapor exposure after volatilization of mercury vapor from mercury-gold amalgams by heating. In addition, neurological effects associated with methylmercury exposure have also been reported among native Indian populations living near gold mining areas of the Amazon Basin (Dolbec *et al*., 2000; Sing *et al*., 1996; 2003), Ecuador (Counter *et al*., 2005), and the Philippines (Akagi *et al*., 2000) secondary to environmental methylation of inorganic mercury used in the mining processes and consumption of fish from these areas.

6 METABOLISM AND TOXIC EFFECTS OF ELEMENTAL MERCURY AND INORGANIC MERCURY COMPOUNDS

There are considerable differences in metabolism between metallic mercury vapor and mercuric mercury. Because of its lipid solubility, mercury vapor penetrates the membranes of the body and is easily absorbed. On the other hand, the lifetime of mercury vapor in the body is very limited because of the rapid oxidation of elemental mercury to mercuric mercury. Reduction of mercuric mercury also occurs in the body (Dunn, 1978). The divalent mercuric ion is most likely to bind to sulfhydryl or selenohydryl groups on proteins and has limited mobility in the body. The toxic effect seen after exposure to mercury vapor can,

according to all available evidence, be attributed to the divalent mercury ion formed through oxidation in the tissue. Just as some Hg-thiol–complexes can mimic endogenous molecules at the site of proteins present in the plasma membrane, these complexes may also mimic molecules at the binding site(s) of intracellular proteins and enzymes. Because Cys-*S*-Hg-*S*-Cys mimics cystine at the site of an amino-acid transporter on the plasma membrane (Bridges *et al*., 2004), it is not surprising to find that this conjugate also acts as a mimic of this amino acid at binding sites of intracellular molecules that use cystine as a substrate. An example of this mimicry has been demonstrated by the preliminary findings of Cooper and colleagues (unpublished data), which indicate that Cys-*S*-Hg-*S*-Cys acts as a mimic of cystine at the binding site of the intracellular enzyme, γ-cystathionase. This enzyme is activated normally by the binding of cystine or cystathionine. Because the binding of Cys-*S*-Hg-*S*-Cys was shown to inactivate the enzyme, rather than activate it, it can be concluded that this conjugate behaves as a structural, but not a functional, mimic of cystine and cystathionine. These findings suggest that the ability of molecular species of metals to mimic endogenous molecules may have serious, deleterious effects on intracellular processes.

More specifically, it has long been known that Hg^{+2} is cytotoxic. SH-groups constitute an important component in proteins. Mercury binding to these groups can produce a change in the proteins' tertiary and quaternary structure and alter binding conditions in prosthetic groups in enzymes (Freitas *et al*., 1996; Palkiewicz *et al*., 1994; Pendergrass & Haley, 1997; Rajanna *et al*., 1995) and block or modify receptor binding (Albrecht & Matyja, 1996; Castoldi *et al*., 1996; Kim and Choi, 1995) and K^- or Ca^- ion flows in the cell membrane's pores and ionic channels (Aschner *et al*., 1996; Busselberg, 1995; Dyatlov *et al*., 1996; Fejtl *et al*., 1994; Leonhardt *et al*., 1996; Rossi *et al*., 1993; Szucs *et al*., 1997; Yallapragada *et al*., 1996). This can affect cell membrane potentials and intracellular and intercellular signals. The release of transmitter substances in nerve cells can be inhibited or accelerated, as is cytokine production in the cells of the immune system and hormone production in endocrine glands. It has been possible to observe these effects in *in vitro* experiments with cell cultures of different types of cells or with the help of intracellular electrodes in single cells and with a 0.1–1 μmol/L mercury concentration in the medium (Berlin, 1999). In cultures of fetal brain tissue, growth inhibition and changing concentration of growth factors have been reported at mercury concentration approximately 0.01 μmol/L (Abdulla *et al*., 1995; Monnet-Tschudi *et al*., 1996; 1998; Soderstrom *et al*., 1995).

On the other hand, mercury has occurred in the environment throughout evolution, and organisms have acquired the ability to manage limited quantities of this metal. Special molecules containing SH-groups or SeH-groups and with an ability to bind strongly to Hg^{+2} have been identified (Nordberg *et al*, 1974; Yoneda and Suzuki, 1997). Glutathione and metallothionein are molecules that can neutralize the mercury ion and prevent it from disturbing the cell's dynamic biochemical systems. Bound to these molecules, mercury can be transported, stored, and eliminated from the body. It has also been shown experimentally that the sensitivity of different cell types to the mercury's cytotoxic effects is related to their ability to synthesize glutathione or metallothionein (Bohets *et al*., 1995; Foulkes, 1993; Kim *et al*., 1995b). Binding to metallothioneins explains, for example, why such high mercury concentrations can be encountered in the kidneys without production of metabolic disturbances.

Because the metabolism and toxic properties of metallic mercury, especially in vapor form, and mercuric mercury differ considerably, they are treated separately below. It should also be noted that many of the studies reported in the older literature should be considered in light of limitations in mercury analyses and quality control.

6.1 Elemental Mercury

6.1.1 Metabolism

6.1.1.1 Absorption Inhalation

Mercury vapor is efficiently absorbed from the alveolar air because of the rapid diffusion of mercury vapor through the alveolar membrane (Berlin *et al*., 1969b) and because of the capacity of red cells to bind and to oxidize mercury to mercuric mercury (Clarkson *et al*., 1961). The oxidation of mercury and the red cells occurs, at least partly, under the influence of catalase (Halbach and Clarkson, 1978; Magos *et al*., 1978). This oxidation can, however, be inhibited by alcohol and aminotriazole (Magos *et al*., 1974; Nielsen-Kudsk, 1965), whereby the absorption and retention by inhalation are reduced. Considering the effect of deadspace, the absorption of mercury vapor at moderate ventilation rates will be approximately 80% at concentration levels encountered in the working environment (Nielsen-Kudsk, 1965). Similar absorption rates were obtained after exposure of volunteers to small amounts of radioactive mercury vapor (Hursh *et al*., 1976).

6.1.1.2 Ingestion

Liquid metallic mercury is poorly absorbed from the gastrointestinal tract. Mercury vapor is slowly released from the surface of metallic mercury at a rate that is related to the surface area present. The tendency of metallic mercury to cover itself with mercury sulfide

further limits the amount of mercury vapor that can be released. As a result of these factors, the amount of mercury released from metallic mercury that is ingested is of no toxicological importance. Cases of mercury poisoning have, however, been described in which mercury was deposited in diverticulas, fistulas, or abscesses in the gastrointestinal tract or after aspiration in the lungs (Geller, 1976).

6.1.1.3 Skin

Hursh *et al.* (1989) studied the uptake of ^{203}Hg-labeled mercury vapor by the forearm skin of volunteers. They found that approximately half of the absorbed amount was trapped in the skin and later shed. The systemic uptake for all skin areas was calculated to be approximately 1% of the uptake from inhalation of the exposure gas.

6.1.1.4 Transport, Distribution, and Biological Half-Time

It has been shown in mice (Berlin and Johansson, 1964) and in primates (Berlin *et al.*, 1969a) that after single exposure to mercury vapor, 10 times more mercury is retained in the brain than after intravenous injection of the same dose of mercuric mercury. It was suggested (Berlin *et al.*, 1966) that this observation was due to physically dissolved mercury vapor in blood being transported to the brain or to the release of mercury vapor from blood cells. The major part of mercury in blood is found in the erythrocytes during ongoing exposure (Cherian *et al.*, 1978), and mercury vapor can be released from the cells. Erythrocytes or hemoglobin serve as a transporter of mercury vapor (Hursh, 1985). Magos (1968) confirmed the findings of Berlin and coworkers and showed—by intravenously injecting mercury vapor and by recovering, within 30 seconds, 19% of the injected dose in the exhaled air of the animal—that physically dissolved mercury would be enough to explain the uptake of mercury in the brain. Nordberg and Serenius (1969) visualized the penetration of mercury vapor to the brain through the capillaries by autoradiography of the guinea-pig brain. It has been shown (Clarkson *et al.*, 1972) that mercury vapor, in contrast to mercuric mercury, also penetrates the placental barrier, causing an accumulation of mercury in the fetus when the mother is exposed to mercury vapor. Mercury vapor has, however, a limited survival time in the body. It is rapidly oxidized to mercuric mercury in tissue and then reacts in the same way as mercuric mercury. Little is known about steady-state distribution during long-term exposure to mercury vapor. Attempts have been made in animal experiments to produce steady state during exposure to mercury vapor. However, the reaching of a steady state has not been proved. Berlin (1976) exposed squirrel monkeys to 1 and 2 mg/m^3 of mercury vapor for 6 hours, 6 days a week for up to 2 months. The animals failed to obtain steady state in the brain, although mercury in blood seemed to reach a steady state during this time span.

From animal studies (Berlin and Johansson, 1964; Berlin and Ullberg, 1963a) with a single exposure to mercury vapor or injection of mercuric mercury salt, it has been shown by autoradiography that in the mammalian organism, mercury is distributed specifically to certain types of cells. Mercury has a special affinity for ectodermal and endodermal epithelial cells and glands. Mercuric mercury is thus accumulated in the epithelial lining of the intestinal tract; in the squamous epithelium of the skin and hair; in glandular tissues like the salivary glands, thyroid, liver, pancreas, and the sweat glands; and in the kidney, as well as in epithelial organs, like the testicles and the prostate. In the brain, most mercury is found in grey matter, more in some nuclei in the brain stem and in some parts of the cerebellum (Berlin *et al.*, 1969a; Nordberg and Serenius, 1969). These studies have also revealed that the retention time of accumulated mercury varies widely among different organs. Biological half-times vary from a few days to months. The organs with the longest retention times are the brain, kidneys, and testicles. These organs are thus likely to show accumulation of mercury on repeated exposure and dominate the distribution at steady state. This has been confirmed in the human brain in cases of occupational exposure to mercury vapor (Takahata *et al.*, 1970; Watanabe, 1971).

Available data from long-term exposure of squirrel monkeys (Berlin *et al.*, 1975a) and a few clinical cases of occupational exposure (Takahata *et al.*, 1970; Watanabe, 1971) indicate that mercury is accumulated in the cerebral cortex, especially in the occipital and parietal cortical areas. By use of a histochemical method, autometallographic silver enhancement, which visualized inorganic mercury, Warfvinge *et al.* (1994) studied the microscopic distribution of mercury in the brain of female squirrel monkeys and their offspring after exposure to mercury vapor during pregnancy. Accumulation of mercury was seen in neurons and astrocytes, and most notably in pyramidal cells of cerebral neocortex in lamina III and V and of pyramidal cells in the hippocampal formation and in cortex piriformis. Mercury was also visualized in neurons of claustrum and the amygdaloid complex. The distribution was similar in offspring and adult monkeys except that the brains of the offspring did not show any laminated distribution in cortex. In the fiber system, the glia of the offspring contained more mercury than the glia of adult brains.

In the cerebellum, the cerebellar nuclei contained the highest amount of mercury, seen in both neuron and glia cells. A high amount was also seen in the Purkinje cell layer in Purkinje cells and Bergmann glia cells. Mercury was also visualized in medullary layer astrocytes, in the molecular layer, and the granule cell layer. Three years after the cessation of exposure, mercury could still be visualized in the cerebellum. No difference between adult and offspring brains in mercury distribution was observed (Warfvinge, 2000).

A part of the brain of the squirrel monkey showing a conspicuous accumulation of mercury is the retina (Khayat and Dencker, 1983; 1984). Warfvinge and Bruun (2000) studied the microdistribution in the retina. They visualized mercury mainly in the optic disc, retinal pigment epithelium, capillary walls, and ganglion cells and found retention of mercury in these structures up to 5 years after exposure. Offspring exposed *in utero* had similar retinal distribution but seemed to eliminate mercury faster than seen in the adult.

The evidence from animal experiments also indicates that the distribution in the kidney and other organs accumulating mercury after exposure to mercury vapor is similar to that seen after exposure to mercuric salts.

6.1.1.4.1 Elimination and Excretion The elimination of mercury after exposure to mercury vapor occurs mainly by excretion of mercuric mercury. However, exhalation of small quantities of mercury vapor has been demonstrated in animals (Clarkson and Rothstein, 1964) and man (Hursh *et al*., 1976). A fraction of the mercury vapor exhaled is the result of reduction of divalent mercuric mercury stored in the tissues (Dunn, 1978).

6.1.1.4.2 Excretion The rate of excretion is dose-dependent, and considerable species differences have been observed. The best mathematical model seems to be a multicompartment model with at least two or more excretion rates and with one small compartment, including the brain, with a very long biological half-time. The limited data from human studies indicate that the bulk of mercury accumulated in the body (80%) is excreted with biological half-time of approximately 60 days (Hursh *et al*., 1976; Rahola *et al*., 1971). Part of the mercury accumulated in the brain is slowly eliminated with a biological half-time that may exceed several years (Kosta *et al*., 1975; Rossi *et al*., 1976). For renal, hepatic, and intestinal excretion of divalent mercury, see the following.

6.1.2 Symptoms and Signs in Poisoning Caused by Exposure to Mercury Vapor

As noted previously, mercury is a potent cell toxin that affects basic functions of the cell by modifying the tertiary and quaternary structure of proteins by bonding strongly with sulfhydryl and selenohydryl groups. This metal may interact with receptor, ion channel, and intracellular signal link functions. As the structure of protein molecules is genetically determined, this leaves ample scope for genetic polymorphism to manifest itself in varying sensitivity and types of reaction to mercury exposure. There are also several target functions for mercury toxicity. In most cases the central nervous system seems to be the most sensitive organ. Peripheral nerve function, renal function, the immune system, endocrine system, and muscle function are also affected. A review (Berlin, 2003) of published cases of accidental mercury poisoning during the 5-year period of 1998–2002 revealed an astonishing variation in symptoms and signs. Several case descriptions were found. These have most likely been published because the symptoms are unexpected. Mercury concentrations were documented with urine and blood values, and the symptoms subsided when the exposure ceased. Accordingly, there is no doubt that mercury caused the symptoms. The symptoms observed included a range of dermal syndromes, such as systemic contact dermatitis (baboon syndrome) (Alegre *et al*., 2000; Bartolome *et al*., 2000). Three cases of nummular dermatitis, which were cured by amalgam removal, are described by Adachi *et al*. (2000) and Pigatto *et al*. (2002). In a review article, Britschgi and Pichler (2000) state that mercury can induce acute generalized exanthematous pustulosis. In another review article, Boyd *et al*. (2000) summarize skin diseases caused by mercury.

One article describes a 5-year-old boy who, after massive mercury exposure, developed tics, extensive blinking, head-twisting, and shoulder-jerking as his sole symptoms (Li *et al*., 2000). There have also been descriptions of several cases in children with hypertension and elevated catecholamine secretion induced by mercury exposure, the symptomatology has resembled phaeochromocytoma (Kosan *et al*., 2001; Laurans *et al*., 2001; Torres *et al*., 2000; Wössmann *et al*., 1999). A 48-year-old man developed aspects of severe, acute polyarthritis (Karatas *et al*., 2002) as a result of massive mercury exposure. Dalén (2000) describes a historical case with symptoms suggesting gastroenteric influence.

This variability in symptoms and signs at the upper end of the dose scale is likely to reflect genetic polymorphism, which most likely will also manifest itself at the lower end. The existence of cases with genetically

determined high sensitivity to mercury and with an incidence <1/100 exposed is very likely and is a problem relevant to mercury vapor exposure from dental amalgam in the population.

6.1.2.1 Acute Poisoning

The lung is the critical organ in acute accidental exposure to high concentrations of mercury vapor. The mercury vapor causes erosive bronchitis and bronchiolitis with interstitial pneumonitis. The patient will eventually succumb to respiratory insufficiency. The symptoms of respiratory distress may be combined with signs caused by effects on the CNS, such as tremor or increased excitability. Garnier *et al*. (1981) reviewed approximately 20 such cases that have been published in the literature.

6.1.2.2 Chronic Poisoning

On long-term exposure to toxic levels of mercury vapor, the CNS is the critical organ. Little is known about the pathogenesis of the brain dysfunction that is seen on exposure to mercury vapor. With increasing dose, signs appear that can together be characterized as a nonspecific, asthenic-vegetative syndrome involving symptoms like weakness, fatigue, anorexia, loss of weight, and disturbance of gastrointestinal functions. This syndrome has been called micromercurialism (Friberg and Nordberg, 1972; Trachtenberg, 1969). At higher exposure levels, the characteristic mercurial tremor appears as fine trembling of the muscles interrupted by coarse shaking movements every few minutes. This begins in peripheral parts like fingers, eyelids, and lips and has the characteristics of intentional tremor. It disappears during sleep. In progressive cases, it may develop into a generalized tremor involving the entire body, with violent chronic spasms of the extremities. Parallel to the development of tremor, mercurial erethism develops. This is characterized by severe behavioral and personality changes, increased excitability, and loss of memory and insomnia, which may develop into depression. In severe cases, delirium and hallucination may occur.

In addition to the effects seen in the CNS, cases of severe poisoning may display inflammatory changes of the gums with ptyalism, possibly severe, with salivation of up to several liters per day.

By use of electrophysiological methods on occupationally exposed cases, changes have been shown in the brain's signals caused through visual, auditory, or somatosensory stimulation (evoked potentials) and reduced peripheral nerve conduction velocity (Andersen *et al*., 1993; Chang *et al*., 1995; Discalzi *et al*., 1993; Ellingsen *et al*., 1993b; Urban *et al*., 1996). Reduced color vision has also been demonstrated (Cavalleri *et al*., 1995), an observation that conforms with animal experimental findings on primates that mercury is accumulated and retained in the retina (Warfvinge and Bruun, 1996). In psychomotor tests, changes in coordination ability, tremor, concentration capability, and mood have been found (Echeverria *et al*., 1995; Liang *et al*., 1993; Netterstrom *et al*., 1996).

White *et al*. (1993) described a worker in a thermometer factory. He was exposed for 3 years and developed neuropsychological symptoms and was referred to a psychiatric clinic for his behavioral problems. His urine mercury level was 690 μg/L. "The MRI showed mild central and cortical atrophy. Punctiform foci of T2 were noted in both frontal regions, especially underlying the precentral gyri and in the subcortical white matter and in the white matter of the gyri. The MRI was interpreted as consistent with diffuse and focal white matter disease. The lesions did not resemble those seen in multiple sclerosis plaques or in microinfarcts."

Another case of short-term, high-exposure to mercury vapor with typical symptoms of mercurialism, SPECT (single-photon emission computerized tomography) showed clear signs of hypermetabolism in the right posterior cingulum cortex but no changes on the MRI image of the brain (O'Carroll *et al*., 1995). Haut *et al*. (1999) studied 13 men (mean age, 45 years) exposed to mercury vapor for 2–4 weeks through welding on material painted with mercury-containing paint. After the exposure ceased, the men's blood mercury concentration averaged 48 μg/L of blood (corresponding to approximately 150 μg/g creatinine), with a range of 21–84 μg/L. One year after exposure had ceased, all the men were subjected to a battery of neuropsychological tests and compared with a control group of 13 nonexposed workers. Compared with the control group, the exposed group displayed cognitive deficits in motor coordination, rapid reception of information with and without motor elements, verbal capacity, verbal memory, visual problem solving, and comprehension. The men exposed also had more emotional problems, such as an increased focus on bodily functions, depression, anxiety, and were more socially withdrawn (Haut *et al*., 1999).

6.1.2.3 Persistent Effects of Mercury Exposure

In cases of slight poisoning, symptoms and signs of mercury poisoning, so called erethism, seem to regress and disappear when exposure has ceased. However, in more severe cases, because of long-term exposure, persistent sequelae related to the nervous system are common. Baldi *et al*. (1953) followed 135 cases of mercurialism in whom exposure had been interrupted for several years. Of these, 69 showed no

improvement, 33 were stationary, 28 regressed, and 5 died of other causes.

An American survey was conducted on 205 workers whose mean age was 71 years. Of these workers, 104 had been heavily exposed to mercury vapor more than 19 years previously, with mercury secretion in excess of 600 μg/L in urine. The other 101 workers had not been exposed. Conduction velocity in peripheral nerves significantly correlated with cumulative mercury exposure, which suggests residual peripheral neuropathy. Motor coordination was also reduced to a statistically significant degree, with a dose-response association (Letz *et al*., 2000).

In several investigations (Andersen *et al*., 1993; Kishi *et al*., 1993; O'Carroll *et al*., 1995), it has been shown that neurological symptoms that arose after occupational exposure to mercury vapor can remain for decades after exposure has ceased, indicating that permanent damage has occurred.

In a Norwegian survey of 75 chloralkali workers compared with 52 controls, a dose-related effect on attention capacity and visuomotor capacity was found 12 years after the termination of exposure. This group's exposure to mercury was considerably lower than that of the aforementioned American cohort. For the Norwegian workers, mean mercury secretion was approximately 100 μg/L urine during their work period (Mathiesen *et al*., 1999).

6.1.2.4 Effects on Fetal Brain Development

Fetal nerve tissue constitutes the cell type that shows most sensitivity to the mercuric ion. Clear effects on nerve growth arise at the concentration level 5–50 nmol/L or 1–10 ng/g tissue (Abdulla *et al*., 1995; Monnet-Tschudi *et al*., 1996; Söderström *et al*., 1995), which is the concentration level found in neonatal infants of amalgam-bearing mothers (Drasch *et al*., 1994; Lutz *et al*., 1996). Animal experimental studies on rats and primates have shown that exposure to mercury vapor gives rise to abortion, increased incidence of neonatal death, and developmental disorders in the brain resembling those seen after exposure to methylmercury. This means migration disturbances and permanent behavioral changes with a reduced learning and adaptation ability (Berlin *et al*., 1992; Danielsson *et al*., 1993; Newland *et al*., 1996; Warfvinge *et al*., 1994). The effects have been seen to arise from a mercury concentration in monkey fetus brains, which is at a 10 times lower concentration than required with exposure to methylmercury. In rats, it has been shown that methylmercury exposure and exposure to mercury vapor have an additive effect on fetal brain development (Fredriksson *et al*., 1996).

There are few reports from human experience. Two cases of mercury vapor exposure during pregnancy with adverse outcomes have been reported (Carmona, 1982; Derobert and Tara, 1950). In one case, the child survived but with signs of brain damage. In the other case, a first pregnancy resulted in abortion and a second pregnancy in neonatal death, but after recovery from overt mercury poisoning the woman gave birth to a healthy child.

A study of 349 pregnant women exposed to mercury vapor at the workplace (no concentration of mercury was specified) showed offspring with decreased birthweight and increased infiltration with lytic lymphocytes in placenta compared with 215 unexposed women (Mishonova *et al*., 1980). By use of a case-reference design, 306 cases of mentally retarded Dutch children with unknown causes were studied. The mothers' occupational exposure during the later stages of pregnancy was compared between the cases and 322 reference children who were mentally retarded for known reasons. A significantly increased odds ratio (8.7) was found for mothers of the cases to have been exposed to mercury during late pregnancy (Roeleveld *et al*., 1993).

6.1.2.5 Sensitive Groups

A large proportion of populations in both developed and developing countries have dental amalgam restorations. In most people, this exposure causes no known adverse health effects. However, mercury, as with other potent substances or pharmaca, is likely to induce more serious side effects among sensitive persons defined on a genetic basis. Several reports in the literature describe patients with neuropsychological symptoms who, during removal of amalgam restorations and for some days after, experience an exacerbation and exhibit conditions of illness (Berlin, 2003). These symptoms fade when the exposure to mercury concomitant from removal of the amalgam has ceased and return after renewed exposure. Such mercury-sensitive patients have been subject to blind provocation tests with inhalation of low concentrations of mercury vapor in air (Stromberg *et al*., 1999) or percutaneous patch tests with mercury or mercury compounds (Marcusson, 1996; Marcusson and Jarstrand, 1998; Marcusson *et al*., 2000). These tests have confirmed the occurrence of high sensitivity to mercury in these patients.

6.1.2.6 Other Organs

There is no evidence that the toxic effects of mercury on organs other than the nervous system, such as the kidneys, the immune system, and endocrine glands, differ between exposure to mercury vapor and exposure to mercuric mercury. Therefore, these effects are discussed later under "Effects of Mercuric Mercury."

6.1.3 Indicators of Exposure and Concentration in the Critical Organ

The mercury concentration in urine per gram creatinine is strongly correlated to the mercury concentration in blood (Smith *et al*., 1970). Thus, the mercury concentration in urine per gram creatinine can be used as an index of recent exposure in workers (Aito *et al*., 1983; Langworth, 1992; Lindstedt *et al*., 1979; Piotrowski *et al*., 1975; Smith *et al*., 1970; Yoshida, 1985). Piotrowski *et al*. (1975) observed a 24-hour variation in mercury excretion that was confirmed by Aito *et al*. (1983). The latter authors, as well as Suzuki *et al*. (1968), found a strong correlation between mercury excretion and creatinine excretion in urine. All these authors reported considerable individual variations in mercury excretion.

It has been shown in animal studies that during continuous exposure, the mercury concentrations in blood and urine are not correlated to concentrations of mercury in brain or kidney (Berlin *et al*., 1969a). Recent exposure is the dominant factor determining mercury concentration in blood and urine (Berlin and Gibson, 1963). Clinical and animal studies have shown that there is a fairly good correlation between ongoing exposure and mercury concentration in urine and blood and that the kidneys contain a large part of the body burden of mercury. It has been shown that renal tubular cells take up mercury from blood, release mercury into the blood, and that mercury is secreted into the proximal tubular lumen and reabsorbed at the distal part of the nephron (see Section 8.2.1) These observations are consistent with the hypothesis that the kidney under physiological conditions maintains a constant relative mercury concentration gradient between blood and distal tubular urine. This also explains why there is a good correlation between blood and urine mercury correlation irrespective of renal load of mercury. The hypothesis is supported by the observation by Cherian *et al*. (1978) that after exposure to radioactive mercury vapor, the specific activity of mercury in urine was lower than that in blood, indicating a dilution with inactive mercury in the renal storage compartment. To what extent renal mercury load contributes to blood mercury concentration and whether the contribution occurs through tubular reabsorption or by direct release into the blood from the storage compartment are so far not studied.

At present, there is no suitable biological index of the mercury concentration in critical organs such as brain and kidney. Studies concerning the relationship between mercury concentration in blood and mercury concentration in brain and/or kidney under conditions of no known exposure are not yet available.

For ongoing exposure, the mercury concentrations in blood plasma and urine are the optimal indices.

6.1.4 Dose-Response Relationships

Dose-response relationships have been studied in which the dose was determined by the degree of exposure or by the mercury concentration in either blood or urine (absorbed dose). Results from all three approaches are summarized in the following. However, it is uncertain to what extent levels of mercury in air reflect actual exposure. Factors such as the release of mercury vapor from mercury-contaminated clothes, temporary, and spot-wise exposure to high levels in connection with special work operations, and variations in physical load that change the rate of lung ventilation and limit the value of mercury concentrations in the air as measurements of exposure. Mercury concentrations in blood and urine are influenced by recent exposure and the body burden of mercury from earlier exposure. The relative contribution of these two parameters for determining levels of mercury in blood and urine is still poorly understood. The level of mercury in urine is also affected by the physiological variation in metabolism.

6.1.4.1 Relationship Between Mercury in Air and Effects

Garnier *et al*. (1981) reviewed approximately 20 cases of acute exposure to mercury vapor, and Milne *et al*. (1970) described four cases of pneumonitis after exposure to mercury vapor. The mercury levels exceeded 1–3 mg/m^3 for a few hours. Available epidemiological investigations (Friberg and Nordberg, 1972) indicate that at long-term exposure to mercury concentrations in air of 0.1 mg/m^3 or higher, the probability of persons manifesting typical mercurialism with tremor and behavioral changes will increase. At concentrations of 0.1 mg/m^3 or lower, the probability of encountering cases of micromercurialism or the asthenic syndrome decreases. There has as yet not been substantial accumulation of evidence or any one conclusive report concerning incidence of poisoning at exposure concentrations <0.01 mg/m^3 mercury in air. Smith *et al*. (1970) reported the prevalence of certain medical findings in relation to mercury exposure as shown in Figure 2.

Gambini (1978) reported on a study on two groups of 145 and 129 workers at chloralkali factories. An increased prevalence of tremor and micromercurialism at urine mercury concentrations exceeding 50 µg/L was found. The author also found that this urine-mercury concentration corresponded to a mercury concentration in air of 0.05 mg/m^3. These figures are consistent with the results reported by Lindstedt *et al*. (1979) and

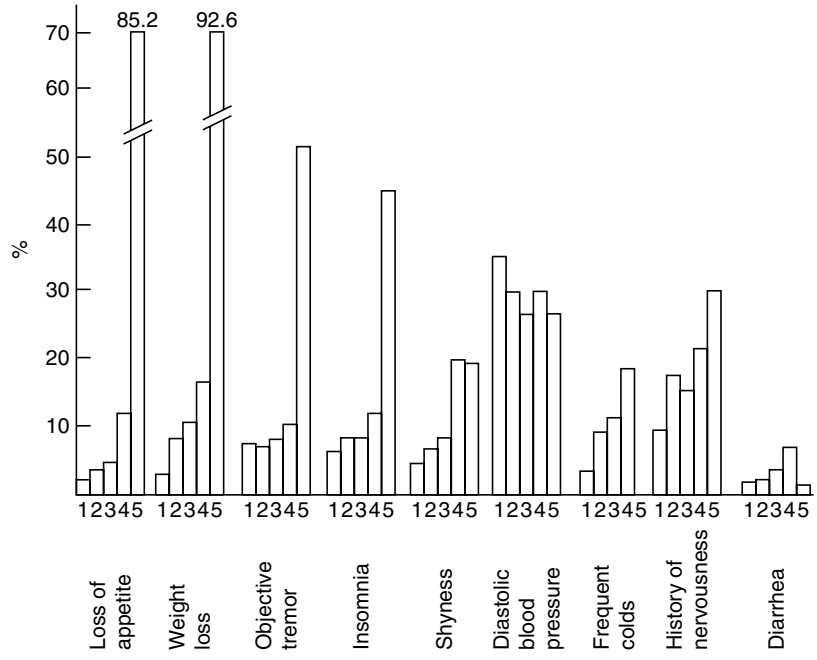

FIGURE 2 Percentage prevalence of certain signs and symptoms among workers exposed to mercury in relation to degree of exposure (From Smith *et al.*, 1970). Data on diastolic blood pressure are probably mean percentages below a certain level. This level is not given in the article. 1: Control. 2: Exposure below 0.01–0.05 mg/m^3. 3: 0.06–0.10 mg/m^3. 4: 0.11–0.14 mg/m^3. 5: 0.24–0.27 mg/m^3.

Tejning *et al*. (1958). Gambini also noted that there was a tendency for the increase of mercury excretion in urine to be greater than the increase of mercury concentration in air. The best fit of the data was an exponential curve. This is probably because the relative contribution of fecal excretion decreases. Lindstedt *et al*. (1979) found that a mercury concentration in air of 50 μg/m^3 corresponded to a group mean value of 30–35 μg Hg/L blood.

6.1.4.2 Relationships Between Mercury in Urine or Blood and Effects

Although recent exposure to mercury vapor shows a close relationship to mercury levels in blood and urine, most epidemiological investigations on exposed populations have not shown more than a weak association between blood or urinary concentrations of mercury and the occurrence of clinical signs or the degree of effects for individual subjects. On a group basis, however, high levels of mercury in urine and blood may be associated with prolonged exposure to high concentrations of mercury vapor and thus a greater likelihood of signs of poisoning. In animal experiments (Berlin and Gibson, 1963) and in epidemiological studies (Benning, 1958; Smith *et al*., 1970), a correlation has been shown between levels of mercury in urine and levels in blood. It is, however, doubtful whether this relationship would be linear over an extended dose range. The quotient between mercury concentration in urine and in blood varies in some studies between 2 and 10 (Chaffin *et al*., 1973; Lindstedt *et al*., 1979; Sexton *et al*., 1978) reported a clear tendency for an increase of urinary excretion in relation to mercury concentration in blood with increasing mercury load. Smith *et al*. (1970) found that group mean values of 100 μg Hg/L blood corresponded to 300 μg Hg/L urine. The relationship between the mercury concentration in air and in urine and between mercury in air and in blood as it appears in the studies by Skerfving and Berlin (1985) can be seen in Figures 3 and 4. Wood *et al*. (1973) reported that tremor associated with mercury poisoning is characterized by an increased frequency and a change in power spectrum, changes that are reversible with decreasing mercury exposure. Vroom and Greer (1972) showed that with mercury poisoning electromyographic changes seem to be mainly characterized by an increase of polyphasic potentials.

Chaffin *et al*. (1973) studied the relationship between mercury excretion in urine and signs of mercury poisoning. They registered the tremor frequency in the forearm, recorded surface electromyograms (EMGs), and performed a number of psychomotor tests. Of these, finger and toe drumming tests seemed to be the most sensitive. These studies were performed on 142 workers in chloralkali factories. A dose-response

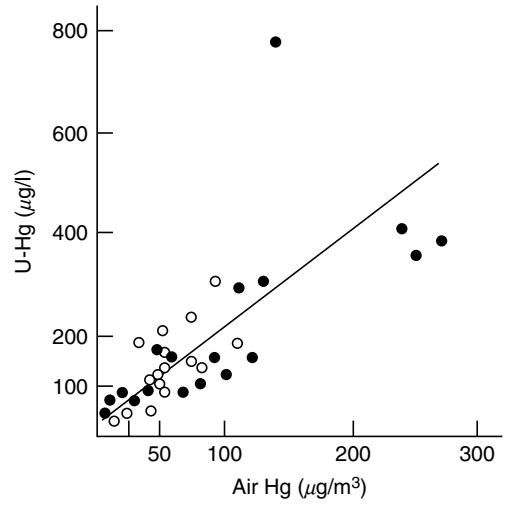

FIGURE 3 Concentration of mercury in urine (uncorrected for specific gravity) in relation to time-weighted a average exposure levels. Open circles are data from Lindstedt et al. (1979) and represent individual mean values. Black circles are data form Smith et al. (1970), representing group mean values. The regression line (y = 1.9x + 25) is that of smith et al. (1970) (Modified from Skerfving and Berlin, 1985).

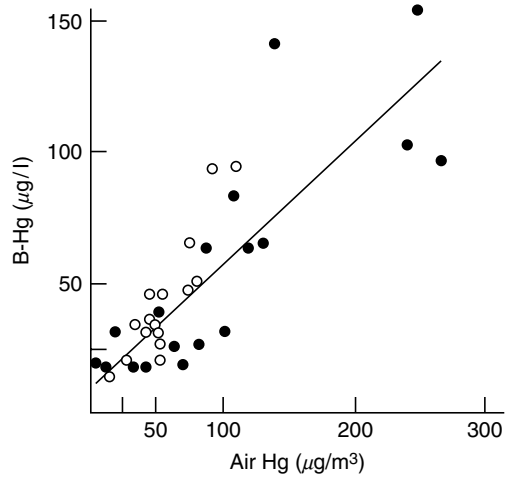

FIGURE 4 Concentration of mercury in blood (uncorrected for specific gravity) in relation to time-weighted a average exposure levels. Open circles are data from Lindstedt et al. (1979) and represent individual mean values. Black circles are data form Smith et al. (1970), representing group mean values. The regression line (y = 0.47x + 10) is that of smith et al. (1970) (Modified from Skerfving and Berlin, 1985).

relationship between tremor frequency and mercury excretion in urine was observed in these workers, as was a correlation between surface EMG and mercury excretion in urine. The mercury excretion in urine varied from a few micrograms per liter to >1000 μg/L. The most sensitive parameter was the tremor frequency, as can be seen in Figure 5, followed by the bandwidth of the surface EMG. It can be seen from the figure that with a urine excretion of Hg corresponding to 50 μg/L, there is a clear increase in tremor frequency. All parameters studied regressed with repeated testing when mercury exposure decreased. In a later study, 135 male workers at a chloralkali factory were followed for 1 year with monthly determinations of mercury concentrations in urine. Action was taken to terminate exposure for workers in which mercury excretion exceeded 500 μg/L in urine or to reduce exposure if the measure exceeded 250 μg/L. A mean excretion of mercury in urine of 250 μg/L with a standard deviation of 210 μg/L was reported, and a dose-response relationship was found between functional parameters such as tremor frequency, power spectrum of surface EMG in the forearm, and psychomotor tests (e.g., finger and toe drumming), and mercury excretion in urine. From a statistical analysis, the dose factor that had the highest correlation with the observed response trend was the number of occasions during the year in which mercury excretion exceeded 50 μg/L in urine. Two other studies in which the exposure was approximately 25 μg/g creatinine mercury in the urine reported dose-related effects (Echeverria *et al*, 1995; Liang *et al*, 1993), but none of these studies could rule out earlier exposure to higher doses.

In early 2002 a meta-analysis that reviewed 44 epidemiological studies of populations occupationally exposed to mercury vapor was reported. Twelve of these studies were included in the analysis, which was made up of 686 exposed persons and 579 controls. In nine neuropsychological performance parameters, statistically significant differences between exposed persons and controls were found, with a dose-response association for exposure corresponding to 18–34 μg Hg/L of urine (Meyer-Baron *et al*., 2002).

In an Italian multicenter study of 122 workers exposed to mercury vapor and 196 controls, a statistically significant decline in motor performance and a significant decrease in blood prolactin concentrations were found, with a dose-response relationship observed. Mean excretion of mercury in urine was 10.4 ± 6.9 μg/L for the exposed subjects and 1.9 ± 2.8 μg/L for the controls (Lucchini *et al*., 2002).

From these data it can be concluded that mercury-vapor exposure that gives rise to group mean values of mercury excretion in urine exceeding 100 μg Hg/L urine is most likely associated with adverse health effects in the form of signs of impairment of neurophysiological functions as tremor in forearms, surface-EMG changes, and changes in psychomotor tests such as finger and toe drumming. The available epidemiological data indicate that such effects can also be found at mercury excretion levels exceeding 10–30 μg Hg/L in urine. However, it

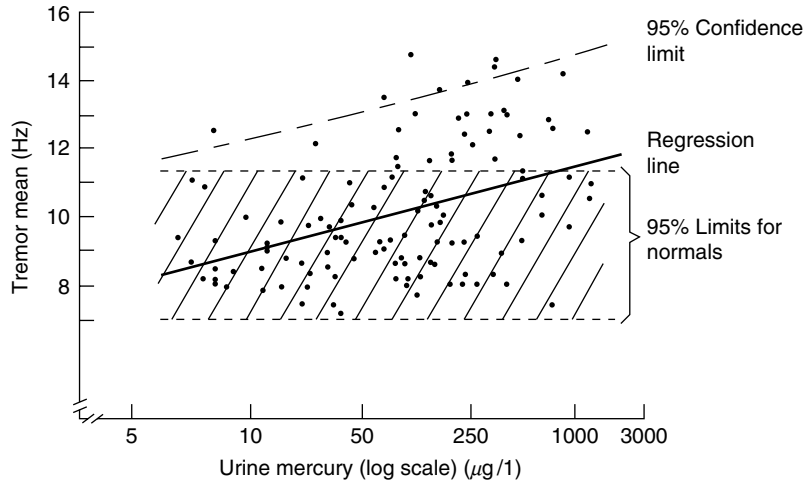

FIGURE 5 The relationship between mercury concentration in urine and mean tremor frequency of the dominating forearm when loaded with 15 pounds in 75 workers from chloralkali and instrument factories (Modified from Chaffin *et al.*, 1973).

is possible that that these results may be explained by accumulation of mercury in the brain at earlier periods of exposure. It is also to be expected that mercury concentrations in the CNS lag considerably behind the mercury excretion in urine. Published studies on a group of workers with low-level mercury exposures could not verify neurological effects at the measured low-level urinary concentration range (Gunther *et al.*, 1996). This may be a reflection of the time lag observed between mercury in urine and the onset of CNS effects. On the other hand, no available data contradict the fact that exposure that gives rise to mercury excretion in urine exceeding 30 μg Hg/L can lead to accumulation of mercury in the CNS and as a consequence gives rise to adverse health effects. It seems, therefore, reasonable to assume that an exposure level giving rise to detectable health effects in a group of exposed workers may fall somewhere between 10 and 30 μg/L.

6.1.4.3 Relationship Between Mercury in the Critical Organ and Effects

Reliable data on concentrations of mercury in the lung as a result of acute hazardous exposure to mercury vapor are not available in the literature. Data on mercury concentrations in the brain after exposure to mercury vapor are also scarce. Brigatti (1949) reported concentrations of 6–9 mg/kg in the brain of two persons exposed for several years to mercury vapor but who died of other causes. Both had shown pronounced signs of mercurialism some years before. Concentrations exceeding 0.5 mg/kg in the brain are not likely to occur in man in the absence of known mercury exposure (Mottet and Body, 1974).

In one case of chronic calomel (calomel releases Hg^0 *in vivo*, see Section 2) intake with signs consistent with mercurialism, Wands *et al*. (1974) reported mercury concentrations in the brain between 4 and 5 μg/g. Pathological changes with a decreased number of Purkinje cells and granule cells in cerebellum were also observed. Davis *et al*. (1974) reported, from a similar case, concentrations in the nucleus olivaris inferior of 106 μg/g and in the nucleus ruber of 14 μg/g. These values should not be considered unrealistic in view of the observations made by Nordberg and Serenius (1969) in guinea pigs exposed to mercury vapor—they reported high concentrations of mercury in these nuclei in the bottom of the fourth ventricle—and the mercury distribution studies in Squirrel monkeys (Warfinge *et al.*, 1994). On the other hand, in view of the difficulties in chemical mercury analysis, such values require further confirmation.

Takahata *et al*. (1970) reported 35 mg Hg/kg brain tissue in occipital cortex of a person with normal nonfatal mercurialism who died of other causes. The highest published value (Takahata *et al*., 1970) in a person without normal signs of classical mercurialism is 18 mg/kg in the substantia nigra. The mercury concentration ranged from 5–18 mg/kg in the rest of the brain. In studies on the rabbit (Ashe *et al*., 1953), concentrations of 1–2 mg/kg were reported to be associated with mild pathological changes in the brain, between 3 and 70 mg/kg with moderate pathological changes, and at <0.5 mg/kg no morphological changes were

observed. Fukuda (1971) exposed rabbits to mercury vapor and found the highest mercury concentrations in the cerebellum, tegmentum, and thalamus. In rabbits with a pronounced tremor, the concentration in brain was 4 μg/g. No morphological changes could be observed at this concentration. Kishi *et al*. (1978) tried to titrate the mercury concentration in the brain associated with behavioral changes in the rat using studies of conditioned avoidance reflexes. They observed clear effects at concentrations of 20 μg Hg/g brain tissue. These effects disappeared at approximately 10 μg/g. In squirrel monkeys, levels up to 8 μg/kg in the occipital cortex were observed (Berlin, 1976) in the absence of any definite morphological changes in the brain. However, the possibility of behavioral disturbances at these levels could not be precluded.

Sällsten *et al*. (1994) analyzed the mercury content in cerebrospinal fluid from 10 chloralkali workers and 16 nonexposed workers. The ongoing exposure was between 20 and 50 $\mu g/m^3$ and average urine excretion was 29 μg/g creatinine for the exposed workers. The concentration in CSF averaged 0.07 ng/mL (range, 0.018–0.17) for the referents and 0.13 ng/mL (range, 0.022–0.30) for the exposed. They found a significant correlation between plasma and CSF levels of mercury and observed a decrease in CSF mercury in two workers 6 months and 19 months, respectively, after the end of exposure.

6.1.4.4 Fetal Brain

Fetal nerve tissue contains the type of cell that shows the most sensitivity to the mercury ion Hg^{2+}. Clear effects arise at the concentration level of 5–50 nmol/L or 1–10 ng/g tissue (Abdulla *et al*., 1995, Monnet-Tschudi, 1998; Monnet-Tschudi *et al*., 1996; Söderström and Ebendal, 1995), which is the concentration level found in neonatal infants of amalgam-bearing mothers (Drasch *et al*., 1994; Lutz *et al*., 1996). Experimental studies on rats and primates have shown that exposure to mercury vapor gives rise to developmental disorders in the brain resembling those seen after exposure to methylmercury. This means migration disturbances and permanent behavioral changes with reduced abilities/capacity to learn and adapt (Berlin *et al*., 1992; Danielsson *et al*., 1993; Newland *et al*., 1996; Warfvinge *et al*., 1994). The effects are seen over a mercury concentration range in a monkey fetal brain of 50–300 ng/g brain tissue, which is 10 times lower than the concentration required with exposure to methylmercury. In rats, it has been shown that methylmercury exposure and exposure to mercury vapor has an additive effect on fetal brain development (Fredriksson *et al*., 1996). The prevalence of disturbed development in the experiments on monkeys ($n = 14$) was 100% in the studied dose range (0.6, 0.2, 0.1, and 0.06 mg Hg/m^3 of air during 24 hours) (Berlin *et al*., 1992; Newland *et al*., 1996; Warfvinge *et al*., 1994). With the two higher exposure levels, 50% of the pregnancies ended with abortion or neonatal death, whereas with the two lower exposure levels, 10% ended in neonatal death. Only normal pregnancy outcomes were observed for the lowest exposure level. All surviving offspring showed behavioral changes compared with unexposed controls. It is of the utmost importance to establish a LOAEL for the human fetal brain. Presently available information is not sufficient to allow risk evaluation. It is a factor of 10 between brain mercury concentration at the lowest exposure dose (50–100 ng/g) and the mercury levels reported for human fetal brains (2–9 ng/g). It is questionable whether this is enough to move from 100% response rate to less than 1%. There is no reason to assume that the human fetal brain would be less sensitive to mercury than other primate brains. Two cases of mercury vapor exposure during pregnancy and with normal pregnancy outcome are reported. In one case, the mother was occupationally exposed and had a urine mercury excretion of 875 μg/L at the 15th gestational week (Melkonian and Baker, 1988). The other case involved a woman exposed in her home to mercury vapor during the first 17 weeks of gestation. The child seemed to have developed normally as an infant (Thorp *et al*., 1992). It can be concluded from the animal experience and human reports that if there is an effect on human fetal brain development from maternal dental amalgams, the effect is likely to be limited to behavioral changes within the normal variation as seen in low-dose lead, alcohol, or PCB exposure. Such effects can only be detected on a group basis with epidemiological methods.

6.2 Mercuric Mercury

6.2.1 Metabolism

6.2.1.1 Absorption, Inhalation

No data are available.

6.2.1.2 Ingestion

Acute poisoning caused by accidental or intentional intake of mercuric chloride was not uncommon at the beginning of this century. Data from reports of such cases (Sollmann and Schreiber, 1936) and experimental studies (Clarkson and Shapiro, 1971; Miettinen, 1973; Rahola *et al*., 1973) indicate that approximately 2% of ingested mercuric chloride is absorbed. On high intake, the corrosive

action of mercuric chloride may alter the permeability of the gastrointestinal tract, enhancing absorption. In newborn rats, a more effective absorption of mercury has been reported (Kostial *et al*., 1978). Data from human newborns are not available. See section on "Intestinal Handling of Hg^{2+}."

6.2.1.3 Skin

It has been demonstrated that on application of mercuric mercury to the human skin, penetration of mercury occurs, but the mechanism for this is unknown. Factors such as type and concentration of mercuric compound, as well as the condition of the skin, determine the rate of absorption (Wahlberg, 1965). In animal studies (Friberg *et al*., 1961), up to 8% of the mercuric chloride applied to the skin was absorbed within 5 hours. Silberberg (1971) studied the penetration of mercury through the human skin electromicroscopically. However, available data are insufficient for an evaluation of the importance of skin absorption relative to absorption by inhalation under conditions of occupational or pharmaceutical exposure.

6.2.1.4 Transport and Distribution

Mercuric mercury in blood is divided between erythrocytes and plasma with perhaps somewhat more in plasma (Suzuki *et al*., 1970). In erythrocytes, mercury is, probably to a large extent, bound to sulfhydryl groups on the hemoglobin molecule (Clarkson *et al*., 1961) and also possibly to glutathione (Nielsen-Kudsk, 1969). The distribution between different plasma-protein fractions varies with dose, time after administration, and type of administration (Cember *et al*., 1968; Farvar and Cember, 1969; Suzuki *et al*., 1967).

Mercuric mercury does not readily traverse the blood–brain barrier or the placental barrier. However, mercuric mercury does accumulate in the placenta (Mitani *et al*., 1978), fetal membranes, and amniotic fluid (Suzuki *et al*., 1977; Wannag and Skjaerasen, 1975). Normally, very little Hg^{2+} enters and accumulates in the placenta (Ask *et al*., 2002; Inouye and Kajiwara, 1990; Suzuki *et al*., 1967), and the mechanism(s) that mediate placental uptake of Hg^{2+} are poorly understood. Interestingly, experiments in brush-border membrane vesicles from human placenta suggest that an amino acid transporter may be involved in the uptake of Hg^{2+} in this organ (Iioka *et al*., 1987). These experiments demonstrated that the Na^+-dependent uptake of alanine was inhibited significantly by $HgCl_2$, indicating that one or more amino acid transporters may be involved in this uptake. Because these studies used $HgCl_2$ rather than an Hg-thiol complex, such as Cys-*S*-Hg-*S*-Cys, the results may not reflect accurately physiological processes that occur *in vivo*. They do provide, however, valuable preliminary data. On the basis of these data and the prevalence of amino acid transporters in the placenta (Jansson, 2001; Kudo and Boyd, 2002), we can postulate that Hg^{2+}, as a thiol-conjugate, mimics a structurally similar amino acid and is used as a substrate by one or more amino acid transporters. This proposed mechanism of molecular mimicry may be similar to that demonstrated in other tissues (e.g., system $b^{0,+}$ in proximal tubular cells, system L in endothelial and glial cells of the blood–brain barrier).

Miyama *et al*. (1968) observed an increased uptake of mercury in the brain after subcutaneous administration of sublimate compared with intravenous administration in the rabbit.

The rate of uptake from blood and different organs varies widely, so does the rate of elimination from different organs. The distribution of mercury within the body and within organs will thus vary widely with dose and time lapse after absorption. So far, no one has attempted to describe the steady-state distribution in any species. The distribution of mercuric mercury in the body is highly differentiated to specific organs and within the organs to specific cells. Under all conditions, however, the dominating mercury pool in the body is the kidney. Here, mercury is distributed at subtoxic doses to the peripheral part of the renal cortex corresponding to the convoluted part of the proximal tubuli (Taugner *et al*., 1966). A large proportion of the mercury in the kidney is soluble and bound to metallothionein (Cherian and Clarkson, 1976; Komska Szumska *et al*., 1976; Piotrowski *et al*., 1974).

The next largest mercury pool in the body is the liver with the highest concentration in the periportal areas (Berlin and Ullberg, 1963a). Other organs or cells where mercury is likely to accumulate are the mucous membranes of the intestinal tract and the epithelium of the skin, the spleen, the interstitial cells of the testicles, and some parts of the brain, as well as plexus choroidei in the brain (Berlin and Ullberg, 1963a).

6.2.1.5 Excretion

As mentioned in the section "Elimination and excretion," mercuric mercury is excreted by the kidney, the fecal route, sweat glands (Lovejoy *et al*., 1974), lacrimal glands (Donat, 1902), mammary glands (Perrin, 1911; Welander, 1886), and salivary glands (Joselow *et al*., 1968). The major part of absorbed mercuric mercury is excreted in urine and feces. Partition between these two routes is dose-dependent, and data indicate a larger fraction being excreted by urine on administration of higher doses (Lombholt, 1928). A small amount of mercuric mercury is reduced to mercury vapor and exhaled (Hursh *et al*., 1976).

Apart from the excretion through saliva, mercury is excreted by the liver through the bile (Klaassen, 1975) and also by the mucous membranes of the small intestines and colon (Berlin and Ullberg, 1963a). More is known about the mechanism of urinary excretion and renal uptake of mercury because < 1% of mercury in plasma is in ultrafiltrable form. It is uncertain whether there is an appreciable amount of mercury filtered through glomeruli at nontoxic doses of mercury. By injection of sublimate in the renal artery of the rat, Wessel (1967) demonstrated that mercury is taken up by the basal cells of proximal tubuli from the capillaries. Richardson *et al*. (1975) postulated that mercury is transported over the cell membranes of the tubular wall as an amino acid complex. They showed that administration of cysteine and glutathione increased the renal uptake of mercury. Transfer of mercuric mercury through the proximal tubules has been demonstrated in the avian kidney (Vostal and Heller, 1968). Animal data thus indicate that mercury is transported from blood to tubular cells and transferred to the tubular lumen. Mercury is stored in the kidney, a process not necessarily linked to the process of excretion. Animal experimental data indicate that excretion is more correlated to blood concentration of mercury than to the mercury load in the kidney (Berlin and Gibson, 1963).

The total excretory capacity of the body is illustrated by the elimination rate for the whole body. It has been demonstrated in rats (Rothstein and Hayes, 1960) that the elimination curve is best described as a multiphase exponential curve, having a rapid phase with a half-time of approximately 5 days, another phase with a half-time of approximately a month, and still another with a half-time of approximately 3 months. Experimental studies do not contradict a similar human elimination pattern, although thus far, only the more rapid phases have been examined. Rahola *et al*. (1971) found a half-time of 42 days for 80% of the absorbed amount after an oral tracer dose in man. The half-time of the remaining 20% could not be determined.

6.2.1.6 Renal Transport and Handling of Hg^{+2}

The kidney is the primary target organ that takes up and accumulates mercuric ions from the blood (rev. by Zalups, 2000a). The uptake and accumulation of mercuric species in the kidneys occur very rapidly after exposure. For example, as much as 50% of a nontoxic dose of inorganic mercury has been shown to be present in the kidneys of rats within a few hours after exposure (Zalups, 1993). Within the kidneys, the segments of the proximal tubule are the principal portions of the nephron responsible for transporting and accumulating mercuric ions.

There is strong evidence implicating multiple mechanisms in the proximal tubular uptake and handling of Hg. At least one of these mechanisms is localized in the luminal membrane (Bridges and Zalups, 2005; Bridges *et al*., 2004; Zalups, 1995; 1997; 1998a,b; Zalups and Barfuss, 1993b; 1998a; Zalups and Lash, 1997b; Zalups and Minor, 1995; Zalups *et al*., 1991; 1998), and at least one other is localized in the basolateral membrane (Aslamkhan *et al*., 2003; Zalups, 1995; 1997; 1998a; Zalups and Ahmad, 2004; Zalups and Barfuss, 1993b, 1995, 1998a; Zalups and Lash, 1997b; Zalups and Minor, 1995; Zalups *et al*., 2004).

Various lines of evidence indicate that the preponderance of the luminal uptake of mercuric ions in the proximal tubule is at least partially dependent on the actions of γ-glutamyltransferase and cysteinylglycinase, which are present in the luminal plasma membrane of proximal tubular epithelial cells. A number of *in vivo* experiments have shown that chemical inhibition of γ-glutamyltransferase reduces significantly the renal (proximal) tubular uptake and increases the urinary excretion of systemically administered Hg (Berndt *et al*., 1985; de Ceaurriz *et al*., 1994; Tanaka *et al*., 1990; Tanaka-Kagawa *et al*., 1993; Zalups, 1995). The findings from these studies have led to the hypothesis that GSH *S*-conjugates of inorganic mercury (G-*S*-Hg-*S*-G) or organic mercury (mainly methylmercury (CH_3Hg-*S*-G) that enter the luminal compartment of the proximal tubule are degraded rapidly and efficiently by γ-glutamyltransferase and cysteinylglycinase to yield thiol *S*-conjugates of Hg^{2+}, including Cys *S*-conjugates of Hg^{2+} (Cys-*S*-Hg-*S*-Cys) and CH_3Hg^+ (CH_3Hg-Cys). Indeed, studies in brush-border membrane vesicles (isolated from the renal cortex and outer stripe of the outer medulla of rats) indicate that mercuric ions are taken up more readily when they are in the form of Cys-*S*-Hg-*S*-Cys than when they are in the form of G-S-Hg-S-G (Zalups and Lash, 1997b). Moreover, studies in suspensions of rabbit proximal tubules (Wei *et al*. 1999; Zalups *et al*., 1993) and isolated perfused proximal tubules from rabbits (Cannon *et al*., 2000; 2001) have provided additional evidence for the luminal uptake of Cys-*S*-Hg-*S*-Cys.

Because Cys-*S*-Hg-*S*-Cys is similar structurally to the amino acid cystine (Cys-*S*-*S*-Cys), Zalups and colleagues hypothesized that Cys-*S*-Hg-*S*-Cys can serve as a molecular mimic of cystine at the site of one or more luminal transporters of this amino acid. Experiments in isolated perfused proximal tubular segments from rabbits provide substantive evidence that amino acid transporters are, indeed, involved in the luminal uptake of Cys-*S*-Hg-*S*-Cys (Cannon *et al*., 2001). The findings from these experiments demonstrate that there is at least one Na^+-dependent and one Na^+-independent carrier involved in the luminal transport of this mercuric conjugate along the proximal tubule.

Possible amino acid transporters involved in the Na^+- dependent transport of Cys-*S*-Hg-*S*-Cys include systems B^0, $B^{0,+}$, and/or ASC, although direct evidence supporting the involvement of these specific carriers is currently lacking. One transporter that seems to be involved in the Na^+-independent transport of Cys-*S*-Hg-*S*-Cys is system $b^{0,+}$. This heterodimeric transporter, which is composed of the subunits, $b^{0,+}$AT, and 4F2hc (Palacin, 1998; 2001), has a high affinity for cystine, as well as other neutral and basic amino acids. Recent direct molecular findings from renal epithelial cells transfected stably with system $b^{0,+}$ indicate that this transport system can, indeed, mediate the luminal uptake of Cys-*S*-Hg-*S*-Cys (Bridges *et al*., 2004). Analysis of substrate specificity showed that the uptake of Cys-*S*-Hg-*S*-Cys and cystine are inhibited by the same amino acids, indicating that these two molecular species are substrates of the same carrier (i.e., system $b^{0,+}$). Additional findings from the transfected cells indicate that this carrier (Bridges *et al*., 2004) does not readily transport mercuric conjugates of GSH (G-S-Hg-S-G), *N*-acetylcysteine (NAC-S-Hg-S-NAC), or cysteinylglycine (CysGly; CysGly-S-Hg-S-CysGly). The ability of system $b^{0,+}$ to transport Hcy *S*-conjugates of Hg^{2+} (Hcy-*S*-Hg-*S*-Hcy) has also been tested recently in these transfected MDCK cells. Because Cys-*S*-Hg-*S*-Cys and Hcy-*S*-Hg-*S*-Hcy are structural homologs, Zalups and Bridges hypothesized that they are both substrates of the same transporter(s). In a separate study that used renal epithelial cells transfected with system $b^{0,+}$, Hcy-*S*-Hg-*S*-Hcy was shown to be a transportable substrate of system $b^{0,+}$ (Bridges and Zalups, 2004). Collectively, these data provide the most concrete lines of evidence to date, which support the hypothesis that Cys-*S*-Hg-*S*-Cys and Hcy-*S*-Hg-*S*-Hcy serve as molecular mimics of the amino acids cystine and homocystine, respectively, at the site of system $b^{0,+}$.

In addition to the uptake of inorganic mercury at the luminal plasma membrane, approximately 40–60% of the renal burden of Hg is taken up by one or more transporters located at the basolateral membrane (Zalups, 1995; 1997; 1998a,b; Zalups and Barfuss, 1995; 1998a,b; Zalups and Minor, 1995). A significant portion of the basolateral uptake of Hg can be inhibited by *para*-aminohippurate (PAH), which is a specific inhibitor of the organic anion transporter (OAT) transporters (Ferrier *et al*., 1983; Pritchard, 1988; Roch-Ramel *et al*., 1992; Shimomura *et al*., 1981; Ullrich *et al*., 1987a,b). Thus, most of the basolateral uptake of Hg is likely mediated by one or more OATs, which are multispecific carriers that mediate the uptake of a wide variety of substrates (Wright and Dantzler, 2003). Current evidence implicates only OAT1 and OAT3 in the uptake of inorganic mercury. Both of these transporters are localized in the basolateral plasma membrane of proximal tubular epithelial cells (Kojima *et al*., 2002; Motohashi *et al*., 2002). The preponderance of current evidence indicates that OAT1 is the major mechanism for the uptake of inorganic mercury at the basolateral plasma membrane of proximal tubular cells (Zalups, 1995; 1997; 1998a,b; Zalups and Barfuss, 1995; 1998a,b; 2000; Zalups and Lash, 1994; Zalups *et al*., 1998).

Recent findings from Madin–Darby canine kidney (MDCK) cells (which are derived from the distal nephron and normally do not express OAT1), which were transfected stably with OAT1, show that various mercuric conjugates of inorganic mercury and methylmercury, including Cys-*S*-Hg-*S*-Cys (Zalups *et al*., 2004), NAC-*S*-Hg-*S*-NAC (Aslamkhan *et al*., 2003), Hcy-*S*-Hg-*S*-Hcy (Zalups and Ahmad, 2004), CH_3Hg-*S*-Cys (Zalups and Ahmad, 2005a), and CH_3Hg-*S*-Cys (Zalups and Ahmad, 2005b) are substrates of this transporter (Figure 6). OAT1 and OAT3 have also been implicated in the transport of Cys-*S*-Hg-*S*-Cys in oocytes from *Xenopus laevis* altered at a molecular level to express these two transporters (Aslamkhan *et al*., 2003; Zalups *et al*., 2004). A significant body of recent molecular evidence indicates that the mercuric conjugates of Cys, Hcy, and NAC are taken up by means of a mechanism involving molecular mimicry.

6.2.1.7 Intestinal Handling of Hg^{2+}

Transport of Hg occurs along the intestine after ingestion of food and/or liquids contaminated with Hg. Consequently, understanding the mechanisms involved in the intestinal absorption, accumulation, and excretion of Hg is important. It has been suggested by Foulkes (2000) that the luminal uptake of inorganic forms of Hg in the intestine depends on the composition of the contents in the intestinal lumen. Inasmuch as there is an abundance of carrier proteins, such as amino acid and peptide transporters, in the enterocytes lining the small intestine (Ganapathy *et al*., 2001), it is reasonable to hypothesize that inorganic and organic forms of mercury are taken up by one or more of these carriers. Surprisingly, even though the intestine seems to be an important site for the entry of Hg into the body, very little is known about the mechanisms involved in the gastrointestinal handling of this metal.

On the basis of experiments in which sections of rat duodenum, jejunum, ileum, and stomach were perfused with $HgCl_2$ for various time intervals, it seems that the duodenum is the primary site for the absorption of mercuric ions within the gastrointestinal tract (Endo *et al*., 1984). Interestingly, biliary ligation has been shown to decrease the absorption of inorganic mercury along the duodenum of rats. Moreover, when bile was delivered into the luminal compartment with

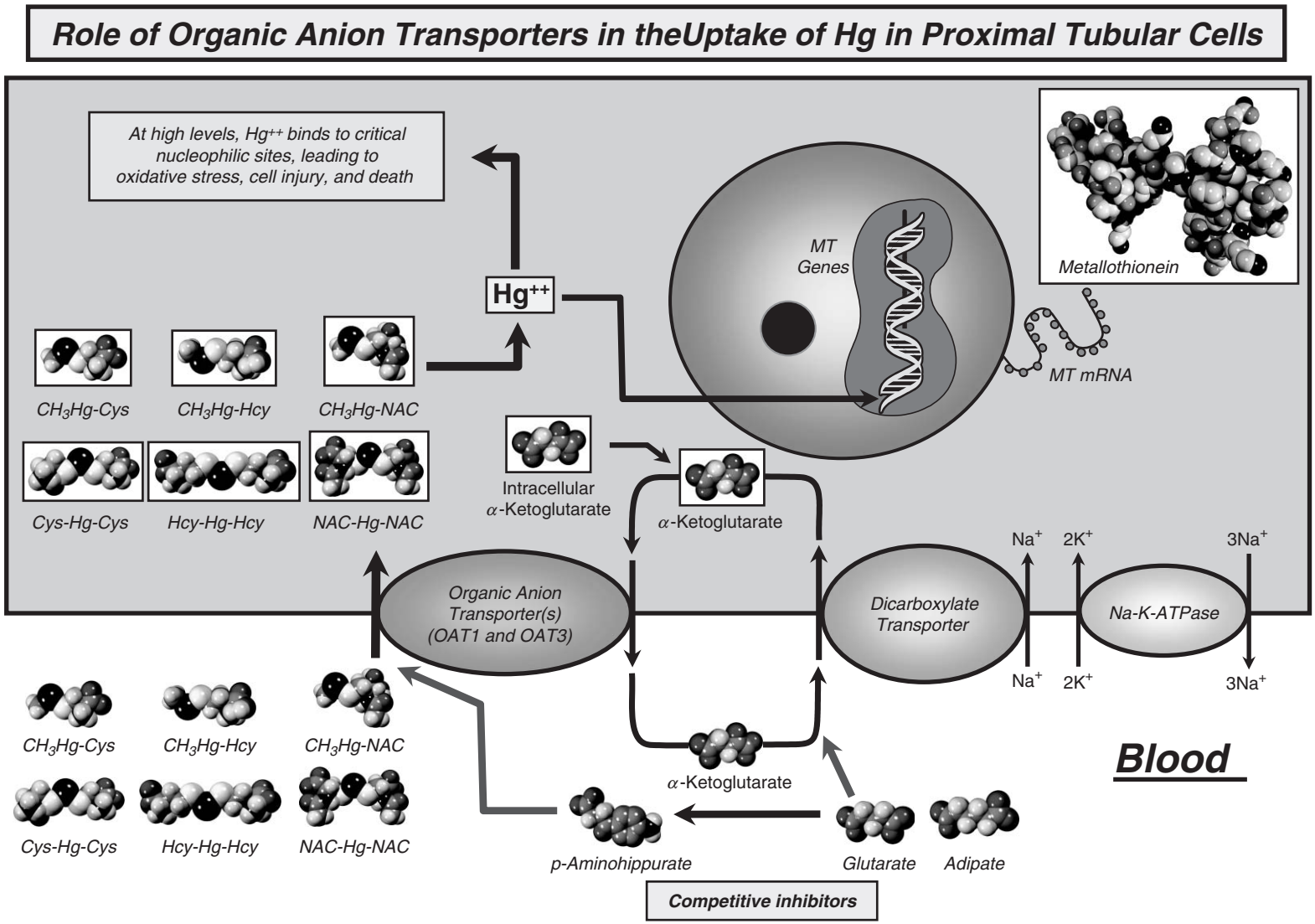

FIGURE 6 Diagrammatic representation of the putative roles of both the organic anion and dicarboxylic acid transport systems in the basolateral uptake of thiol *S*-conjugates of inorganic mercury and methylmercury along the proximal tubule. On the basis of the current line of evidence regarding the organic anion transport system, intracellular generation of α-ketoglutarate (resulting from normal metabolic processes) creates a chemical gradient facilitating the movement of this dicarboxylate out of the cell. When intracellular concentrations of α-ketoglutarate are high enough, this dicarboxylate exits proximal tubular cells at the basolateral membrane by exchanging with organic anions. After it is transported out of proximal tubular cells, α-ketoglutarate is reclaimed through a symport involving the cotransport of sodium. This symport is driven by the sodium-gradient generated by the Na^+-K^+–ATPase. Current evidence indicates that both inorganic mercury (Hg^{2+}) and methylmercury (CH_3Hg^+) can enter proximal tubular epithelial cells as conjugates of cysteine (Cys), homocysteine (Hcy), or *N*-acetylcysteine (NAC) through the organic anion transport system in exchange for intracellular α-ketoglutarate. Support for this notion comes from the fact that the basolateral uptake of mercury can be inhibited by *p*-aminohippurate (PAH), probenecid, and certain dicarboxylic acids. Once inorganic or organic mercuric ions gain entry into the proximal tubular epithelium, they can induce metallothionein I and II, which are believed to provide a protective sink for certain divalent cations to bind. When the cellular burden of mercury becomes too great for the proximal tubular epithelial cells to handle, cellular intoxication and then death occur.

$HgCl_2$, the duodenal absorption of inorganic mercury increased to levels similar to those observed in control rats. An alkaline pH of the luminal contents also seems to promote the uptake of inorganic mercury in the duodenum (Endo *et al*., 1984; 1986). This suggests that alkalinity increases the absorption of mercuric ions across the intestinal epithelium, although the mechanism for this effect is currently not known.

Recent studies in blue crabs have provided some insight into the mechanisms involved in the intestinal uptake of inorganic mercury. The findings from these studies indicate that there are passive and active mechanisms involved in the uptake of mercuric ions across plasma membranes of enterocytes (Andres *et al*., 2002; Laporte *et al*., 2002). It is likely that amino acids and peptides present in digested food bind to inorganic mercury and that the complexes formed are taken up by active and/or facilitated mechanisms involving amino acid and/or peptide transporters. Because amino acid transporters have been implicated in the transport of Cys-*S*-Hg-*S*-Cys in renal proximal tubular cells, it seems reasonable to postulate that a similar mechanism may play a role in the intestinal absorption of Hg. However, direct evidence supporting such a mechanism has yet to be provided.

The intestine also plays an important role in the elimination of Hg. Two mechanisms seem to be involved in the fecal elimination of Hg^{2+}: (1) transcellular and/or paracellular secretion of Hg^{2+} through enterocytes, and (2) delivery of Hg^{2+} into the intestinal lumen through bile (Zalups and Lash, 1994). Data from *in vivo* studies in rats with cannulated or ligated (Zalups, 1998c) bile ducts indicate that intestinal secretion of Hg^{2+} from the blood into the lumen of the intestine accounts for a substantial fraction of the total pool of Hg^{2+} that is excreted in the feces. Until these studies, it had been assumed that biliary secretion of Hg^{2+} was the principal mechanism involved in the fecal elimination of systemic Hg^{2+}. The intestinal secretion of Hg^{2+} may involve the transport of an Hg^{2+}-thiol complex, which could act as a mimic or a structural homolog of an endogenous molecule normally secreted by enterocytes. Amino acid transporters are potential mechanisms for this secretion. Given that many of these transporters are actually counterexchangers, they have the potential to transport substrates both into and out of cells. Consequently, Hg^{2+}-thiol complexes, such as Cys-*S*-Hg-*S*-Cys, may use these carriers to enter and exit enterocytes.

6.2.1.8 Hepatic Handling of Hg^{2+}

The transport of Hg^{2+} across the sinusoidal membrane into hepatocytes is not well defined. Hepatocytes contain some of the same transporters that have been implicated in the transport of Hg^{2+} in other organs, including a transporter of G*SS*G and numerous amino acid carriers. Therefore, it can be postulated that these transporters may be involved in the uptake of Hg^{2+} across the sinusoidal membrane of the hepatocytes. It has been established that Hg^{2+} forms complexes with GSH and/or amino acids, such as Cys and Hcy. Although transporters for G*SS*G (multidrug resistance proteins 1 and 2) have been identified in the canalicular membrane (Akerboom *et al*., 1984; 1991; Keppler *et al*., 1998; Leslie *et al*., 2001), they have not been localized in the sinusoidal membrane. Furthermore, various amino acid carriers have been identified in the liver (Bode, 2001; Wagner *et al*., 2001); however, it is presently unclear whether these carriers are present on the sinusoidal membrane.

Much more information is available on the transport of Hg^{2+} across the canalicular plasma membrane of hepatocytes. Several studies have provided important data regarding potential mechanisms involved in the hepatocellular export of Hg^{2+} across the canalicular membrane. The prevailing theory regarding this export implicates a mechanism by which the transport of Hg^{2+} depends on the cytosolic concentration of GSH (Ballatori and Clarkson, 1983; 1984; 1985a,b; Dutczak and Ballatori, 1992). Subsequent *in vivo* studies, in which hepatocellular concentrations of GSH were reduced by pretreatment with buthionine sulfoximine (BSO) or diethylmaleate (DEM), offer additional evidence supporting the role of GSH for the transport of Hg^{2+} into the canalicular compartment (Zalups and Lash, 1997a). It seems that in the absence of adequate levels of cytosolic GSH, mercuric ions are unable to exit the hepatocyte, which leads to increased cytosolic accumulation of Hg^{2+}. Although the actual mechanism(s) responsible for the transport of mercuric ions from within hepatocytes across the canalicular plasma membrane has/have not been demonstrated directly, experimental evidence indicates that Hg^{2+} bonds GSH to form G-*S*-Hg-*S*-G, which seems to be transported into the biliary canalicular compartment. Because G-*S*-Hg-*S*-G is similar structurally to G*SS*G, this mercuric complex may serve as a molecular mimic of G*SS*G at the site of a GSSG transporter. However, a definitive role for a transporter of G*SS*G in the transport of Hg^{2+} has yet to be explained. This transporter may be one of the multiple drug-resistance proteins (MRPs), which apparently mediate the transport GSH S-conjugates (Leslie *et al*., 2001; Suzuki and Sugiyama, 1998).

6.2.2 Symptoms and Signs in Poisoning Caused by Mercuric Salts

6.2.2.1 Acute Poisoning

The critical organs for accidental or suicidal ingestion of sublimate or other mercuric salts are the kidneys and the intestinal tract. The corrosive effect of concen-

trated mercuric salt solution on the mucous membranes of the gastrointestinal tract causes extensive precipitation of proteins. Gastric pain and vomiting may ensue. If the salt is allowed to pass the lower regions, general abdominal pain and bloody diarrhea with necrosis of the intestinal mucosa will occur. This may lead to circulatory collapse and death. If the patient survives the gastrointestinal damage, the critical organ will be the kidney. Within 24 hours, renal failure caused by necrosis of the proximal tubular epithelium, which develops into anuria and uremia, occurs. Apart from the mechanisms underlying the tubular necrosis, there may be a local vasospasm, because of activation of the angiotensin system (Barnes *et al*., 1980a,b).

6.2.2.2 Chronic Poisoning

Chronic poisoning caused exclusively by mercuric mercury salts is uncommon. Most chronic exposure involves a mixture of mercury vapor and mercuric mercury. This is the case at least in occupational exposure. In some parts of the world, calomel is still used as a purgative. Cases of chronic poisoning with calomel intake have been described that exhibited signs and symptoms similar to those of poisoning with mixed exposure to mercury vapor and mercuric mercury (Wands *et al*., 1974). It can be assumed that mercurous mercury is oxidized in the gastrointestinal tract to mercuric mercury under the formation of mercury vapor, which can be physically dissolved in blood and transported to the brain. At present, there are only indirect indications of such a process. With chronic exposure to mercuric mercury, the kidney is the critical organ from a human health and survival point of view. However, it may not always be the target in the body that has the lowest LOAEL. This is especially true for sensitive groups because of genetic polymorphism. Other targets in the body are the immune system, muscle tissues, and the thyroid. Symptoms can also appear in the form of increased salivation and inflammatory changes of the gums and black lines on the teeth because of precipitation of mercuric sulfide.

6.2.2.3 Renal Effects

Two types of renal damage can occur:

1. A glomerular injury caused by the toxic effect of mercury on the cells of the basal membrane of the glomeruli. An autoimmune reaction is induced because of the formation of antigen against the glomerular tissue, and a nephrotic syndrome develops with proteinuria and the classical signs of glomerular nephritis. Hunter *et al*. (1932) observed, in animal experiments on rabbits and monkeys, that injection of mercuric chloride gives rise to morphological changes in the glomerular basal membrane. Sapin *et al*. (1977) reported that mercuric chloride administration induced antibodies against the basal membrane of the glomeruli of the rat. The same effects have been observed in the cat (Shull *et al*., 1981). Weening *et al*. (1980) identified mercury-induced antibodies in the rat against glomerular tissue as a nuclear protein belonging to the non-histone-chromatin protein fraction.

2. The other type of renal injury is tubular damage. Mercury accumulation causes necrosis and damage of the distal and middle portion of the proximal tubuli. If glomerular damage develops parallel to this, the resorption of mercury from protein-bound filtered mercury may also take place in distal parts of the renal tubules, with pronounced necrosis also in these parts as a consequence (Barnes *et al*., 1980a,b; Grizka and Trump, 1968; Wessel, 1967).

In the renal tubules, an early effect of mercury is an inhibition of the protein synthesis. Ultrastructurally, a swelling of the endoplasmic reticulum with disaggregation of polyribosomes has been seen in the mouse (Pezerovit *et al*., 1981). Experiments on rats indicate a considerable sex difference in sensitivity of the kidney to mercury. In female rats, the number of sulfide groups present is greater than in male rats. This seems to be related to the testosterone concentration in blood. Thus, male rats developed renal damage at lower doses than female rats (Muraoka and Hoh, 1980).

Glomerular damage has also been observed in man. Kazantzis *et al*. (1962) reported three cases of nephrotic syndrome in a group of mercury workers. They interpreted these cases as immunological reactions because the subjects did not show any signs of mercurialism or high mercury excretion in urine. In two cases, renal biopsies were carried out and characteristic light microscopic changes in the glomeruli were seen. Later, several reports appeared in the literature describing cases of nephrotic syndrome associated with exposure to mercury compounds in which biopsies have shown typical changes of glomeruli nephritis (Becker *et al*., 1962; Cameron and Trounce, 1965; Strunge, 1970). Electron microscopic studies support an immunological genesis to glomerular nephritis after mercury exposure (Hilton *et al*., 1968; Kibukamusoke *et al*., 1974; Lindqvist *et al*., 1974; Mandema *et al*., 1963). Deposition of IgG- and CT-complement in the glomeruli membrane has been demonstrated by use of immunofluorescence techniques (Kibukamusoke *et al*., 1974; Lindqvist *et al*., 1974).

Renal tubular acidosis has been described in children caused by exposure to mercuric salts from the application of ointment containing ammoniated mercury. A similar syndrome has been described in connection with so-called teething powder (Husband and McKellar, 1970).

6.2.2.4 The Immune System

A more detailed discussion of the effect of mercuric mercury on the immune system can be found in Chapter 12. Only some clinical reports are reviewed here.

Idiosyncratic reactions to mercury and mercury compounds on local contact have been described by many authors. Cell-mediated immune responses with skin manifestation have been seen in connection with mercury applied locally to skin and mucous membranes. Typical manifestations are erythema and contact dermatitis. Thiomersal and ammoniated mercury are common causes (North American Contact Dermatitis Group, 1973). Exposure to metallic mercury may give rise to sensitization, for example, from amalgam teeth fillings and other dental exposure (Feuerman, 1975; Finne *et al*., 1982; Frykholm, 1957; Spreng, 1963; Strassburg and Schubel, 1967; White and Brandt, 1976). One side effect of amalgam fillings that is not particularly unusual is oral lichen. Larsson (1998) describes accumulation of mercury in the tissue affected and accumulation of dendritic cells. Little *et al*. (2001) showed that a culture of human oral keratocytes, on exposure to subtoxic concentrations of $HgCl_2$ (10 μmol/L), expresses ICAM-1, which in turn induces T-cell binding, release of TNF-α, and interleukin-8 and downregulation of interleukin-1α. This induces activation of the immune system, which is not seen in experiments with cutaneous keratocytes. Bronchial asthma has also been reported in connection with exposure to phenylmercury (Mathews, 1968).

In an examination of 48 workers exposed to mercury with an average elimination in the urine of 24 μg/g creatinine, a significant reduction of chemotactic migration and chemical reduction capacity in neutrophile leucocytes was found (Perlingeiro and Queiroz, 1995; 1994).

An increase in the T-cell concentration in blood, CD3–, CD4–, and CD8– cells has been reported in workers exposed to mercury (Moszczynski *et al*., 1996). In a group with low exposure, a decrease of the TNFα-concentration in serum has been reported (Soleo *et al*., 1997).

Effects on the immune system of occupational exposure to mercury vapor have been studied in several worker populations. The workers were exposed to mercury levels below and around the threshold value for permitted exposure, which corresponds to a urinary secretion rate of mercury of some 50μg/g creatinine. These results were summarized by Moszczynski (1999) and reported statistically significant deviations in the number of cell elements, cytokine concentrations, and immunoglobulin concentrations. Both stimulating and inhibitory effects were found.

In a later study, 20 workers exposed to mercury vapor had mean urinary secretion of mercury of 45 μg/L. The study reported that the number of CD4+ and CD45RA+ and the total number of CD4+ T lymphocytes were significantly lower than in the controls. The numbers of CD57+ and CD16+ NK (natural killer) cells were also found to be negatively correlated with the mercury concentration in urine (Park *et al*., 2000).

Another group of 19 workers exposed to mercury vapor had a mean urinary secretion of mercury of 9.7+5.5 μg/L. In this group, Vimercati *et al*. (2001) found an inverse correlation between mercury in urine and the numbers of CD13+ and CD15+ leukocytes and NK cells. A reduced capacity for chemotaxis in polymorphonuclear leukocytes was also found. Loftenius *et al*. (1998) studied the effect of amalgam removal on mononuclear lymphocytes from 10 patients. They found no statistically significant change in the number of cell types. However, they found a rise in IL-6 in plasma after 48 hours. The mercury concentration in plasma rose by some 10%.

In 47 chloralkali workers with mercury exposure corresponding to 5.9 nmol/mmol creatinine, an increase in autoantibodies against myeloperoxidase and proteinase 3 was observed. This increase was correlated with the mercury concentration in urine (Ellingsen *et al*., 2000a).

For acrodynia (pink disease) and Kawasaki disease see Chapter 12.

6.2.2.5 Thyroid

A study on 41 chloralkali workers with a mean urinary excretion of 27fg/g creatinine showed a significantly higher concentration of both free T4 and the free T4/free T3 ratio compared with unexposed referents. Moreover, serum-free T3 was inversely associated with cumulative mercury exposure. The authors suggested that the effect indicated an inhibitory effect by mercury on 5′-deiodinases, selenoprotein enzymes (Barregard *et al*., 1994). Ellingsen *et al*. (2000b) reported finding impaired thyroid function in a group of 47 chloralkali workers compared with 47 controls. The exposed workers showed a statistically significant rise in reverse T3 (rT_3)—a rise that was dose-related. The mean urinary concentration of mercury was 10.6 μg/g creatinine, with a range of 2.0–30.1.

6.2.2.6 Testicles

Exposure to mercury vapor causes mercury to accumulate in the testicles, where it is eliminated very slowly. Lee and Dixon (1975) observed inhibition of spermatogenesis in mice given mercuric chloride, 1 mg/kg intraperitoneally. Daily administration of $HgCl_2$ to mice in a dose that did not affect body weight caused a reduced sperm count, modified sperm morphology, and lower fertility. It was possible to offset this effect by administering vitamin E (Rao and Sharma, 2001).

Monsees *et al*. (2000) studied the *in vitro* effect of $HgCl_2$ on Sertoli cells from rat. They observed that concentrations <1 µmol/L of $HgCl_2$ sharply reduced inhibin production. Clinical observations have prompted suspicions of associations between acrodynia (pink disease) and epididymis obstruction (de Kretser *et al*., 1998). Clinical data on effects of mercuric mercury on human spermatogenesis are lacking.

6.2.2.7 Muscles

Atrophy and capillary damage in thigh muscle were observed in five of six workers in dental care, who had a urinary mercury-secretion rate of 13–67 µg/L at the time of the biopsy. These changes may, according to the authors, have been induced by the effect of the mercury on the nervous system or on capillaries. There might also be a direct effect on muscle fibers (Nadorfy-Lopez *et al*., 2000).

6.2.2.8 Other Effects

Wada *et al*. (1969) reported a dose-response relationship between mercury excretion in urine and cholinesterase activity in serum with a pronounced decrease in enzyme activity at mercury concentrations in urine of 150–200 µg/g creatinine. A similar trend was observed for ALA-D activity in erythrocytes.

Zabinski *et al*. (2000) reported that enzyme activity for several enzymes in erythrocytes—G-6PD, AchE, GR, and SOD—was significantly reduced in a group comprising 46 chloralkali workers, with a urinary mercury concentration of 77 µg/L. Bulat *et al*. (1998) observed reduced activity for GPx and SOD in erythrocytes in a group of 42 chloralkali workers with a urinary secretion rate of 23.2 ± 11.3 nmol/mmol creatinine.

In a group of 16 workers exposed to mercury vapor, reduced levels of glutathione and elevated catalase activity in red blood cells were observed. Mean urinary secretion of mercury in this group was 18.5 ± 8.8 µg/L (Queiroz *et al*., 1998).

6.2.3 Indicators of Exposure and Concentration in the Critical Organ

There are no available data relating concentrations of mercury in blood and urine to data on exposure and absorption of mercuric mercury. From theoretical considerations, it can be assumed that, as is the case for exposure to mercury vapor, concentrations of mercury in blood and urine may, on a group basis, be related to the degree of absorption of or the exposure to mercuric mercury. Renal biopsy has been used as a diagnostic procedure to determine mercury concentration in the critical organ (Kazantzis *et al*., 1962). Several indices of impaired renal function have been suggested. Lauwerys and Buchet (1973) found increased concentrations of ß-galactosidase in plasma and urine of workers exposed to mercury vapor. In further studies, the increased ß-galactosidase excretion in urine was found to be correlated to the degree of mercury exposure (Buchet *et al*., 1980). Foa *et al*. (1976) studied a population of 81 workers in a chloralkali industry. They found an increase in plasma of lysosomal enzymes such as ß-galactosidase, ß-glucosidase, ß-*N*-acetylglucosaminidase, and ß-glucosidase but no change of β-cholinesterase activity. This increase was significantly correlated with exposure level. The authors interpreted this increase as being caused by a general effect of mercury on lysosomes and lysosomal membranes. Dierickx (1980) found a strong correlation between mercuric chloride dose to rats and excretion of γ-glutamyltransferase, an enzyme especially well represented in the kidney. Clinical observations concerning γ-glutamyltransferase excretion in urine during an epidemic of mercury poisoning in Argentinian children caused by diapers treated with mercury-containing fungicides, revealed an increased urinary excretion of γ-glutamyltransferase in mercury concentrations in urine exceeding 150 µg/g creatinine (Gotelli, 1982).

6.2.4 Dose-Effect and Dose-Response Relationships on Exposure to Mercuric Salts

The lethal dose from acute poisoning is in the order of 1–4 g (Winek *et al*., 1981). Lethal poisoning has been reported after intake of 0.5 g (Gosselin *et al*., 1976). In persons not additionally exposed to mercuric mercury—apart from the daily intake from food, water, and air—the mercury concentration in the kidneys can be expected to range from less than 0.1–3 mg/kg (Berlin, 1972). In cases of poisoning caused by mercuric salt, 10–70 mg/kg in the kidney has been reported (Clennar and Lederer, 1958; Kazantzis *et al*., 1962; Winek *et al*., 1981). These values are comparable with the concentrations seen in kidneys of mercury-poisoned rats (Ashe *et al*., 1953).

It is obvious from the available literature that the prevalence of immune reactions caused by mercury exposure increases with increasing exposure to mercury or mercuric salts. However, great difficulties arise when an attempt is made to quantify the prevalence of common immune responses caused by mercury at different dose or exposure levels. This also includes the prevalence of glomerular nephritis caused by mercury. Available data illustrate the order of magnitude or the prevalence of immune reactions to mercury only. However, it has to be kept in mind that in these cases an association between mercury exposure and clinically manifested immune or allergic responses has been observed. Mercury exposure could always be identified and quantified. Information is lacking for

the cases in which allergic manifestations caused by minor exposure to mercury appeared and that could not be identified by available methods, mainly urine analysis.

Several cases of oral and skin manifestations, as well as of allergic enteritis and other side effects, see previously, caused by mercury in amalgam dental fillings have been reported (Berlin, 2004; Feuerman, 1975; Spreng, 1963). The number of cases, however, is very small considering the very large number of people with amalgam dental fillings. Several epidemiological studies have been carried out in which the health status among amalgam-bearers or dental service personnel with low exposure and nonamalgam bearers and persons with no occupational exposure were compared, including studies on twins discordant with regard to amalgam fillings in the teeth (Ahlqwist *et al*., 1999; Bates *et al*., 2004; Bjorkman *et al*., 1996; Kingman *et al*., 2005; Langworth *et al*., 2002; Saxe *et al*., 1995). In none of these or earlier studies have any health effects related to the mercury exposure been demonstrated. From these studies we can draw the conclusion that the prevalence of side effects from mercury in amalgam probably does not exceed 10%.

White and Brandt (1976) studied the development of mercury hypersensitivity using the so-called prick test technique on dental students and dental technicians. In new students, they found positive reactions to mercury in 2% compared with 18.8% in senior students, and an increasing prevalence with increasing years of study. The North American Contact Dermatitis Group stated that thiomersal is the third most common sensitizing material after nickel and chromium.

Under normal conditions mercury excretion in urine is less than 10 μg/L in children (Husband and McKellar, 1970). In the cases of infantile renal tubular acidosis described by Lightwood and Butler (1963) and Husband and McKellar (1970), the mercury concentration in urine exceeded 10 μg/L, and by provocation with BAL (British antilewisite; dimercaprol) and penicillamine excretion increased up to 100 μg/L. Warkany and Hubbard (1953) and Warkany (1966) summarized the reports in the literature concerning pink disease and concluded that in these cases > 50 μg Hg/L urine is excreted.

Warkany estimated the incidence of pink disease in mercury-exposed children to be 1 in 500. However, this figure was not on the basis of regular epidemiological investigations. In adults, the excretion of mercury in urine is normally <5 μg/L (Sandborgh-Englund *et al*., 1998. Kazantzis *et al*. (1962) described four cases of albuminuria among 72 mercury-exposed workers, with a mercury excretion in urine averaging approximately 300 μg/L; 10% of these workers excreted more than 1000 μg/L. Barr *et al*. (1972), in a case reference study on patients with nephrotic syndrome in Nairobi, observed that 53% of all cases used skin-bleaching ointment containing mercury. The mercury excretion in urine from use of such ointment varied between 100 and 250 μg/L with a mean of 150 μg/L. However, the authors stated that the upper normal limit for mercury excretion in urine with the method they used was 80 μg/L, which may mean that they generally overestimated the concentrations. In contrast to cases of glomerular nephritis from other causes, the prognosis for these patients was generally good. The authors gave no information about the size of the population that used the skin-bleaching ointment from which they drew their patient sample. They stated that the prevalence of nephrotic syndrome was clearly greater than what could be expected.

Foa *et al*. (1976), in a study of a group of 81 workers in a chloralkali factory, found 15 cases of glomerular nephritis. The urinary excretion of mercury in this population averaged 116 ug/L; excretion up to 762 μg/L occurred. Buchet *et al*. (1980) examined 63 mercury-exposed workers with a mean excretion in urine of 60 μg/g creatinine. Fifteen of these workers had a mercury excretion averaging 145 μg/g creatinine. In this group, the prevalence of glomerular damage was between 5 and 10%. Foa and coworkers, as well as Buchet and collaborators, observed a dose-related increase of ß-galactosidase activity in blood and urine; the mercury excretion in urine exceeded 50 μg/g creatinine.

6.2.5 Factors Interacting with the Toxicity of Mercuric Mercury

A number of factors may affect the dose-response relationship for mercuric toxicity. Factors like age, state of nutrition, gender, and earlier exposure that may give rise to sensitization are all likely to affect the relationship between dose and effect. In the following, some specific factors that have been observed to interfere with the metabolism and the toxic effects of mercury are discussed.

Selenium has been found to affect the distribution of mercuric mercury in mice (Eybl *et al*., 1969), in rats (Parizek *et al*., 1967), in rabbits (Imura and Naganuma, 1978; Naganuma and Imura, 1980), as well as in pigs (Hansen *et al*., 1981). As a consequence of this redistribution, decreased toxicity has also been observed (Johnson and Pond, 1974; Parizek and Ostadalova, 1967). Mercury forms a mercury-selenium protein complex with selenium (Burke *et al*., 1974) and is likely to bind to selenohydryl (SeH) groups on proteins. This complex can be identified in plasma and blood cells (Chen and Fang, 1974; Imura and Naganuma, 1978). Given together with selenium, mercury is retained longer in

blood and, as a consequence, lessens accumulation in the kidney. The mercury taken up by the kidneys is bound to the protein selenium complex; binding to metallothionein is diminished or negligible on administration of equivalent amounts of selenium (Björkman *et al*, 1994; Komska-Szumska and Chmielnick, 1977; Mengel and Karlov, 1980). Prolonged oral exposure to mercury and selenium in drinking water produces distinctive crystalline mercury-selenium intranuclear inclusion bodies in renal proximal tubule cells of rats (Carmichael and Fowler, 1979) with an Hg/Se ratio of 1:2 by X-ray microanalysis. Another consequence of the changed binding of mercury in blood brought about by selenium is that transport of selenium and mercury over the placental membranes is inhibited (Parizek *et al*., 1971).

Studies of selenium interaction with mercuric mercury have mainly been done on animals, especially rodents. A change in mercury distribution caused by selenium has been verified in pigs. Information concerning the effects of selenium in man is, however, lacking. Selenium metabolism in man is different from that in most animals. The selenium dependence of man is comparatively less than that of rodents. Observations in workers exposed to mercury vapor indicate, however, that there is a strong relationship between selenium concentration and mercury concentration in organs such as brain, thyroid, and pituitary, with a molar ratio of 1:1. Kosta *et al*. (1975) reported mercury concentrations up to 13 ppm in the brain of mercury-exposed workers who showed no obvious clinical signs or symptoms of mercury poisoning or any morphological changes in the brain. The highest concentration was found in one man exposed to mercury for 33 years, followed by 16 years of retirement without exposure. Similar reports have been given by Takahata *et al*. (1970).

7 METABOLISM AND TOXIC EFFECTS OF ORGANIC MERCURY COMPOUNDS

7.1 Organic Compounds Relatively Stable in the Mammalian Body

Organic mercury compounds that resist chemical degradation by the biochemical processes in the body are the short-chain alkyl compounds and some mercurials used in pharmaceutical practice. Of the alkyl compounds, methylmercury compounds occur naturally, and it is to them that most accumulated knowledge pertains. Available data indicate that ethylmercury compounds have toxicological properties qualitatively similar to those of the methylmercury compounds but is more rapidly degraded *in vivo*, resulting in a much shorter half-time than that of methylmercury. This presentation will be limited to methylmercury compounds (MeHg) and ethylmercury compounds (EthylHg). For the toxicology of the organic mercury compounds used for pharmaceutical purposes, the reader is referred to handbooks on pharmacology (e.g., Goodman and Gilman, 2005).

7.1.1 Metabolism Absorption—Inhalation

MeHg compounds can be absorbed by inhalation. Vapors of MeHg salts readily penetrate the membranes of the lung, and the absorption rate is estimated to be approximately 80%. In cases of exposure to alkylmercury salt aerosols, the absorption rate would be dependent on particle size and on the rate of deposition in the respiratory tract.

7.1.1.1 Ingestion and Skin

MeHg ingested with food is likely to be bound to proteins in the intestinal tract. Experiments on man (Aberg *et al*., 1969; Miettinen, 1973) and primates (Berlin *et al*., 1975a) have shown that it is efficiently absorbed through the intestinal tract.

Absorption of alkylmercury compounds through the skin is likely to occur. The rate will depend on the type of compound, the concentration, and the condition of the skin. Friberg *et al*. (1961) and Wahlberg (1965) have demonstrated skin absorption of an aqueous solution of MeHg in guinea pigs. Cases of poisoning caused by local application of MeHg-containing ointment to the skin have been described (Suzuki *et al*., 1970). To what extent the absorption can be explained by inhalation cannot be estimated from the reports.

7.1.1.2 Transport, Distribution, and Biotransformation

MeHg absorbed into the body is bound to protein sulfhydryl groups or, to a lesser extent, to sulfhydryl groups of amino acids, or peptides, like cysteine and glutathione. Thus, in blood plasma, MeHg is mainly bound to sulfhydryl groups of plasma proteins and is actively transported through the cell walls bound to L-cysteine by the large neutral amino acid carrier. Structurally the L-cysteine complex is similar to the amino acid L-methionine (Kerper *et al*., 1992). In blood, MeHg is accumulated to a large extent (>90%) in the red cells bound to cysteinyl residues on the beta-chain of the hemoglobin molecule (Doi, 1991). MeHg is slowly distributed from the blood to the organism. In man, equilibrium between blood and body is not reached until after 4 days (Kershaw *et al*., 1980).

Studies of the uptake of CH_3Hg^+, as CH_3Hg-*S*-G, in erythrocytes have provided additional information regarding the cellular handling of this conjugate.

Experiments carried out at 5°C indicated that the following systems were involved in this transport: (1) one or more OATs, which seem to be the primary mechanism of uptake, (2) a D-glucose diffusive transporter, (3) a cysteine facilitated transporter, and (4) a Cl^- transporter (Wu, 1995). On the basis of these findings, it seems that CH_3Hg-*S*-G mimics an endogenous substrate of each of these transporters to gain access into the erythrocyte. In later studies, Wu (1996) measured the uptake of CH_3Hg-*S*-G at 5°C and 20°C and concluded that an OAT is the primary mechanism of CH_3Hg-S-G uptake at both temperatures. Additional studies in which probenecid inhibited the uptake of CH_3Hg-S-G confirmed previous data, indicating that this conjugate is a transportable substrate of OAT (Wu, 1997). These data support the hypothesis that molecular mimicry at the site of one or more transporters plays a role in the transport of CH_3Hg-*S*-G in erythrocytes.

In Squirrel monkeys the adult brain and the fetal brain show a special affinity for MeHg, with levels of alkylmercury at least 3–6 times higher in brain than in blood, depending on dose (Berlin *et al*., 1975a). In studies of the distribution of MeHg after tracer doses of radiolabeled MeHg in man, Aberg *et al*. (1969) found 10% of the body burden of labeled mercury in the head. Miettinen (1973) found approximately 1% in 1 liter of blood. These values correspond to MeHg concentrations that are approximately six times higher in brain than in blood. This estimate is consistent with the finding in a case of lethal poisoning. (Niernberg *et al*., 1998) The uptake in brain is slower than in other organs, as has been demonstrated in the mouse (Berlin and Ullberg, 1963b). In the rest of the body, MeHg is rather evenly distributed in the tissues compared with other mercury compounds with an intracellular distribution. Other organs that concentrate MeHg are the liver and the kidneys. From the blood of pregnant women, MeHg is transported through the placenta to the fetus (Reynolds and Pitkin, 1975). In the fetus, MeHg is accumulated and concentrated, especially in the brain. The distribution in the fetus is close to that of the mother, although fetal brain levels of mercury may be higher (Berlin and Ullberg, 1963b). Tejning (1970) reported higher mercury concentrations in fetal than in maternal blood cells. This may be due to the different chemical structure of fetal and adult hemoglobin. Twenty percent of MeHg in brain is in water-soluble form and a part of it is bound to glutathione (Winroth *et al*., 1981). In the rat kidney, MeHg is bound to a large extent to glutathione (Richardson and Murphy, 1975).

7.1.1.3 Handling of CH_3Hg^+ in Brain

The brain and central nervous system (CNS) are the primary target sites where the adverse effects of CH_3Hg^+ are manifest (ATSDR, 2003; WHO, 2000). Accordingly, a great number of studies have focused on mechanisms by which this organic form of Hg gains access to the CNS, and more specifically, how it crosses the blood–brain barrier. As with inorganic mercuric ions, the methyl mercuric ion (CH_3Hg^+) does not exist as a free, unbound cation in biological systems (Hughes, 1957) but, rather, is found conjugated to thiol-containing biomolecules, such as GSH, Cys, Hcy, or NAC (Clarkson, 1993). In fact, initial studies that used homogenates of rat cerebrum demonstrated that the primary nonprotein thiol bound to CH_3Hg^+ is GSH (Thomas and Smith, 1979). Subsequent studies in rats and primary cultures of bovine brain endothelial cells revealed a possible role for Cys in the transport of CH_3Hg^+ across the blood–brain barrier (Aschner and Clarkson, 1988; 1989; Hirayama, 1980). In particular, coadministration of Cys with CH_3Hg^+ has been shown to increase the uptake of CH_3Hg^+ into capillary endothelial cells of the blood–brain barrier. Interestingly, experimental findings from rats have demonstrated that the uptake of CH_3Hg^+ is inhibited significantly by the neutral amino acid, phenylalanine (Hirayama, 1980; 1985; Thomas and Smith, 1982). These data led to the hypothesis that the Cys *S*-conjugate of CH_3Hg^+ (CH_3Hg-*S*-Cys) is a transportable substrate of a neutral amino acid transporter in the capillary endothelium of the blood–brain barrier. *In vivo* studies in rat brain (Aschner and Clarkson, 1988) and *in vitro* studies in bovine cerebral capillary endothelial cells (Aschner and Clarkson, 1989) indicate that the uptake of CH_3Hg-*S*-Cys is inhibitable by several neutral amino acids, providing additional support for the theory that this complex is taken up by a neutral amino acid carrier. The investigators in these studies suggested that CH_3Hg-*S*-Cys behaves as a "mimic" of an amino acid to cross the blood–brain barrier. Indeed, it is similar structurally to the amino acid methionine (Jernelov, 1973; Landner, 1971), which is a substrate of the neutral amino acid carrier, system L.

System L is present in the basolateral plasma membrane of many types of transporting epithelia. Interestingly, in the endothelial cells lining the blood–brain barrier, this carrier is found in both the apical and basolateral plasma membranes (Betz and Goldstein, 1978). Not surprisingly, system L is considered a major carrier of large neutral amino acids into the substance of the brain. In addition, this transporter has a broad substrate specificity (Oldendorf, 1973), which may allow it to use CH_3Hg-*S*-Cys as a substrate. Indeed, *in vivo* studies in rats (Kerper *et al*., 1992) and *in vitro* studies that used primary cultures of rat astrocytes (Aschner *et al*., 1990, 1991) provide evidence that CH_3Hg-*S*-Cys is a transportable substrate of system L. The involvement

of this transporter in the uptake of CH_3Hg-*S*-Cys has also been demonstrated in another study that used cultured endothelial cells from the brain (Mokrzan *et al*., 1995). Interestingly, this study measured the uptake of CH_3Hg^+, as a conjugate of Hcy (CH_3Hg-*S*-Hcy) or as CH_3Hg-*S*-Cys and found that these two complexes were transported similarly. Although the authors of this study suggest that system L is involved in the uptake of CH_3Hg-*S*-Cys, they did not conclude that this carrier also mediates the uptake of CH_3Hg-*S*-Hcy.

Since these initial studies, two isoforms of the system L family have been identified at the molecular level: the L-type, large neutral amino acid transporters, LAT1 (Kanai *et al*., 1998; Prasad *et al*., 1999) and LAT2 (Pineda *et al*., 1999). These transporters are heterodimeric proteins, composed of a heavy chain, 4F2hc, and a light chain, LAT1 or LAT2, bound together by a disulfide bond (Chillaron *et al*., 2001). With this knowledge, it has become possible to identify and characterize further the specific mechanisms involved in the uptake of CH_3Hg-*S*-Cys. To illustrate this point, Simmons-Willis *et al*. (2002) used oocytes from *Xenopus laevis* to study directly the involvement of LAT1 and LAT2 in the transport of this conjugate. These investigators provide the first line of direct molecular evidence implicating CH_3Hg-*S*-Cys as a transportable substrate of LAT 1 and 2 (Simmons-Willis *et al*., 2002). These data also provide substantive evidence for the phenomenon of molecular mimicry, where CH_3Hg-*S*-Cys seems to mimic methionine at the site of system L.

7.1.1.4 Biotransformation

MeHg undergoes biotransformation to inorganic mercury by demethylation in the body. Suda and Takahashi (1992) studied the rate of demethylation in the rat. They found that the reticuloendothelial system is a site of MeHg demethylation with a dominating role of spleen and liver. Their results suggest that the demethylation is accomplished by OH^- radicals produced by P-450 reductase or by HOCl radicals. They also demonstrated that MeHg is taken up and demethylated in phagocytic cells. (Suda and Hirayama, 1992; Suda *et al*., 1993). In the brain, inorganic Hg is slowly accumulated in astrocytes and microglia after exposure to MeHg.

Considerable levels of inorganic mercury have been demonstrated in kidney, liver, feces, bile, and urine after administration of MeHg to primates (Berlin *et al*., 1975a, Charleston *et al*., 1995). Biotransformation seems also to occur in the brain at a slow rate. Studies on *Maccaca fascicularis* have shown an increasing accumulation of inorganic mercury in astrocytes and microglia in the brain after long-term exposure to MeHg (Charleston *et al*., 1995). Twenty percent of MeHg in brain is in water-soluble form, and a part of it is bound to glutathione (Winroth *et al*., 1981). In the rat kidney, MeHg is bound to a large extent to glutathione (Richardson and Murphy, 1975). The distribution in the fetus is close to that of the mother, although fetal brain levels of mercury may be higher (Berlin and Ullberg, 1963b).

7.1.1.5 Elimination and Excretion

The main routes of elimination of MeHg are through the liver into the bile and through the kidney into urine. The net excretion in humans amounts to approximately 1% of the body burden, corresponding to a biological half-time of 70 days, when this burden is nontoxic (Swedish Expert Group, 1971). Clinical observations from MeHg poisoning epidemics in Japan (Swedish Expert Group, 1971) and Iraq (Bakir *et al*., 1973) support an elimination in man under conditions of intoxication of the same order of magnitude. The major part of the excretion is by the fecal route. Much of the MeHg excreted in the bile is absorbed in the gut, producing an enterohepatic circulation of MeHg. In the rat, MeHg in the bile is bound to glutathione and cysteine (Refsvik and Norseth, 1975). A part of the mercury in the bile (approximately 30–80%) of the monkey (Berlin *et al*., 1975a) is inorganic mercury, derived from the demethylation of MeHg in the body. This part, less effectively absorbed in the gut, is excreted. The relative amount of inorganic mercury in bile may be dose dependent. In the gut, MeHg can be decomposed by the microflora to inorganic mercury (Rowland *et al*., 1977). As inorganic mercury is absorbed to approximately 5–10%, this factor contributes to an increased excretion. Approximately 90% of the total excretion of MeHg in man is by the fecal route. MeHg is also excreted in breast milk, the concentration being approximately 5% of the concentration in the maternal blood (Bakir *et al*., 1973). Twenty percent of the mercury in breast milk is MeHg, provided the load of MeHg is nontoxic (Skerfving, 1974a). Bakir *et al*. (1973) reported 60% MeHg in Iraqi cases of MeHg poisoning; thus, the fraction of MeHg excreted in breast milk may be dose-dependent.

MeHg is also taken up in hair during hair formation. The amount incorporated is proportional to the blood concentration of mercury at the time of incorporation. Thus, the ratio of blood concentration/hair concentration in man is 1/250 under steady-state conditions (Skerfving *et al*., 1974; Tsubaki, 1971). The quotient may vary with age (Suzuki *et al*., 1970).

The capacity of MeHg elimination varies in the population. Analyses of consecutive hair segments from MeHg-exposed populations in Iraq (Al-Shahristani and Shihab, 1974) suggest that a part (10%) of the population eliminates MeHg at a rate considerably lower (biological half-time, 110–190 days) than 1% per day.

Whether this observation may be due to the existence of genetic enzyme anomalies in the population or to other causes is unknown.

Information concerning variations in excretion rate of MeHg with age is lacking. In newborn mice, the excretion of MeHg is smaller than after weaning (Choi *et al*., 1981a; Robinson *et al*., 1982). This is probably because before weaning the liver has not acquired the ability to excrete MeHg bound to glutathione in bile. In newborn rats this ability is developed during the second or fourth week (Ballatori and Clarkson, 1982). It is likely that the content of the diet can also influence the excretion rate of MeHg through interference with the reabsorption in the lower part of the intestinal canal. Landry *et al*. (1979) showed that in mice, three different types of diets resulted in considerably different elimination rates of MeHg.

7.1.1.6 Renal Handling of CH_3Hg^+

Although the primary target of CH_3Hg^+ is the central nervous system, it also induces significant deleterious effects in other organs, including the kidneys (Fowler 1972a,b; Fowler and Woods, 1977; Friberg, 1959; Magos and Butler, 1976; Magos *et al*., 1981; 1985; McNeil *et al*., 1988; Norseth and Clarkson, 1970 a,b; Prickett *et al*., 1950; Woods and Fowler, 1977; Zalups *et al*., 1992). Until recently, it was unclear as to how this organometal complex is taken up by renal tubular epithelial cells. Richardson and Murphy (1975) demonstrated that the renal tubular uptake of CH_3Hg^+ depends on the cellular concentration of GSH. Moreover, several studies have shown that when CH_3Hg^+ is coadministrated with GSH, the renal uptake and accumulation of CH_3Hg^+ increase (Alexander and Aeseth, 1982; Tanaka *et al*., 1992).

It has been proposed that γ-glutamyltransferase and cysteinylglycinase, which are present in abundance on the luminal (brush-border) plasma membrane of proximal tubular cells, act on GSH *S*-conjugates of CH_3Hg^+ (CH_3Hg-*S*-G) to yield CH_3Hg-*S*-Cys (Zalups, 2000a). *In vitro* evidence indicates that the methyl mercuric ion remains bonded to the sulfur atom of Cys during the catabolism of GSH (Naganuma *et al*., 1988). Experimental evidence supporting the role of γ-glutamyltransferase in the renal tubular uptake of CH_3Hg^+ comes from studies in which the activity of this enzyme was inhibited by the alkylating agent acivicin. After the pretreatment with acivicin, the renal tubular uptake of CH_3Hg^+ was shown to decrease, whereas the urinary excretion of GSH and CH_3Hg^+ was shown to increase (Berndt *et al*., 1985; de Ceaurriz and Ban, 1990; Di Simplicio *et al*., 1990; Gregus *et al*., 1987; Kostyniak, 1985; Mulder and Naganuma *et al*., 1988; Tanaka *et al*., 1990, 1991; 1992; Yasutake *et al*., 1989). The observed changes in the renal cellular uptake and excretion of CH_3Hg^+ indicate that the catabolism of the CH_3Hg-*S*-G complex is a necessary step in the renal proximal tubular absorption of CH_3Hg^+.

Findings from some studies indicate that a fraction of the CH_3Hg^+ that enters into systemic circulation is oxidized to Hg^{2+} either before and/or after it enters the proximal tubular epithelial cells of the kidney (Dunn and Clarkson, 1980; Gage, 1964; Norseth and Clarkson, 1970a,b; Omata *et al*., 1980; Zalups *et al*., 1992). These findings lead one to suggest that some or all of the mercuric ions taken up in the kidneys after exposure to CH_3Hg^+ may be due to the transport of some chemical form of Hg^{2+} rather than CH_3Hg^+.

Tanaka *et al*. (1992) demonstrated in mice the existence of one or more luminal and basolateral mechanisms involved in the renal tubular uptake of CH_3Hg^+. These investigators found that the luminal mechanism(s) are greatly dependent on the actions of γ-glutamyltransferase. The role of cysteinylglycinase, however, was not studied. Collectively, their data indicate that the species of CH_3Hg^+ taken up is most likely in the form of a cysteinylglycine *S*-conjugate of CH_3Hg^+ (CH_3Hg-S-CysGly) or CH_3Hg-*S*-Cys. The mechanism(s) responsible for the uptake of CH_3Hg-*S*-Cys in the proximal tubule has/have not yet been identified. However, we can draw parallels from the information available for the transport of Hg^{2+}, as Cys-*S*-Hg-*S*-Cys. Inasmuch as Cys-*S*-Hg-*S*-Cys seems to mimic cystine at the site of an amino acid transporter in proximal tubular cells (Bridges *et al*., 2004), it is possible that CH_3Hg-*S*-Cys behaves in a similar way. In addition, because CH_3Hg-*S*-Cys has been implicated as a molecular mimic of methionine at the site of system L in endothelial and glial cells, this complex may also mimic methionine at the site of one or more carriers of this amino acid in the kidney.

Uptake of CH_3Hg^+ at the basolateral membrane also seems to involve the multispecific carrier, organic anion transporter 1 (OAT1). As mentioned previously, in the kidneys, this transporter is localized exclusively in the basolateral membrane of proximal tubular epithelial cells (Kojima *et al*., 2002; Motohashi *et al*., 2002). There is evidence from studies in *Xenopus laevis* oocytes implicating this transporter in the cellular uptake of NAC and DMPS *S*-conjugates of CH_3Hg^+ (CH_3Hg-*S*-NAC and $(CH_3Hg$-*S*$)_2$-DMPS, respectively; Koh *et al*., 2002). More substantive evidence implicating OAT1 in the basolateral uptake of methylmercuric thiol-conjugates comes from recent studies that used MDCK cells stably expressing hOAT1. The findings from these studies indicate that NAC, Cys, and Hcy *S*-conjugates of CH_3Hg^+ are all potential transportable substrates of OAT1 (Zalups and Ahmad, 2005a,b,c).

The mechanism(s) by which CH_3Hg^+ is transported out of the proximal tubular cell into the tubular lumen have not been tested directly. However, Koh *et al*. (2002) proposed that the efflux of CH_3Hg^+ across the luminal plasma membrane is mediated by MRP2. MRP2 is an ATP-binding cassette (ABC) transport protein that is localized in the luminal membrane of the proximal tubule (Schaub *et al*., 1997; 1999) and has been shown to be involved in the transport of glutathione-*S* conjugates of other metals (Leslie *et al*., 2004). Clearly, a great deal about this potential mechanism remains to be clarified.

7.1.1.7 Intestinal Handling of CH_3Hg^+

Ingestion of food and/or water contaminated with CH_3Hg^+ is the primary route of human exposure to this compound. Thus, a thorough understanding of the mechanisms involved in the intestinal absorption of CH_3Hg^+ is an important determinant of understanding the transport and toxicity of CH_3Hg^+ in the body. Studies in ligated rat intestinal segments demonstrate that the uptake of CH_3Hg-*S*-Cys and CH_3Hg-*S*-CysGly is 1.5 times greater than the uptake of CH_3Hg-*S*-G (Urano *et al*., 1990). Interestingly, when the activity of γ-glutamyltransferase in the striated border of the intestine was inhibited (by acivicin), the uptake of CH_3Hg^+ (when presented as CH_3Hg-*S*-G) in the enterocytes was reduced by 50%. These data indicate that CH_3Hg-*S*-Cys and/or CH_3Hg-*S*-CysGly is/are the most likely species of CH_3Hg^+ taken up at the luminal plasma membrane of enterocytes. Because the luminal plasma membrane of enterocytes contains dehydropeptidases, a fraction of CH_3Hg-*S*-CysGly formed in the lumen is likely degraded to yield CH_3Hg-*S*-Cys. Any CH_3Hg-*S*-CysGly that escapes degradation, however, may be transported by a peptide transporter present in the luminal membrane. In the intestine, dipeptide and tripeptide transport is the primary means for the absorption of amino acids. Given this, and the similarity between CH_3Hg-*S*-CysGly and a small peptide, it is possible that this complex mimics an endogenous dipeptide or tripeptide to gain access into enterocytes. In addition, CH_3Hg-*S*-Cys has been identified as the primary species of CH_3Hg^+ that is delivered into the lumen of the intestine by bile. Once in the lumen, CH_3Hg-*S*-Cys is reabsorbed rapidly by the enterocytes possibly by acting as a mimic of methionine at the site of an amino acid carrier (Ballatori *et al*., 1998; Dutczak and Ballatori, 1992; Norseth and Clarkson, 1971; Wang *et al*., 2000) present in the luminal plasma membrane. Indeed, the findings from an *in vitro* study of isolated, perfused catfish intestines, indicate that system L mediates at least some of the luminal transport of CH_3Hg-*S*-Cys in enterocytes (Leaner and Mason, 2002). The investigators in this study suggested that there is/are one or more active-transport carrier proteins involved in this uptake. Competitive inhibition experiments provided indirect evidence that a neutral amino acid transporter, possibly system L, mediates (at least some of) the uptake of CH_3Hg-*S*-Cys in intestinal enterocytes.

Additional data from experiments in ligated segments of rat intestine demonstrate that treatment with probenecid (an inhibitor of OAT1), in addition to acivicin, significantly reduces the luminal uptake of CH_3Hg^+ (as CH_3Hg-*S*-G; Urano *et al*., 1990). It was concluded that there are two independent transport systems for the uptake of CH_3Hg^+ across the luminal plasma membrane of enterocytes. One of these mechanisms depends on the activity of γ-glutamyltransferase, whereas the other seems to be inhibited by probenecid. These findings lead one to postulate that one or more OATs may also be involved in the intestinal uptake of CH_3Hg^+. It is important to note, however, that probenecid is not a specific inhibitor of OAT and may act on other transporters. Furthermore, there is currently no direct evidence to suggest that OATs are present in the intestine.

The efflux of CH_3Hg^+ across the basolateral membrane of the enterocyte into the extracellular compartment is less clear than the luminal transport of this molecule. Foulkes (1993) suggested that the intracellular concentrations of GSH play a role in regulating the efflux of CH_3Hg^+ out of the intestine, although data supporting this notion are lacking. The apparent similarities between the structures of CH_3Hg-*S*-G and GSH may lead one to postulate that this conjugate is a transportable substrate of a GSH transporter present in the basolateral plasma membrane of enterocytes, which would facilitate its transport into blood.

7.1.1.8 Hepatic Handling of CH_3Hg^+

After CH_3Hg^+ is absorbed by the intestine, it is delivered to the liver through portal blood. Although, little is known about the mechanisms involved in the hepatocellular uptake of CH_3Hg^+ from sinusoidal blood, the data from an *in vivo* study in rodents showed that the uptake and accumulation of CH_3Hg^+ are enhanced after coadministration of or subsequent administration with Cys or GSH (Thomas and Smith, 1982). These data also indicate that CH_3Hg-Cys is the most likely species of CH_3Hg^+ taken up at the sinusoidal membrane of hepatocytes (Thomas and Smith, 1982). Overall, these findings are consistent with the notion that an amino acid transporter, such as system L, is involved in this uptake.

The current evidence indicates that the transport of CH_3Hg^+ from the hepatocytes into the biliary

canaliculus depends on GSH (Ballatori and Clarkson, 1982; 1983; 1985a,b; Refsvik, 1982; Refsvik and Norseth, 1975). Indeed, early studies in hepatic tissues indicate that the preponderance of CH_3Hg^+ within hepatocytes is bound to GSH (Omata *et al*., 1978). Magos *et al*. (1978) demonstrated that increasing hepatic levels of GSH enhanced the biliary excretion of GSH and CH_3Hg^+. In contrast, Refsvik (1978) showed that compounds that reduce significantly the hepatic and biliary levels of GSH cause the accumulation of CH_3Hg^+ in the liver to decrease. It seems that the intracellular concentration of GSH has a significant effect on the transport of CH_3Hg^+. Indeed, studies in mice deficient in γ-glutamyltransferase have demonstrated that the distribution and accumulation of CH_3Hg^+ in liver is affected by the actions of γ-glutamyltransferase and cysteinylglycinase (Ballatori *et al*., 1998). Furthermore, experiments in cultured human hepatocytes (HepG2 cells) in which γ-glutamyltransferase was inhibited indicate that the transport of CH_3Hg^+ in these cells depends on the intracellular concentration of GSH (Wang *et al*., 2000). One can hypothesize that CH_3Hg-*S*-G is formed within the hepatocytes and is subsequently transported into the bile at the canalicular membrane. It is reasonable to hypothesize that CH_3Hg-*S*-G may act as a mimic of GSH at the site of a GSH transporter in the canalicular membrane of hepatocytes. Accordingly, Dutczak *et al*. (1993) have suggested that a GSH transport system on the canalicular membrane serves a primary role in the biliary secretion of CH_3Hg-*S*-G. A GSH-transporter has since been identified on the canalicular membrane of hepatocytes (Ballatori and Dutczak, 1994; Ballatori and Truong, 1995; Fernandez-Checa *et al*., 1992; 1993; Garcia-Ruiz *et al*., 1992), and it most likely plays a crucial role in the export of CH_3Hg^+.

After being secreted into the bile, CH_3Hg^+ may be reabsorbed along the biliary tree as a conjugate of GSH or one of its metabolites, CysGly, and/or Cys (Ballatori, 1994). Experimental evidence indicates CH_3Hg^+ is absorbed more readily by ductal epithelial cells when it is administered as a complex of GSH or Cys (Dutczak *et al*., 1991). Once in the biliary tree, CH_3Hg-*S*-G seems to be catabolized to yield CH_3Hg-*S*-Cys, which can be reabsorbed by cells lining the bile ducts and the enterocytes in the intestine (Dutczak and Ballatori, 1992). Although the actual mechanism(s) involved in this uptake have yet to be determined, it is reasonable to hypothesize that CH_3Hg-Cys acts as a mimic of an amino acid at the site of an amino acid transporter, such as system L. A number of amino acid transporters, including system L (LAT3; Babu *et al*., 2003), have been identified in the liver (Bode, 2001; Wagner *et al*., 2001); however, the exact localization of any one of them has not yet been determined.

7.1.1.9 Handling of CH_3Hg^+ in Placenta

One of the most publicized and serious consequences of CH_3Hg^+ exposure is the deleterious neurological effect observed in fetuses whose mothers were exposed to methylmercury during pregnancy (Amin-Zaki *et al*., 1974; Harada, 1978; 1995; Inouye and Kajiwara, 1988; Kajiwara and Inouye, 1986; 1992; Matsumoto *et al*., 1965). CH_3Hg^+ crosses the placenta readily and accumulates in the fetus (Inouye and Kajiwara, 1988; Inouye *et al*., 1985; Suzuki *et al*., 1967) and placenta (Ask *et al*., 2002) at levels higher than that in maternal tissues and blood. Yet, little is known about the mechanism(s) by which this metal is taken up and transported across this organ. Kajiwara *et al*. (1996) have shown that CH_3Hg^+ is transported across the rat placenta by a neutral amino acid carrier in a time- and dose-dependent manner. These investigators demonstrated that coinjection with methionine increased the uptake of CH_3Hg^+. In addition, they proposed that this increase might be the result of the intracellular conversion of methionine to Cys, which may subsequently combine with CH_3Hg^+ to form the readily transportable conjugate, CH_3Hg-*S*-Cys. This conjugate may then mimic methionine at the site of system L to gain access to the placenta. Accordingly, the authors concluded that the neutral amino acid carrier, system L (Kajiwara *et al*., 1996), mediated the uptake of CH_3Hg^+ in placenta. The exact species of CH_3Hg^+ that was transported was not determined in this study, nor was there direct evidence supporting the conclusion that system L was involved in this transport. However, because system L has been shown to mediate the transport of CH_3Hg-*S*-Cys across the epithelial cells of and the astrocytes associated with the blood–brain barrier (Aschner *et al*., 1990; Kerper *et al*., 1992; Mokrzan *et al*., 1995), it is reasonable to postulate that this same carrier is also responsible for the uptake of CH_3Hg-*S*-Cys in placenta. System L has been identified in the placenta (Kanai *et al*., 1998; Pineda *et al*., 1999; Prasad *et al*., 1999) and is an important participant in the transfer of nutrients from the maternal to the fetal circulation.

It is important to note that a number of other protein carriers have been identified in the placenta. These include MRPs, organic anion-transporting polypeptides (OATPs), OATs, organic cation transporters (OCTs), and zinc transporters (Leazer and Klaassen, 2003). The localization of most of these transporters in the placental membrane has not been determined. However, one can suggest that one or more of them may play a role in the uptake and/or efflux of CH_3Hg^+ complexes.

Interestingly, MRP1, 2, and 3 have been identified in the apical membrane of the syncytiotrophoblast. MRP1

and MRP3 were also present in the endothelial cells of placental blood vessels (St. Pierre *et al*., 2000). Given the role of MRPs in detoxification (Leslie *et al*., 2001), it is logical to hypothesize that these carriers mediate the efflux of unwanted substances from the fetal circulation. Thus, in cases of CH_3Hg exposure, some of the CH_3Hg may be transported back across the placenta into the maternal circulation.

7.1.2 Toxic Effects and Mechanisms

MeHg reacts *in vitro* with DNA and RNA and probably binds to sulfhydryl groups, resulting in changes of the secondary structure of the DNA and RNA molecules (Gruenwedel and Lu, 1970; Livingstone *et al*., 1976; Millar, 1967).

It has been suggested that this effect could give rise to disturbances in protein, DNA, and RNA synthesis. *In vivo* and *in vitro* studies on cell cultures or tissue material from mammalians support this assumption. Gruenwedel and Cruikshank (1979) observed a dose dependence of macromolecule synthesis in HeLa S cells with MeHg concentrations in the culture media between 1 and 10 µmol/L. Verity *et al*. (1975) observed a 50% inhibition of protein synthesis in synaptosomes from rat brain cortex when the MeHg concentration in the media was 12 µmol/L. *In vivo* studies on mammals have shown a decrease of RNA content with increased dose of MeHg in the dorsal root ganglia (Chang *et al*., 1972). These authors also found a decreased incorporation of tritium-labeled uridine in RNA of nerve cells from MeHg-treated mice. Farris and Smith (1975) reported a decreased protein synthesis in the brain of rat when the content of MeHg in the brain was approximately 50 ppm. This observation was made by measurement of C^{14}-leucine incorporation. Brubaker *et al*. (1973), however, found an increased DNA, RNA, and protein synthesis in the brain of rats with a MeHg content of 10 ppm. They also observed ultrastructural changes in the dorsal spinal ganglia characterized by a continuous destruction of the endoplasmic reticulum and disappearance of ribosomes, described by Herman *et al*. (1973). Studies in livers of fetal rats exposed to methylmercury *in utero* (Fowler and Woods, 1977) showed marked inhibition of mitochondrial biogenesis and a number of biochemical functions related to decreased biosynthesis of mitochondrial structural membrane proteins. The observed decreases in mitochondrial structural protein synthesis were correlated with statistically significant decreases in the volume density of mitochondria and loss of mitochondrial respiratory function. The occurrence of sulfhydryl groups in the cell membrane is reason to suspect that MeHg, except for interference with membrane-bound enzymes, may also interfere with the cell-membrane structure. Studies on HeLa S cells in culture support this suspicion. In a scanning electron microscope, changes in the membrane structure appear when MeHg is added to the media before effects on intracellular activity such as DNA, RNA, and protein synthesis are observed (Gruenwedel *et al*., 1979). These effects were related to dose-related concentrations of methyl and inorganic mercury in livers of these fetal animals.

Alterations in the heme biosynthetic pathway and mercury porphyrinurias after exposure to methylmercury have been extensively studied by Woods and coworkers (Fowler and Woods, 1977; Miller and Woods, 1993; Pingree *et al*., 2001; Woods and Fowler, 1977; Woods *et al*., 1991) and also shown to occur after exposure to mercury vapor (Woods *et al*., 1993). The role of porphyrins in catalyzing formation of reactive oxygen species (ROS) has also been evaluated by these investigators (Miller and Woods, 1993; Woods and Sommer, 1991). The potential roles of porphyrins in exacerbating the toxicity of mercurials by increasing formation of ROS may facilitate cell death through apoptotic pathways and lead to the observed attenuation of NF-kappa B in renal tubule epithelial cells (Diaguez-Acuna *et al*., 2004; Woods *et al*., 2002).

Considering that the CNS is the critical organ for MeHg exposure in primates, it is of special interest to discuss in further detail the observed effects on nerve tissue elements. Studies have been performed in tissue cultures of glioblastoma and neuroblastoma cells, fetal brain cells, and on preparations of nerve tissue. Kim (1971) found that MeHg was toxic for cultured myelinating cells from embryonal mouse brains. Kasuya (1972) tested the toxicity of a number of organic mercury compounds and mercury chloride and found that MeHg had the greatest toxicity on cultures of cells from dorsal root ganglia of chick embryos. The lowest concentration in the media producing an effect was 5 µmol/L. Half this concentration resulted in inhibition of the growth of the cell culture from the spinal cord (Kasuya, 1975a). Prasad *et al*. (1979a,b) found that glioma cells from rat glioma are more sensitive to MeHg than neuroblastoma cells from mice. The difference in sensitivity appeared at the concentration difference of a factor 2 in the culture media of MeHg. Effects were observed at a concentration level <1 µmol/L of MeHg in the media (Prasad *et al*., 1979a,b). Koerker (1980) observed a toxic effect on mouse neuroblastoma cells at a concentration of MeHg-hydroxide in the media of 1 µmol /L.

Choi *et al*. (1980) exposed a culture of human fetal astrocytes to MeHg in a concentration of 6 µmol /L and observed a reduction of the DNA synthesis, a reduction that was reversible if the media were exchanged for mercury-free media. The same authors studied

the effect of 200 μmol/L MeHg on a culture of human fetal neurons. The normal migration of neurons from the center of the colony was inhibited followed by a tdegeneration of neurites with fragmentation of neurites from the cell body. Injuries to the microtubuli were observed in the electron microscope and at an early stage also on the plasma membranes. Sager and Doherty (1982) observed in *in vitro* studies that 40 μmol/L of MeHg in the media inhibited the polymerization of tubulin by 60%. Tubulin is the basic element in the microtubuli of the brain cells. Microtubular fragmentation has been seen in cultured primary rat cerebellar granular neurons at a MeHg concentration as low as 0.5–1 μmol/L (Castoldi *et al*., 2000). So far, no author has determined the intracellular concentration of MeHg after onset of effect. This shortcoming prevents a comparison of concentrations between different experiments, because the protein concentration in the media varies in different investigations.

Effects on mitochondria respiration in the brain tissue have been observed *in vivo* as well as *in vitro* (Verity *et al*., 1975; Von Burg *et al*., 1979). However, these effects appeared at a MeHg concentration in brain in an order of magnitude above the concentration at which toxic effects appear (i.e. 10–100 μmol /L MeHg) in the tissue.

Verity and coworkers, Von Burg and coworkers, and Wakatayashi *et al*. (1976) studied the effects of MeHg, given parenterally to rats, on the axonal flow in the sciatic nerve. At a mercury concentration of 20 μg/g in the brain, they found an increased axonal flow. Local injection of MeHg in the vicinity of the sciatic nerve resulted in total blockage of axonal flow locally. Asano *et al*. (1979) studied the enzyme activity in lysosomes of brains of MeHg-poisoned rats. They found a decreased activity at highly toxic doses.

MeHg induces generation of reactive oxygen radicals in rat glia cells and neurons (Yee and Choi, 1996). If the generation of such radicals is inhibited, the toxicity of MeHg is decreased (Sarafian *et al*., 1994). MeHg in a few micromolar concentrations has also been reported to disrupt Ca^{2+} homeostasis causing increased intracellular Ca^{2+} concentration (Oyama *et al*., 1994). Administration of blockers of voltage-dependent Ca^{2+} channels to rats dosed with MeHg prevents the appearance of neurological signs (Sakamoto *et al*., 1996).

Studies have also been performed on the effects of MeHg on neurotransmitter concentrations in the brain, on specific transmitter receptors, and on the synaptic performance. A concentration of MeHg-chloride of 5–10 μmol/L added to mouse brain homogenates resulted in an increased release of transmitter substances such as dopamine, glutamate, GABA, glycine, and choline (Bondy *et al*., 1979). Juang (1976) observed that MeHg increased the presynaptic release of acetylcholine on a preparation of sciatic and sartorius nerves. These effects were observed at a concentration of 10 μmol/L MeHg. Hrdina *et al*. (1976) reported a decrease of cortical acetylcholine and serotonin in the brain stem of MeHg-poisoned rats. Concentrations of MeHg in the brain were not reported (Hrdina *et al*., 1976). Studies of the electrical organ of the *Torpedo oscilata* have shown that MeHg with high specificity blocks the acetylcholine receptor (Eldefrawi and Mansour, 1976; Shamoo *et al*., 1976).

Astrocytes accumulate MeHg that causes swelling of the cell and inhibition of uptake of excitatory amino acids like glutamate and aspartate. This effect is seen in astrocyte cultures with MeHg concentration approximately 10 μmol/L (Aschner *et al*., 1993; 2000). Park *et al*. (1996) demonstrated the importance of excitotoxic effects for the neurotoxicity of MeHg on a culture of rat cerebral neurons, showing that antagonists of *N*-methyl-D-aspartate (NMDA) receptor inhibit the toxic effect of MeHg (Park *et al*., 1996).

Extensive studies on animals have been performed to reveal the toxicology of MeHg. From the preceding, it is clear that the toxicokinetics of MeHg vary considerably in different species, especially the fraction of MeHg, which is absorbed and accumulated in the CNS. This fraction seems to increase phylogenetically in the animal species with advanced brain development. In the rat, 1% of the body burden of MeHg is retained in the brain, whereas in man 10% is retained. In other mammals, percentages lie between these two extremes. There are not only quantitative differences but also considerable qualitative ones among species. The CNS is the critical organ in primates, whereas in rodents, kidneys and peripheral nerves are damaged at lower doses than those that affect the brain (Magos and Butler, 1972). When the CNS is damaged by MeHg, the damage occurs in different parts of the brain for different species. Typically, the MeHg concentration in the nerve tissue is between 5 and 10 μg/g when the first morphological damage appears. In the rat, the first morphological changes appear in the dorsal root ganglia at a concentration of approximately 10 μg/g. These changes are combined with degenerative changes in the peripheral nerves. Twice these mercury concentrations in the brain are necessary to cause changes in the cerebellum, as well as in the granular cells and the brain stem. At much higher mercury concentrations, more extensive damage can be seen in other parts of the brain. These changes are partly secondary to vascular damage that is mainly seen in the arterioles (Diamond and Sleight, Fehling *et al*., 1975; 1972; Herman *et al*., 1973; Klein *et al*., 1972). Such seemingly primary vascular damage has also been observed at very high doses of MeHg in primates (Shaw *et al*., 1979).

In the mouse, the first morphological changes observed by Berthoud *et al*. (1976) were in the corpus striatum, above all the putamen, thalamus, and hypothalamus. These authors observed no changes in the cerebellum. MacDonald and Harbison (1977) who used another strain of mice reported first changes in the cerebellum and no other degenerative changes in the CNS. None of these authors reported on investigations of peripheral nerves (Berthoud *et al*., 1976; MacDonald and Harbison, 1977). The discrepancy between these observations can possibly be explained by genetic differences among the mouse strains. Jacobs *et al*. (1977) and Carmichael *et al*. (1975) studied the effects of MeHg on the CNS of the rabbit. They found, similar to what has been seen in the rat, that the first morphological changes appeared in the dorsal root ganglia, but at increasing doses degenerative changes in the rabbit appeared in small neurons and in granular cells of the cerebellum and of the parietal and occipital parts of the cortex. In the cat, the first morphological changes are seen in the cerebellum with degeneration of the granular cells, first in the vermis and at higher doses in the deep part of the cortical sulci. Purkinje cells also show degeneration. At still higher doses, changes appear in the occipital, parietal, and temporal cortex (Charbonneau *et al*., 1974; Grant, 1973). The toxicity of MeHg has also been studied in the pig in which no changes were seen in the cerebellum (Davies *et al*., 1976; Tryphonas and Nielsen, 1973). The neurotoxic effects in the dog are similar to those in the pig. The first changes appear in the brain cortex with the most pronounced ones in the visual cortex. No changes are seen in the cerebellum or other parts of the CNS or peripheral nerves (Miller, E. *et al*., 1972; Mozai, 1975).

The extensive studies performed on primates show consistently that the primary injury is located in the cerebral cortex, especially in the visual cortex and other cortical sensory centers. Changes in the cerebellum or the peripheral nerves have been observed at moderate toxic doses in marmosets (Eto *et al*., 2002). The changes in the occipital cortex in primates appear at a concentration of MeHg in the brain of 5–10 μg/g (Berlin *et al*., 1975b; Shiraki, 1979).

Accumulation of MeHg in the brain occurs in the fetal brain, in the growing and maturing brain as well as in the adult brain, and gives rise to neuronal damage that is especially located in the cortical sensory centra. The mechanism of this injury is still unknown; thus, it is still unclear whether the primary injury is inhibition of membrane function or whether vital processes in the cell nucleus are interfered with. There are research results supporting both possibilities. A further discussion of the neuropathological findings of MeHg poisoning is found in Shiraki (1979).

The aforementioned studies at the cellular level, involving *in vitro* cultures and *in vivo* systems, illustrate that MeHg is primarily toxic for the cells of the brain, neurons, and glia cells, and the compound seems to interfere with membrane function and structure, protein, and macromolecule synthesis. On this basis it could be expected that in all species, MeHg causes a consistent type of injury involving a large part of the nervous system. However, this is not the case. The injury found is very differentiated and specific and involves mainly sensory and cortical areas in some species and basal ganglias in others. In some species, the cerebellar cortex is involved and in others it is not. In affected cortical areas, some neurons are injured and others are not. This pattern is difficult to explain unless transport of MeHg by the interneuronal axonal flow in an anterograde direction is postulated. This assumption could explain why sensory centra of the brain are first affected. In these centra, afferent paths converge, and, as a consequence, the MeHg concentrations increase continuously with each synaptic step.

Motor centers are protected because the paths are efferent, and MeHg taken up in the system is transported peripherally out of the motor nuclei. This hypothesis can also explain the long latency between maximal mercury load in the body and the appearance of signs seen in man and mammals, because the axonal flow is relatively slow. The finding that toxic doses of MeHg are accumulated in subcortical association pathways (Berlin *et al*., 1975a) and the fact that in MeHg-poisoned monkeys, degeneration of the axons of the large efferent motor bundles in the spinal cord is observed (Berlin *et al*., unpublished data) both support this hypothesis. The hypothesis can also explain the observed species differences. It is reasonable to assume that in different species, different nerve paths and centra are dominant, depending on the function of the species. In primates, visual centra have a very dominating position in the brain. It has been shown that MeHg at low concentrations inhibits polymerization of tubulin to microtubuli. These observations offer a possible cellular mechanism that can explain the neural damage, because the microtubuli are likely to have a central function in axonal transport, especially in the anterograde direction. In tissue cultures (Choi *et al*., 1981b), as well as in the brain cortex of Squirrel monkeys (Berlin *et al*., unpublished data), the primary injury seems to be in the periphery of the neuron at the end of the neurite, the axon, or the dendrite. The available information concerning the effect of MeHg on the axonal

transport is very scarce and is referred to previously. However, this evidence does not contradict the suggested hypothesis. It has been suggested (Eto *et al*., 2001; Shaw *et al*., 1979) that areas close to deep sulci are preferably injured because of development of toxic edema in subcortical white matter resulting from accumulation of MeHg. This edema causes obstruction of the complex vascular network resulting in hypoxia and neural injury. Eto *et al*. (2001) supported the hypothesis with results from MRI studies of MeHg-exposed marmoset monkeys. They could visualize the edema subcortically and the distortion of the vascular bed around the calcarine fissures. Further investigations are necessary to confirm or reject this hypothesis concerning the mechanisms of MeHg neurotoxicity and distribution in the CNS.

Methylmercury inhibits the normal migration of nerve cells from the central parts of the neurotube toward the peripheral parts of the brain cortex of the human fetus and thus inhibits the normal development of the fetal brain (Choi *et al*., 1978).

In *in vitro* experiments on cultures of fetal neurons from the human fetus, Choi *et al*. (1980) showed that 20 μmol/L MeHg in the culture media inhibited the normal migration of neurons in the culture. When MeHg was given to newborn mice (Choi *et al*., 1981a) in doses that did not give rise to any acute toxic injuries on nerve cells, a considerable decrease of the embranchment of the dendrite tree of the Purkinje cells in the cerebellum of the treated animals was observed compared with what could be seen in untreated animals. Sager *et al*. (1982) performed similar studies on newborn mice and found that at a concentration of 1.8 μg Hg/g of cerebellum tissue, an inhibition of cell mitosis and a decrease of cellular density in the cortical cell layers occurred. This observation could be made distinctly 19 days after the start of MeHg exposure. With a doubling of the dose of MeHg concentration in the cerebellum, the difference was even more conspicuous. No information is available about the effect of MeHg exposure on the development and maturation of the brain during the postnatal period and early childhood.

7.1.3 Symptoms and Signs in Poisoning Caused by Exposure to Alkylmercury

Available data point to great similarities in symptoms and signs of poisoning caused by ethylmercury and MeHg. Most detailed information deals with MeHg. There is no sharp difference between acute and chronic poisoning from exposure to MeHg compounds. Once a toxic dose has been absorbed in the body, it is retained for a long time, causing functional disturbances and damage. On the other hand, a single toxic dose does not produce signs or symptoms until after a latency period, which may vary from one to several weeks.

Two clinical types of intoxication may be discerned, a prenatal and a postnatal type. These give rise to different kinds of signs and symptoms.

7.1.3.1 Prenatal Poisoning

The clinical picture in prenatal MeHg poisoning is that of an unspecific infantile cerebral palsy (Swedish Expert Group, 1971) involving ataxic motor disturbances and mental symptoms. On autopsy, the brain is found to be hypoplastic, with a symmetrical atrophy of cerebrum and cerebellum. Decreased numbers of neurons and distortion of the cytoarchitecture in the cortical areas are histological features (Choi *et al*., 1978; Takeuchi, 1977). The changes coincide with those of cerebral palsy of unknown etiology. Similar findings have been reported in cases of ethylmercury poisoning (Bakulina, 1968).

In less severe cases, psychomotor retardation has been observed, as revealed in delayed walking and talking by more than 12 months and an increased incidence of seizures (Marsh *et al*., 1980). In fish-consuming populations, a moderate increase in hair-mercury levels during pregnancy has been associated with impaired psychomotor test performance of the child at 4–7 years of age (Grandjean *et al*., 1997; Kjellstrom and Kennedy, 1985).

7.1.3.2 Postnatal Poisoning

The clinical signs of postnatal intoxication caused by MeHg are characterized by sensitivity disturbances with paresthesia in the distal extremities, in the tongue, and around the lips. These are early signs occurring after slight intoxication. In more severe intoxication, ataxia, concentric constriction of the visual field, impairment of hearing, and extrapyramidal symptoms may appear. In severe cases, clonic seizures have been observed. The pathological changes in the CNS are characterized by general neuron degeneration in the cerebral cortex with gliosis, most pronounced in the calcarine, the precentral, and postcentral areas. These changes are accompanied by atrophy of the cerebral cortex. In the cerebellar cortex, less pronounced changes, involving a loss of granular cells in the neocerebellum, may be encountered (Hunter and Russell, 1954; Okinaka *et al*., 1964; Takeuchi, 1968; Tsuda *et al*., 1963).

Considering that MeHg is excreted through breast milk, postnatal poisoning of the nursed child can easily arise. Symptoms for this type of poisoning are similar to those of the adult. It is, however, unclear to what extent poisoning during this period may, except for neuron damage, give rise to inhibition of the development and maturation of the brain.

Some reports seem to indicate that regeneration and compensatory adaptation during this period are better than during adulthood. When neurological signs because of MeHg poisoning appear, the duration of exposure is of importance for recovery and rehabilitation. The outlook for recovery and for rehabilitation seems to be better in the case of acute exposure compared with prolonged exposure (Amin-Zaki *et al*., 1978).

In the rat, MeHg has been observed to inhibit spermatogenesis (Katsuragi, 1978; Lee and Dixon, 1975; Sakai, 1972). Studies of spermatogenesis of MeHg-exposed men have, however, not been reported. MeHg in moderate toxic doses has an inhibiting effect on both primary and secondary immune response with a decreased resistance against viral infections in rodents as a result (Koller, 1980). Human data are not available regarding effects of MeHg on spermatogenesis.

7.1.3.3 Genetic Effects

Because MeHg reacts with DNA and RNA, it can be expected that genetic effects can arise from MeHg exposure. MeHg has been proved to be mutagenic under experimental conditions (Ramel, 1972). Little evidence of such an effect is available from clinical experience. However, Skerfving *et al*. (1974) reported chromosome aberration in lymphocytes of consumers of MeHg-contaminated fish, and Verschaeve *et al*. (1976) reported aberration and aneuploidy in ethylmercury-exposed workers. C. T. Miller *et al*. (1979) reported decreased DNA-repair activity in the leukocytes from cats exposed to MeHg at dose levels that were not neurotoxic. They also observed chromosome aberrations in leukocytes and bone-marrow cells. Mitsumori *et al*. (1981) reported that ICR mice given MeHg chloride in drinking water at toxic doses developed renal cancer (adenocarcinoma), which is a rare tumor in this animal. However, a large number of chronic toxicity studies have been made on laboratory animals like rats, cats, and monkeys, as well as epidemiological studies on MeHg-exposed human populations, and yet no evidence of tumor induction or promotion has appeared.

7.1.4 Indicators of Exposure and Concentration in the Critical Organ

Experimental studies in man and primates have shown that mercury concentration in blood is, under steady-state conditions, linearly correlated to intake of MeHg and to the concentration of MeHg in the critical organ (the brain), at nontoxic body burdens. Because >90% of MeHg in blood is to be found in the erythrocytes, the mercury concentration in red cells is the most reliable index of MeHg body burden and brain concentration. However, there are large species differences in the rate of MeHg binding to hemoglobin because of varying numbers of cysteinyl groups (Doi, 1991), resulting in varying blood–brain ratios in MeHg content. This is also true for primates. Thus, the blood/brain ratio in Squirrel monkeys is about twice that of *Macaca fascicularis* (Evans *et al*. 1977; Stinson *et al*. 1989) and the human ratio is about twice that of Squirrel monkeys (see previously), although the MeHg concentration in brain tissue is the same at equivalent toxic effect.

MeHg is deposited in the hair during the formation of the pile. The deposition of the MeHg in the pile is proportional to the mercury concentration in blood at the time of pile formation. Thus, the mercury concentration in the hair pile constitutes a calendar of mercury concentrations in blood that occurred during the formation of the pile. The MeHg concentration in the hair can be used as an indicator of mercury concentration in blood, and in the critical organ, or body burden of mercury, provided that allowance is made for the growth rate of the hair pile—approximately 1 cm a month, depending on age (Pelfini *et al*., 1969) and for the time lag between hair formation and extrusion. As mentioned previously, the quotient between MeHg concentration in human blood and hair is 1:250. Under occupational conditions, the possibility of external contamination of hair should be kept in mind.

Because urinary excretion of MeHg is very small, MeHg concentration in urine is easily masked by the presence of inorganic mercury. Thus, urine concentration of Hg is not a good index of MeHg body burden or of MeHg concentration in the critical organ.

7.1.5 Dose-Response Relationships

Most of our knowledge about dose-response relationships in MeHg is derived from studies of the epidemics of MeHg poisoning in Iraq (Bakir *et al*., 1973) and Japan (Swedish Expert Group, 1971) and from studies of populations eating mercury-contaminated fish (Clarkson *et al*., 1975; Skerfving, 1974b). When considering the dose-response relationships in MeHg poisoning, three main effects can be discussed: the neurotoxic effect, the embryo toxic effect, and the genetic effect as shown in chromosomal aberrations of the lymphocytes. The dose-response relationship for each of these effects is discussed separately in the following.

The dose of MeHg can be expressed in terms of level of intake, amount absorbed as reflected by concentration of MeHg in blood or hair, and finally in terms of

concentration in the critical organ (the brain). Each approach is used below for each effect whenever data are available.

7.1.5.1 Incidence of Signs and Symptoms of Prenatal MeHg Poisoning Related to Concentration of MeHg in the Hair of the Mother

In the Minamata epidemic of MeHg poisoning, 23 cases of prenatal intoxication were diagnosed. Among the Japanese cases, the mothers of the affected children had commonly shown no clinical signs of poisoning. Scanty data available on mercury levels in biological materials like blood and hair (Swedish Expert Group, 1971) indicate that the difference in sensitivity between the fetus and the adult organism is less than a factor of 5 and closer to a factor of 2. In a follow-up study of children in the Minamata area 20 years later, a strong association between prevalence of mental retardation and mercury concentration in the umbilical cord, customarily saved in Japanese families, was encountered (Harada *et al*., 1977). Marsh *et al*. (1980) studied, in an epidemiological investigation in Iraq, the relationship between mercury concentration in the mothers' hair during pregnancy and prevalence of signs and symptoms of neurological damage or mental retardation in the children; neurological signs in the mothers themselves were also examined. The mercury exposure during pregnancy was assessed by scanning hair samples from the mothers, approximately 9 cm of hair, representing the entire pregnancy period. The children were followed up for 4–5 years, and milestones like onset of walking or speech as well as prevalence of mental retardation and seizures were recorded. All the children were neurologically examined by specialists.

Among children of mothers who, during any month of the pregnancy, had mercury concentrations in hair exceeding 50 mg/kg, there was a significant increase in the prevalence of severe psychomotor retardation involving delayed onset of walking and speech, as well as the occurrence of seizures. In two children with severe psychomotor retardation, the highest mercury concentration in the hair of the mother during 1 month was approximately 50 mg/kg. This material, so far comprising 29 mothers and infants, does not preclude that in the interval—10–70 mg/kg MeHg in hair during 1 month of pregnancy—there may be an increased risk of psychomotor retardation for the children.

It is, at present, unclear whether the effect of MeHg on the CNS of the fetus is related to the mercury exposure during the entire pregnancy or whether adverse effects can appear as a result of high MeHg exposure during a short period of the pregnancy. Neither is any information available about what period of the pregnancy is the most sensitive to MeHg exposure. It should be pointed out that in these epidemiological studies of the Iraqi epidemics, the recorded and observed signs were signs of severe neurological impairment and retardation resulting from high level of exposure for a limited period. So far, no such information is available for the Iraqi population concerning the level of methylmercury exposure during pregnancy in which period more subtle changes may occur, changes detectable only in psychomotor tests on a group basis. A study in New Zealand (Kjellstrom and Kennedy, 1985) indicates that more subtle effects may occur at hair-mercury levels exceeding 6 mg/kg (ppm), as demonstrated in a population of 31 mother-child pairs, in which the fish consumption during pregnancy resulted in hair-mercury levels within the range of 5–20 ppm. The 31 children were tested with the Denver test at 4 years of age, and the test results were compared with those of matched controls. A significant dose-related difference was found (Kjellstrom and Kennedy, 1985). Forty-six children were tested at 6 years of age and showed exposure-associated deficits in Wechsler intelligence scale for children-revised or in language development test compared with referents at maternal hair levels of 13–15 ppm (Crump *et al*., 1998). However, in both studies, there were differences between exposed and referents not fully controlled for.

A large cohort of 917 children, 7 years old, with mothers exposed to Me Hg and PCBs, mainly from consumption of Pilot whale, was studied in the Faroe Islands. The geometric mean maternal MeHg hair level was 4.5 ppm (range, 0.6 – 39.1 ppm). A comprehensive test battery including neurophysiological and neuropsychological tests showed pronounced mercury exposure–related deficient performance in attention, memory, and language in children of mothers with <10 ppm MeHg in hair and also an exposure associated delay in peak III of the brain stem auditory evoked potentials. (Grandjean *et al*., 1997) This increased latency has also been seen in a cross-sectional study of 149 approximately 7-year-old children in Madiera when maternal MeHg in hair from fish consumption exceeded 10 ppm (Murata *et al*., 1999) A follow-up of the Faroe cohort at 14 years of age showed that the findings at 7 years of age were persistent (Debes *et al*., 2006).

Attempts to control for PCB levels in cord blood did not support a contribution of PCB exposure to the effect of mercury in the Faroe Island cohort (Budtz-Jörgensen *et al*., 1999). In another study by the Faroe Group of 182 neonatals with prenatal exposure to MeHg and PCBs, similar to the Faroe cohort described previously, the neurological optimality score (NOS) was assessed. A significant negative correlation between cord blood Hg and NOS was found (Steuerwald *et al*., 2000).

A prospective longitudinal cohort study of 711 children followed to 9 years of age in the Seychelles did

not show any adverse effects of the maternal exposure from fish consumption (Davidson *et al*., 1998; Huang *et al*., 2004; Myers *et al*., 2003). The average maternal Hg content in hair during pregnancy was 6.9 (SD, +4.5) ppm; range, 0.5–26.7 ppm. The cohort was tested with an age-specific comprehensive neuropsychological test battery every third year. There are several possible explanations to the differences in results between the two largest cohort studies. The question about the size of the risk of neurodevelopment interference by MeHg exposure resulting in maternal hair levels below 10 ppm remains open. It is, however, important to point out that all the mentioned epidemiological studies have been performed on populations with frequent amalgam bearers. The release of mercury vapor from dental restorations may be an important confounder, so far uncontrolled for. Mercury vapor causes in animal experiments the same toxic signs and brain pathology at fetal exposure as MeHg has an additive effect at concomitant exposure to MeHg and is approximately 10 times more toxic in terms of brain concentration of Hg needed for adverse effect (see also preceding).

7.1.5.2 The Relationship Between Daily Intake and Biological Index for MeHg

The relationship between daily intake of MeHg and the concentration of MeHg reached at steady state is still under debate. Epidemiological studies involving studies of food intake have resulted in values of 0.3–0.8 for the relation between MeHg intake and MeHg concentration in blood expressed in μg/L or day at 70-kg body weight (reviewed by the WHO in 1976). In experimental short-term studies on man, this quotient has been found to be close to 1 (Kershaw *et al*., 1980; Miettinen; 1973).

7.1.5.3 The Influence of Exposure Duration on the Incidence of Signs and Symptoms of Postnatal MeHg Poisoning

Most epidemiological data concerning MeHg poisoning are derived from studies of acute epidemics that occurred in Japan and Iraq. In two epidemiological studies, data concerning more long-term exposure (Tsubaki and Irukayma, 1977; Tsubaki *et al*., 1978) have been collected from a follow-up study of the Niigata epidemics 10–15 years after the exposure event. They reported that signs appeared with very long latency at low levels of exposure. Similar experiences have arisen from animal experiments on primates. In another study on Canadian Indians, who over a very long period were exposed seasonally to MeHg and who had blood concentrations of MeHg probably not exceeding 700 μg/L and seldom exceeding 200 μg/L, a statistically significant association between consumption of contaminated fish and prevalence of neurological symptoms, such as ataxia, was found (Health and Welfare, Canada, 1979; Methylmercury Study Group, 1980). The results from these two epidemiological studies indicate that exposure over decades may result in brain damage, although blood concentrations of MeHg never rise to those observed in more acute epidemics of MeHg poisoning.

7.1.5.4 Incidence of Signs and Symptoms of MeHg Poisoning Related to Concentration of MeHg in the Brain

Few data on concentrations of MeHg in the brains of fatalities from the epidemic in Japan have been reported. Almost no data are available from the extensive epidemic in Iraq. The lowest concentrations reported from Japan (Swedish Expert Group, 1971) are in the order of 5 mg/kg in brain tissue. This value can be estimated to correspond to approximately 800 μg/L in blood, and approximately 200 mg/kg in hair. These values are rather low in comparison to dose-response relationships for early signs like paresthesia. No basis for the estimation of the mortality rate exists at this level. The MeHg concentration in the brain that corresponds to 200 ng/g in blood or the lowest concentration at which neurological signs have been observed can be calculated to lie between 1 and 2 mg/kg on the basis of the data cited previously. This concentration can be compared with data from animal experiments in which the lowest concentration seen gives rise to objectively recorded changes in the CNS. Mattson *et al*. (1981) found that the lowest concentration in a dog at which clear effects on "visual evoked response" in the visual cortex could be observed was 1.28 mg/kg. These authors also found pronounced histopathological changes in the brain tissue at 8–15 mg/kg in the dog. Such changes in the "visual evoked response" have also been observed in patients from Niigata and Minamata (Tsubaki and Irukayama, 1977). A sudden 50% increase in brain/blood mercury concentration ratio was observed with increasing MeHg levels in the blood of Squirrel monkeys with brain concentration approximately 2.5 mg/kg and MeHg in blood approximately 1000 μg/L. This change coincided with a subcortical autoradiographic accumulation of radioactive mercury in the brain and development of neurological signs (Berlin *et al*., 1975). This observation indicates a change in toxicokinetics, which may be due to change of the blood–brain barrier, a saturation of elimination routes, or both. The subcortical accumulation seems to support the second alternative. Brain concentrations of Hg approximately 2 mg/kg was found in offspring prenatally exposed and with pronounced brain pathology with migration disturbances (Lögdberg *et al*., 1993).

7.1.5.5 Incidence of Genetic Effects as Related to Concentration of MeHg in the Blood

Two studies related to chromosome aberration in lymphocytes of alkylmercury-exposed populations have been published. Skerfving *et al*. (1974) observed a dose-related increase of chromosome aberration in lymphocytes in consumers of MeHg-contaminated fish. They found increased chromosome aberrations in persons with MeHg concentrations in blood approximately 100 µg/L. Verschaeve *et al*. (1976) reported chromosome aberrations and increased prevalence of aneuploidy in ethylmercury-exposed workers. However, these authors did not account for the exposure levels.

7.1.5.6 Factors Interacting with MeHg Toxicity

In experimental studies on animals and in studies on nerve tissue cultures (Kasuya, 1976) selenium has been shown to interfere with the metabolism and the toxic effect of MeHg. Selenium and MeHg create in the blood, probably under the influence of glutathione, bismethylmercury selenide ($[CH_3Hg]_2Se$) (Naganuma and Imura, 1980). This compound seems to penetrate the brain barrier and give rise to mercury accumulation to a larger extent than MeHg alone (Naganuma et al., 1980).

Bismethylmercury selenide seems to decompose and be reformed in the tissue in the presence of selenium and MeHg. Yamane *et al*. (1977) injected ^{14}C-labeled MeHg in rats and found that the exhalation of ^{14}C increased considerably if sodium selenide was given to the rats. These results indicate that selenium can accelerate demethylation of MeHg. Several authors have reported that administration of equivalent amounts or lower doses of selenium to rats or other rodents decreased toxicity of MeHg. These reports have been reviewed by Skerfving (1978). Sugiura *et al*. (1976; 1978) point out that MeHg can be bound to selenium or selenohydryl ligands with a stability that exceeds that of sulfhydryl bonds. Several authors have reported changed distribution of MeHg after administration of selenium with an increased accumulation of selenium in the brain, liver, kidneys, and blood. Selenium administration seems to increase mercury retention in adult and fetal brain (Iijima *et al*., 1978; Satoh and Suzuki, 1979). The benevolent effect of selenium administration on MeHg toxicity seems to be mainly limited to the membrane-damaging effect of MeHg. This effect depends on the type of cells (Alexander *et al*., 1979; Potter and Matrone, 1977) and the type of selenium compound that is administered.

Ohi *et al*. (1976) reported that administration of sodium selenide more effectively counteracts the neurological signs in rats compared with the selenium in fish flesh. These selenium compounds were, however, equivalent in counteracting the inhibition of growth of the rats by MeHg. Lee *et al*. (1979) found no effect of selenium administration on MeHg-induced palate defects in mice. This toxic effect of MeHg may be an expression of interaction of MeHg with protein synthesis. For further discussion of selenium and mercury interaction on membrane structures, the reader is referred to Ganther (1980).

MeHg inhibits the production of antibodies (Koller, 1975) but given together with selenium to mice, a stimulation of antibody formation occurs that is more pronounced than after administration of selenium only (Koller *et al*., 1979). Excretion of mercury through bile after administration of MeHg in the rat is markedly inhibited by selenium, probably because of inhibition of the formation of the MeHg-glutathione complex (Alexander and Norseth, 1979).

Another factor that can interact with membrane stability in nerve cells and other cells is vitamin E. Studies on the effect of vitamin E administration on MeHg toxicity in rat have shown that administration of vitamin E increased tolerance to MeHg (Welsh, 1979). Similar observations have been made by Chang *et al*. (1978) in studies on hamsters. In tissue cultures of nerve tissue, a benevolent effect of vitamin E has been observed (Kasuya, 1975b). It has long been known that organic solvents interfere with the membrane function in the CNS. It is therefore of interest to note that ethanol potentiates the toxic effect of MeHg in rat (Turner *et al*., 1981).

It is, at present, difficult to evaluate the experimental animal reports on interaction between selenium or vitamin E and MeHg with respect to health risks for man because of MeHg exposure. Although the enzyme glutathione reductase, which contains selenium, has been demonstrated in man, the need for selenium and the selenium metabolism in man are different from what has been found in rodents. Human dose-response data for selenium are lacking, and it is uncertain whether further administration of selenium or vitamin E above the normal intake through food has any importance for the MeHg toxicity in man. The mechanism for MeHg toxicity and the metabolism of MeHg is different in man compared with rodents. However, it cannot be precluded that the administration of selenium or vitamin E can modify or counteract MeHg toxicity in man.

7.1.5.7 Toxicity of Dimethylmercury

Dimethylmercury is effectively absorbed by inhalation or by skin penetration. When absorbed, dimethylmercury is transformed in the organism to methylmercury. Severel lethal cases of poisoning have been described in chemists (Hunter, 1957; Nierenberg *et al*., 1998). The symptomatology and toxicokinetics are identical to that seen after exposure to methylmercury compounds. A remarkable delay of 5 months between

a single exposure and debut of neurological signs has been reported in a lethal case of poisoning (Nierenberg *et al*., 1998) Such a long delay may indicate that dimethylmercury can be distributed in fat depots and subsequently be slowly released demethylated.

7.1.5.8 Toxicity of Ethylmercury Compounds

The ethylmercury moiety in water-soluble EthylHg compounds will bind to SH groups in the biological environment like MeHg and is also distributed in mammals in a similar manner. The uptake of ethylmercury in the brain suggests active transport, although the transporter has so far not been identified. The *in vivo* dealkylation and elimination rates of EthylHg are faster than of those MeHg. The half-time in blood is approximately a third of that of MeHg in man (Magos, 2001). The toxicokinetics of EthylHg has been previously reviewed in detail by Magos (2003). On the basis of animal experiments (Magos *et al*., 1983), the accumulation of inorganic Hg in the brain is likely to be larger than after exposure to MeHg as a consequence of faster biotransformation. This has been recently confirmed in a nonhuman primate, *Maccaca fascicularis* (Burbacher *et al*., 2005).

Poisoning from EthylHg exposure has occurred in industry from overdosing pharmaceuticals containing EthylHg preservatives like thimerosal and by ingestion of contaminated food with EthylHg fungicides. Magos (2001) has previously reviewed the cases reported in the literature. It seems that the clinical signs and symptoms at EthylHg poisoning are very similar to those for MeHg poisoning, including dysarthria, ataxia, constricted visual fields, and paraesthesias. The brain pathology is also similar. Results from animal studies are consistent with clinical observations. The clinical material does not allow dose-response estimations, but it seems that blood Hg of approximately 1 μg/mL involves risk of adverse effects and with approximately 2 μg/mL, severe poisoning develops from exposure to EthylHg (Magos, 2001).

Epidemiological studies on populations of infants have reported some positive and some negative associations between vaccines containing thimerosal and autism (See IOM, 2004 for a summary). The hypothesis linking thimerosal to autism is biologically plausible but controversial. On the one hand, methylmercury and ethylmercury have been shown to be neurodevelopmental toxicants, so it is possible that with repeated doses of ethylmercury, neurodevelopmental interference could occur. On the other hand, because the incidence of autism is low in vaccinated infant populations, it is possible that only a rare sensitive genotype may be affected such that statistical significance of an association would be difficult to establish. Recent gene expression studies on lymphocytes from autistic and nonautistic children indicated no increases in metallothionein expression in response to thimerosal and no differences in up-regulation of metallothionein genes by zinc between the two groups (Walker *et al*., 2006). Marked up-regulation of a number of heat shock proteins in response to thimerosal exposure was observed in lymphocytes from both groups. Autistic children have also been reported to deviate from normal children in terms of plasma concentrations of metabolites involved in the transsulfuration and methionine cycle pathways (Geier and Geier, 2006a). In a recent ecological study, Geier and Geier, (2006b) observed that the reported incidence of neurodevelopmental disorders over an 11-year period, including 642 cases of autism in the United States, has significantly declined after removal of thimerosal from child vaccines. Another ecological study from Canada included 27,749 school children and reported approximately 60 cases of autism vaccinated as birth cohorts between 1987 and 1998 (Fombonne *et al*., 2006). Thimerosal in vaccines was discontinued in Canada in 1996. On the basis of the findings of their study, the authors concluded that there was not any association between pervasive developmental disorder (e.g., autism) and levels of ethylmercury exposure similar to those found in the United States during the 1990s or with single or multiple MMR vaccinations. It is not possible to draw any conclusions from these ecological observations, but they underline the need for further research to determine possible risks with exposure to thimerosal. To bring this controversy to closure, further research is also clearly needed to evaluate whether specific differences in genotype exist between autistic and nonautistic children. The situation with ethylmercury thus is in line with the general note of a recent review (Clarkson and Magos, 2006) on mercury regarding it as an "element of mystery."

8 PREVENTION, PROGNOSIS, AND TREATMENT

The prognosis of poisoning from mercury compounds depends on the type of mercury compound involved and the dose. All types of specific therapy are aimed at lowering the concentration of mercury at the site of action in the organism and removing the mercury compound from the organism. This can be achieved by different methods common to all kinds of mercury poisoning. Chelating agents such as DMSA (Frumkin *et al*., 2001) and DMPS (Aposhian, 1998; Bose-O'Reilly, 2003; Garza-Ocanas *et al*, 1997; Gonzalez-Ramirez *et al*., 1998; Pingree *et al*., 2001; Torres-Alanis *et al*., 2000) have been used for both diagnostic purposes and treatment with varying degrees of effectiveness. In all severe cases, the first choice should be hemodialysis and infusion of chelating agents for mercury, such as *N*-acety1cysteine or cysteine and oral administration of DMPS. In less severe cases, mobilization

and redistribution of mercury can be achieved by aid of chelating agents such as DMPS (2,3- dimercaptopropane-1-sulfonate) (Wannag and Aaseth, 1980) and DMSA (dimercaptosuccinic acid) (Magos, 1985).

The excretion of methylmercury can be increased by catheterization and drainage of the choledochal duct or surgical establishment of gallbladder drainage, thereby breaking the enterohepatic circulation of mercury. This method is most efficient with regard to those types of compounds for which enterohepatic circulation plays an important role.

8.1 Mercury Vapor

The prognosis in pronounced intoxication by mercury vapor involving severe tremor and mental changes is, according to what is found in the literature, remarkably good with complete regression if exposure ceases. Cases of successful treatment with DMPS have been reported in the literature (Campbell and Clarkson, 1986; Mant, 1985). However, after years of exposure, serious sequelae with neurological symptoms, mental impairment, and dementia have been reported (Andersen *et al*., 1993; Baldi *et al*., 1953, Kishi *et al*., 1993; O'Carroll *et al*., 1995). Whether the regression is due to a compensatory mechanism—remaining neurons taking over the function of damaged ones—or whether it is a complete regression cannot be answered with the present knowledge; nor is it known whether mercury deposited in the brain can be mobilized by therapeutic measures, or whether regression is due to mercury eliminated from the brain or converted to and deposited in an inert form. Exposure of the fetus or during infancy to mercury vapor should be avoided to prevent interference with brain development. A frequent cause of acute mercury poisoning is the use of vacuum cleaner to collect spilled mercury liquid. The vacuum cleaner generates and disperses mercury vapor in the surrounding area.

8.2 Inorganic Mercuric Mercury

Severe sublimate poisoning with renal tubular damage is a fatal condition, but this prognosis can be considerably improved by therapeutic measures. In acute poisoning, DMPS will efficiently mobilize mercury from the kidney and reduce biological half-life for mercury (Ashton and House, 1989; Mant, 1985; Nadig, 1985; Toet *et al*., 1994).

The experimental evidence is that the damaged tubules can regenerate to a considerable degree. In cases of acrodynia or pink disease, DMPS or DMSA should be used for mobilization of mercury from the body.

8.3 Short-Chain Alkylmercury

Signs and symptoms of intoxication with alkylmercury compounds do not appear until several weeks after absorption. Moreover, the appearance of signs of intoxication is secondary to severe damage of the cerebral cortex. If exposure is stopped, considerable regression of signs and symptoms may occur, according to the experiences in the Iraqi epidemic (Bakir *et al*., 1973). To date, therapeutic improvement of the prognosis on manifest intoxication has not been seen clinically. Good experimental and clinical evidence shows that hemodialysis with *N*-acetylcysteine or cysteine infusion combined with oral administration of DPMS can drastically reduce the concentration of alkylmercury in the brain and the body (AI-Abbasi *et al*., 1978; Kostyniak *et al*., 1974; 1975; Lund *et al*., 1984). This therapy should be the first choice in any progressive state of intoxication. This treatment can be followed by continuous therapy with DPMS to reduce the body burden of alkylmercury further. If hemodialysis cannot be applied, bile drainage should be considered, with oral replacement of the bile salts and electrolytes lost in the drain. From a theoretical point of view, bile drainage should be the most efficient way to eliminate methylmercury excreted in bile. However, there are no reports on the practical application of this treatment. Facemask and skin protection are necessary when handling dimethylmercury and methylmercury compounds. Common protective clothing and gloves are not sufficient. Special material impenetrable to alkyl mercury compounds has to be used (Nierenberg *et al*., 1998).

8.4 Phenylmercury Compounds or Methoxyethylmercury Compounds

Severe renal damage from these types of compounds should be treated with oral administration of DPMS (Buchet and Lauwerys, 1989). There are no clinical cases of this therapy reported. BAL is contraindicated because it forms lipid-soluble complexes with the organic mercury compounds and redistributes mercury to the brain, whereby it may cause severe disturbance in the CNS (Berlin and Levander, 1965).

8.5 Long-Term Therapy in Chronic Exposed Cases

There is no clinical evidence that long-term exposure to chelators is safe. On the contrary, there is some evidence that chelators alone or in combination with mercury can have side effects and also mobilize essential metals (Ewan and Pamphlett, 1996). Inorganic mercury accumulated in the brain cannot be mobilized

with available chelators according to experimental experience (Buchet and Lauwerys, 1989).

Sulfur taken orally was used for treatment of mercury poisoning during the 19th century (Dyes, 1865). Berglund and Carlmark (1997) reported that a daily dose of 50 mg sulfur in tablets or capsules increased the fecal excretion 2–6 times in five amalgam bearers. They also reported a follow-up study on 153 treated patients with duration of treatment of a few days up to 6 years diagnosed with side effects from the mercury released from dental amalgam. Many of the patients reported subjective improvement of symptoms; a few reported adverse effects in terms of constipation and itching.

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CHAPTER

34

Molybdenum

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ABSTRACT

Molybdenum is an essential element for humans, and dietary recommendations have been established. Dietary intakes in humans are usually within the range of the recommendations. Soluble molybdenum compounds are readily absorbed when ingested. The highest molybdenum concentrations are found in the kidneys, liver, and bone. Excretion, primarily through the urine, is rapid. The biological half-life ranges from a few hours to a few days. Turnover is much more rapid when intake is high than when intake is low.

The metabolism of molybdenum is affected by copper and sulfur intake in some species. In ruminants, copper generally has a beneficial effect on the symptoms caused by excessive molybdenum, and thiomolybdates increase the excretion of copper. Both positive and negative effects of the interaction between these three elements have been reported. The effects and their magnitude vary between animal species.

In livestock, chronic molybdenum poisoning known as "teart disease" is caused by a diet high in molybdenum and low in copper. Symptoms include anemia, gastrointestinal disturbances, bone disorders, and growth retardation. In laboratory animals, excessive molybdenum may give rise to morphological and functional changes in the liver, kidneys, and spleen. It has a growth-depressing action, and deformities of bone may occur.

A few cases of pneumoconiosis have been reported among workers exposed to metallic molybdenum and molybdenum trioxide. Increased blood uric acid values and goutlike symptoms have been reported among workers exposed to molybdenum in a copper-molybdenum plant, as well as among the general population living in an area with high molybdenum and low copper content in soil and vegetables.

Molybdenum chemistry, metabolism, toxicity, and essentiality have been reviewed by Friberg *et al*. (1975), Chappell and Petersen (1976; 1977), Mills and Davis (1987), Johnson (1997), Turnlund (2002), Institute of Medicine (2002), and Anke (2004).

1 PHYSICAL AND CHEMICAL PROPERTIES

Molybdenum (Mo): atomic weight, 95.9; atomic number, 42; density, 10.2 g cm^{-3}; melting point, 2617°C; boiling point, 4612°C; crystalline form silver-white metal or grey-black powder, cubic; most important oxidation states are +2, +3, +4, and +6.

More than 50 inorganic forms of molybdenum are known, including the insoluble metallic molybdenum, molybdenum disulfide, and lead molybdate. Soluble molybdenum compounds include ammonium molybdate, calcium molybdate, molybdic oxide, sodium molybdate, and molybdenum trioxide.

A number of organometallic compounds containing molybdenum are found in biological systems, and these compounds have been known to be important to biological systems for more than 75 years. Plants, microorganisms, and animals contain molybdoenzymes, and they are required by bacteria, archaea, fungi, plants, and animals (Stiefel, 2002). All but one of the molybdoenzymes (nitrogenase, an enzyme needed by nitrogen-fixing plants) includes a pterin-containing molybdenum cofactor. Detailed reviews of the many important roles of molybdoenzymes in nitrogen fixation, the carbon

†Deceased

cycle, and sulfur metabolism can be found in Sigel and Sigel (2002). Three of the molybdoenzymes are found in animals and humans. Their roles are described in Section 5.5 of this chapter.

2 METHODS AND PROBLEMS OF ANALYSIS

Colorimetric methods, among them the thiocyanate and the dithiol methods, have been used for decades for the determination of molybdenum concentrations. Differential pulse polarography, X-ray fluorescence, and neutron activation analysis have also been used. Duval (1971) in a comparison of different methods found detection limits for the thiocyanate and polarographic methods to be 0.01 mg/kg dry weight, and for neutron activation 0.02 mg/kg. For a detailed discussion of the determination of molybdenum concentrations by these methods, refer to reviews by Meglen and Glaze (1974), Kosta (1980), and Cantone *et al*. (1995).

Flame atomic absorption (AA), graphite furnace atomic absorption (GFAA), inductively coupled plasma atomic emission spectroscopy (ICP), inductively coupled plasma mass spectrometry (ICP-MS), and thermal ionization mass spectrometry (TIMS) are the analytical methods used most frequently now. A description of the first four methods can be found in Perkin-Elmer (2000). Reported detection limits in µg/L (or ppb) are 45 for flame AA, 0.03 for GFAA, 0.5 for ICP, and 0.001 for ICP-MS. Detection limits have not been reported for TIMS, but molybdenum has been measured reliably in purified samples containing 10 ng of molybdenum (Giussani *et al*., 1995).

The mass spectrometric methods listed previously, ICP-MS and TIMS, have the best precision and accuracy (Crews *et al*., 1994; Giussani *et al*., 1997). They are used in conjunction with stable isotope dilution in studies of molybdenum metabolism (Keyes and Turnlund, 2002; Luong, *et al*., 1997; Turnlund *et al*., 1993) to attain the highest accuracy and precision. Recent advances in analytical methods and the use of stable isotopes to trace the metabolic fate of molybdenum have advanced the understanding of molybdenum metabolism greatly in recent years.

3 PRODUCTION AND USES

3.1 Production

Molybdenum does not occur naturally in the native state but is obtained from the ores molybdenite, wulfenite, ferrimolybdate, and jordicite. The overwhelming majority comes from molybdenite, the largest deposit of which is found at Climax, Colorado (Lander, 1977). It is also recovered as a by-product of copper and mining operations. Worldwide production in 2003 was 127,000 tons (Magyar, 2004). The United States, Chile, and China produce 75% of the total amount.

3.2 Uses

The primary use of molybdenum is as an alloying agent in steel and cast iron. As such it is utilized in the arms industry, in aeronautical engineering, and in the automobile industry. Approximately 75% of its use is in the iron and steel industry. In the chemical industry, molybdenum compounds are used as catalysts and as chemical reagents. There are also many pigments that contain molybdenum compounds. Molybdenum is an essential element in plant and animal nutrition. Requirements are very low, but it may be added to soil, plants, or water for specific purposes. Sodium molybdate is used in the animal feed industry but is allowed only in sheep feed. It reduces copper toxicity in sheep.

4 ENVIRONMENTAL LEVELS AND EXPOSURE

4.1 Food and Daily Intake

The estimated daily intake of molybdenum has been revised downward with recent analytical techniques. Dietary intakes were reported to range from 50–126 µg/day in the United States (Pennington and Jones, 1987). Mean intakes of 113 µg/day were reported in Great Britain (Buss and Rose, 1992) and 87 µg/day in Belgium (Van Cauwenbergh *et al*., 1997). Holzinger *et al*. (1998) reported intake in Germany of 95 µg/day with a mixed diet, 179 µg/day with a vegetarian diet, and intake in Mexico of 160–200 µg/day. The foods contributing the most molybdenum to the diet are grains (31–39% of molybdenum intake of adults), milk and dairy products (28% of intake of infants and 18% of intake of toddlers), and legumes (4–19% of intake of adults) (Pennington and Jones, 1987).

Molybdenum concentrations in plants range from <0.5–>100 mg/kg dry weight. Generally, high concentrations are found in leafy vegetables and legumes, whereas edible roots have a lower content. Animal products are generally low in molybdenum (Mills and Davis, 1987). The variations in foodstuffs, especially plants, greatly depend on species and soil characteristics. The molybdenum content of animal feeds and forages is highly variable, depending on the molybdenum content of the soil and pH of the soil. When pH of soil

increases, uptake of molybdenum increases. For example, a Scottish study demonstrated that grasses grown in soil with pH 5.5 have a molybdenum content of 1.1 mg/kg dry weight and the molybdenum content increases progressively reaching 5.2 mg/kg when the pH is 7.5 (Mills and Davis, 1987).

4.2 Water, Soil, and Ambient Air

The concentration of molybdenum in the ocean is approximately 0.01 mg/L (Kuroda and Tarni, 1974; Muzzarelli and Rocchetti, 1973; Sugawara *et al*., 1962). Levels in American rivers range from 5–30 mg/L (Turekian and Scott, 1967). Molybdenum in drinking water ranges from 0.11–6.21 µg/L (Bostrom and Wester, 1967; Chappell *et al*., 1979; Hrabovska and Groch, 1978; Osmolovskaya, 1967; Valcuk and Copik, 1974). In mineral water, molybdenum levels are between 2.3 and 3.3 µg/L (Nevoral, 1975). In areas where molybdenum ore is mined, considerable contamination may occur, which can cause high concentrations in drinking water, leading to daily intakes of more than 1000 µg (Chappell, 1974).

Molybdenum in normal soil varies between 0.1 and 10 mg/kg (Brogan *et al*., 1973; Reddy, 1964). In mining areas and near molybdenum-emitting industries, considerably higher values have been reported (Chappell, 1974). Molybdenum in sewage sludge was found to vary between 2 and 30 mg/kg dry weight (Berrow and Webber, 1972).

In ambient air in urban areas, molybdenum ranged from 0.01–0.03 µg/m^3, and in nonurban areas it varied between 0.001 and 0.0032 µg/m^3 (Schroeder, 1970).

5 METABOLISM

5.1 Absorption

5.1.1 Inhalation

Little data are available on absorption of molybdenum after inhalation. Guinea pigs showed no noticeable absorption after exposure through inhalation to 285 mg Mo/m^3 as molybdenum disulfide. Hexavalent molybdenum compounds were absorbed to an appreciable extent, even though it was not possible to measure the absorption (Fairhall *et al*., 1945). There are no human data on absorption of molybdenum after inhalation.

5.1.2 Ingestion

Gastrointestinal absorption is high in animals and humans. Animal data from single exposure studies demonstrated that hexavalent molybdenum is readily absorbed from the gastrointestinal tract. Absorption is between 40 and 85% in guinea pigs, rats, and goats (Anke *et al*., 1971; Fairhall *et al*., 1945; Neilands *et al*., 1948; Van Campen and Mitchell, 1965).

Molybdenum is very well absorbed by humans over a broad range of intakes. Absorption, determined by use of stable isotopes as tracers, ranged from 88–93% with dietary intakes of 22–1400 µg/day (Turnlund *et al*. 1995a). An absorption study with intrinsically labeled foods suggested that the efficiency of absorption may be less from some foods, as absorption was 57% from soy compared with 87% from kale (Turnlund *et al*., 1999).

5.2 Distribution

Studies of the distribution of molybdenum in guinea pigs and rats after a single oral administration of molybdenum trioxide show that there was an immediate accumulation in the kidneys, liver, and bone. Similar distribution is seen in rats, cows, and goats after prolonged exposure. The highest concentrations are found in the kidneys (Anke *et al*., 1971; Fairhall *et al*., 1945; Huber *et al*., 1971; Robinson *et al*., 1964).

In mice, after a single intravenous injection, the highest concentrations were found in the kidneys, liver, and pancreas, 1–24 hours after exposure. A decrease with time took place in the kidneys and pancreas, whereas the concentrations of molybdenum in the liver remained constant during the observation period (Rosoff and Spencer, 1973).

Fourteen days after intragastric and subcutaneous application of 25–40 µg/kg body weight to rats, most of the molybdenum was present in the kidneys, liver, hard tissues, and skin. Concentrations of the element in tissues were dose dependent (Lener and Bibr, 1973).

Molybdenum reacts with skin collagen and alters the mechanochemical properties of collagen fibers (Bibr *et al*., 1977; Lener *et al*., 1976). It accumulates in long bone, in growth cartilage, and in diaphysis spongiosis (Lener *et al*., 1974). The maximum concentration in teeth occurs in dentin, cement, and to a lesser extent in enamel (Lener, 1978).

In the blood, molybdenum is bound in the form of molybdate, specifically to α_2-macroglobulin, and in erythrocytes to proteins of the erythrocyte membrane, especially spectrin (Bibr *et al*., 1983; Kselikova *et al*., 1980). Molybdenum concentrations in the blood of humans increase as dietary intake increases. In controlled human studies, concentrations increased from 4–44 nmol/L in plasma when intake increased

from 22–1400 µg/day (Turnlund and Keyes, 2004). Concentrations are usually <10 nmol/L (Versieck, *et al*., 1981). Blood concentrations rise after meals, then return to basal levels (Cantone *et al*., 1995). Infused tracer doses disappear rapidly, with 2.5–5% remaining after an hour (Cantone *et al*., 1995, Rosoff and Spencer, 1964).

5.3 Excretion

Animal data indicate low retention and more or less complete excretion of molybdenum, primarily through urine, during the first 2 weeks after single exposures in guinea pigs, rats, goats, and swine (Anke *et al*., 1971; Bell *et al*., 1964; Bibr and Lener, 1973; Fairhall *et al*., 1945; Kselikova *et al*., 1974; Neilands *et al*., 1948). The main excretion route in cows was reported to be through feces (Bell *et al*., 1964). An important excretion route related to the gastrointestinal excretion is the bile (Lener and Bibr, 1979). Small amounts are excreted through milk and hair in goats (e.g., approximately 2% and 0.2%, respectively) (Anke *et al*., 1971). A negligible amount of molybdenum is excreted in the saliva (Lener, 1978).

The pattern and route of molybdenum excretion in man is influenced by dietary intake. The primary route of excretion is through the urine (Rosoff and Spencer, 1964; Turnlund *et al*., 1995a; 1995b). Excretion was studied by Rosoff and Spencer (1964) after a single intravenous injection of radioactive molybdenum to terminally ill patients. The cumulative ^{99}Mo excretion over 14 days was 24% in one subject and 29% in another, whereas the corresponding fecal excretion was 6.8% and less than 1%, respectively. Controlled human studies demonstrated that when intake is very low, approximately 60% of the amount of ingested molybdenum is excreted in the urine, and approximately 40% is eliminated in the stools. When intake is high, > 90% is excreted in the urine and <10% in the stools (Turnlund *et al*., 1995a,b). The source of molybdenum in the stools is partly unabsorbed dietary molybdenum and partly endogenous molybdenum excreted into the gastrointestinal tract through the bile. The excretion of urinary molybdenum in the United States averaged 69 µg/day (Paschal *et al*. 1998) and in Germany ranged from 28–52 µg/day. German women excreted 10 µg molybdenum/day in milk on the 35th day of lactation (Anke, 2004).

Molybdenum excretion is influenced by copper and sulfates, but the effect is not consistent and varies among species studied (Mills and Davis, 1987). The interaction is discussed in Section 7.3.

5.4 Biological Half-Life

The excretion of molybdenum and its rapid clearance from the liver, kidney, spleen, testis, and hard tissues in animal studies show that the biological half-life for the major part of the absorbed molybdenum must be in the order of hours and extending to a maximum of approximately 1 day in laboratory animals and cows (Bibr and Lener, 1973; Fairhall *et al*., 1945; Neilands *et al*., 1948; Robinson *et al*., 1964; 1968).

In healthy adults, the half-life is influenced by dietary intake and is much shorter when intake is high than when it is low (Turnlund *et al*., 1995a). Half-lives of plasma clearance in humans were estimated to be between 4 and 70 minutes for a fast component and between 3 and 30 hours for a slow component (Werner *et al*., 2000). When a small dose was injected, 34% of the dose was excreted in the urine within 1 day, and 60% was excreted when a large dose was injected. Residence time in slow turnover tissues, based on 120-day studies, was estimated at between 56 and 267 days (Thompson and Turnlund, 1996; Thompson *et al*., 1996).

Studies in rats demonstrated that after a single subcutaneous injection, the concentration of molybdenum in blood decreases rapidly. After 24 hours, only approximately 1% of the maximum concentration remained. During the next 10 days, concentrations in blood diminished with a half-life of approximately 7 days (Kselikova *et al*., 1974). After application of doses up to 3 µg/kg, the biological half-life was 47 hours. At doses higher than 3 µg/kg, the biological half-life was 3 hours for subcutaneous and 6 hours for intragastric application (Bibr and Lener, 1973). After single subcutaneous application of 0.02–1 µg Mo/kg to rats, the biological half-life in the kidneys, liver, spleen, jejunoileum, and skin varied between 2.5 and 4.7 days (Bibr and Lener, 1974).

5.5 Molybdenum Deficiency

Molybdenum is an essential element for animals (Rajagopalan, 1988). It is a constituent of three mammalian enzymes, xanthine oxidase, aldehyde oxidase, and sulfite oxidase. These enzymes all contain the molybdenum cofactor, a complex of molybdenum and an organic component, molybdopterin (Higdon, 2003; Johnson, 1997; Turnlund, 2002). Sulfite oxidase oxidases sulfite to sulfate, reducing molybdenum VI to molybdenum IV, and is required for the metabolism of the sulfur amino acids. Xanthine oxidase catalyzes the breakdown of nucleotides to uric acid. Xanthine oxidase and aldehyde oxidase catalyze a number

hydroxylation reactions and play a role in the metabolism of drugs and toxic substances. Of these enzymes, sulfite oxidase is the only one that is essential to human health.

Only one case of molybdenum deficiency has been attributed to dietary molybdenum (Abumrad *et al*., 1981). A man with Crohn's disease who was on total parenteral nutrition (TPN) for 18 months developed symptoms including tachycardia, headaches, and nightblindness 6 months before his death. Biochemical abnormalities included elevated plasma methionine, low serum uric acid, high urinary thiosulfate, low urinary uric acid, and low urinary sulfate. These abnormalities were reversed after administration of ammonium molybdate.

Metabolic defects have been related to molybdenum metabolism but are not associated with dietary intake. These defects are rare. More than 100 infants have been identified who lack functioning sulfite oxidase. The patients had either a defect in the gene coding for the sulfite oxidase enzyme or, more often, a genetic deficiency in the molybdenum cofactor (Johnson, 1997; Turnlund, 2002). Both defects are autosomal recessive traits. Those with molybdenum cofactor deficiency are deficient in all three molybdoenzymes due to lack of functional molybdopterin. The serious symptoms are due to lack of functional sulfite oxidase. Symptoms include severe brain damage, resulting in death at an early age. The defects can be diagnosed in early pregnancy through chorionic villus sampling (Higdon, 2003). Biochemical abnormalities listed in the preceding paragraph are found in these conditions, as well as seizures, mental retardation, brain atrophy and lesions, and dislocated lenses (Johnson, 1997). Classic xanthinuria is also an autosomal recessive trait and is due to either a defect in xanthine oxidase or both xanthine oxidase and aldehyde oxidase, but not sulfite oxidase. It is much more common than molybdenum cofactor deficiency but is not a lethal defect.

Molybdenum deficiency has been produced in goats when on a diet with a molybdenum content of only 24 μg/kg dry weight (Anke *et al*., 1985). It has been induced in chicks and rats, but only after tungsten was added at a ratio of 1000:1 (Anke *et al*., 1985).

Symptoms of molybdenum deficiency were observed in lambs in areas of New Zealand with low molybdenum concentrations in the soil and simultaneous protein deficiency. In these animals, signs of renal lithiasis were observed, and the renal stones were composed predominantly of xanthine. For this reason the syndrome was called "xanthine disease" (Ferrando, 1971).

On chicken farms, birds displayed a number of symptoms characterized by the loss of feathers, disorders in the ossification of long bones, and changes in joint cartilage, leading to complete immobility. This seemed to be due to a molybdenum deficiency, because addition of molybdenum to the diet in doses of 0.2–2.5 mg/kg eliminated the preceding symptoms (Bains and Mc Kenzie, 1975; Payne and Bains, 1975), but results require experimental examination.

5.6 Dietary Requirements and Recommendations

The first dietary molybdenum recommendations for humans were made in the United States in 1980 (National Research Council, 1980). Because inadequate information was available to establish a Recommended Dietary Allowance (RDA), an Estimated Safe and Adequate Daily Dietary Intake (ESADDI) was introduced. It was based on usual intake, which was estimated to be 150–500 μg/day. The ESADDI was revised downward to 75–250 μg/day after lower estimates of dietary intake were reported (National Research Council, 1989). On the basis of controlled human studies in young men, the minimum dietary requirement was estimated at 25 μg/day (Turnlund *et al*., 1995b), which agrees with estimates based on extrapolation from nonruminant animal studies (Anke *et al*., 1985). In 2002 Dietary Reference Intakes (DRIs) were introduced for molybdenum (Institute of Medicine, 2002). The DRIs include an Estimated Average Requirement (EAR), the intake that meets the requirement of half of the healthy individuals in a age and gender group; a Recommended Dietary Allowance (RDA), the average intake that meets the requirement of nearly all healthy individuals intake; and a Tolerable Upper Intake Level (UL), the highest intake that is likely to pose no risk of adverse health effects for almost all individuals. The EAR for adults was based on a requirement of 25 μg/day, with an added factor for bioavailability, or 34 μg/day. The RDA was set at 45 μg/day for adults of all ages, 50 μg/day for pregnant and lactating women, and from 17 μg/day for children 1–3 years of age to 43 μg/day for those 14–18 years of age. When data are not available to establish an RDA, an Adequate Intake (AI) is established. An AI of 2 μg/day was set for infants 0–6 months and 3 μg/day for those 7–12 months based on the usual intake of breast-fed infants. Because of lack of availability of human data, the UL was based on extrapolation from studies of reproductive effects in rats and mice and using an uncertainty factor (UF) of 30. The UL for adults was thus set at 2 mg/day. The ULs for children 1–18 years of age ranged from 0.3–1.7 mg/day, and no UL was set for infants because of lack

of data. This is generally consistent with human data showing no adverse effects of 1.5 mg/day in controlled studies (Turnlund, 1995b). The European Commission's Scientific Committee on Food (SCF, 2000) used a more conservative UF of 100 and derived a UL of 0.6 mg/day for adults. The ULs for children ranges from 0.1–0.5 mg/day.

6 BIOLOGICAL MONITORING

6.1 Biomarkers of Exposure

A biochemical marker for molybdenum status has not been identified. Levels in many tissues and excretion reflect dietary intake in animals and humans. However, none of these has yet been directly related to molybdenum status. The mammalian molybdoenzymes are involved with catabolism of sulfur amino acids, purines, and pyrimidines. Defects in these enzymes result in changes in metabolites of sulfur amino acids, purines, and pyrimidines. S-sulfocysteine, taurine, xanthine, and hypoxanthine increase and uric acid is low (Turnlund, 2002).

In normal adults, median values for the liver, kidneys, and adrenal glands have been found to be 1, 0.3, and 0.7 mg/kg wet weight, respectively, when examined spectrographically (Tipton and Cook, 1963). Gurskaya (1966) found that molybdenum content in the human amnion was on average 3.5 mg/kg and in the chorion 0.6 mg/kg wet weight. Plantin (1973) determined molybdenum in the liver and kidney cortex by neutron activation analysis in eight autopsy cases in Sweden (age range, 51–63 years). The average concentrations were 0.88 and 0.2 mg/kg wet weight. Pribluda (1964) reported on the molybdenum concentration in the liver and kidneys of 90 subjects aged 17–77 years in the U.S.S.R. Arithmetic mean values for the liver (thiocyanate method) were 0.5–0.6 mg/kg and for kidneys approximately 0.2 mg/kg wet weight. The molybdenum content of the liver was shown to increase with age, up to 10–20 years (Schroeder *et al.*, 1970; Tipton and Cook, 1963). The age of German men and women did not affect molybdenum concentrations in the liver, which averaged approximately 4 mg/kg dry weight (Anke, 2004).

Data on daily urinary excretion of molybdenum in "normal" human subjects have been presented by Meltzer *et al*. (1962) and Wester (1974). On average, excretion in the groups varied between 49 and 71 µg/day. Urinary excretion is directly related to recent dietary intake (Turnlund *et al*., 1995a). However, the values do not necessarily reflect molybdenum status (Institute of Medicine, 2002).

A wide range of plasma molybdenum concentrations were reported before 1973, ranging from 5.6–257 µg/L (Versieck *et al*., 1978). However, more recent estimates on the basis of NAA and ICP-MS resulted in concentrations of from 0.3–1.1 µg/L (Luong *et al*., 1997; Versiek and Cornelis, 1980). Similar to urinary excretion, blood plasma concentrations also reflect recent dietary intake, but not molybdenum status. They are considerably higher, from 2–4 µg/L, when intake is unusually high, from 500–1500 µg/day (Turnlund and Keyes, 2004).

6.2 Biomarkers of Effects

Symptoms associated with molybdenum deficiency, which is rare, or defects in molybdoenzymes in humans include seizures, mental retardation, dislocated lenses, and brain atrophy, and lesions (Turnlund, 2002).

The effects of molybdenum toxicity on health are described in the next section.

7 EFFECTS AND DOSE-RESPONSE RELATIONSHIPS

7.1 Local Effects and Dose-Response Relationships

7.1.1 Animals

Inhalation of molybdenum compounds in high concentrations may be irritating to the upper respiratory tract. A few reports on inhalation or intratracheal administration have been published. Pneumoconiosis-like effects in the lungs have been reported in rats and rabbits. In one study, rabbits were given a suspension of powdered molybdenum intratracheally in doses of 70–80 mg/kg. After 9 months, diffuse pneumoconiosis with interstitial pneumonia was observed on histological examination (Dzukaev, 1970; Mogilevskaya, 1963).

7.1.2 Humans

Pneumoconiosis with X-ray findings and subjective symptoms has been reported in 3 of 19 workers exposed to metallic molybdenum and molybdenum trioxide. Exposure varied between 1 and 19 mg/m^3 for 4–7 years (Mogilevskaya, 1963).

In the U.S.S.R., of 500 workers from a molybdenum and copper mine, many revealed nonspecific symptoms and changes in the CNS. The molybdenum dust levels in the mines were in some cases 10–100 times greater than 6 mg Mo/m^3—the U.S.S.R. maximum permissible level (Eolayan, 1965).

In 25 workers from a roasting plant in Denver, where molybdenum sulfide is converted to molybdenum oxides, nonspecific complaints have been observed. The most frequent complaints were joint pains, headaches, backaches, and nonspecific hair and skin changes. The exposed workers when compared with the control group demonstrated significant increases in serum ceruloplasmin and milder increases in serum uric acid levels. Hyperuricosuria was not found. Eight-hour time-weighted average of exposure to molybdenum has been calculated to be 9.47 mg Mo/m^3; the minimum daily body burden of molybdenum has been calculated to be 10.2 mg Mo (Walravens *et al.*, 1979).

7.2 Systemic Effects and Dose-Response Relationships

7.2.1 Laboratory Animals

Acute as well as prolonged exposure to excessive molybdenum may give rise to morphological changes in the liver, kidneys, and spleen of rats, guinea pigs, and rabbits. Proteinuria and functional disturbances of the liver have been reported. Molybdenum has a growth-depressing action. Other symptoms after prolonged exposure are anemia, diarrhea, and deformities of joints and long bones, as well as mandibular exostoses (Arrington and Davis, 1953; Ferguson *et al.*, 1943; Halverson *et al.*, 1960; Mc Carter *et al.*, 1962; Ostrom *et al.*, 1961; Mills and Mitchell, 1971; Valli *et al.*, 1969).

Effects in rats include mandibular exostoses, growth retardation, and anemia after a 5-week diet containing 400 mg Mo/kg as sodium molybdate (Ostrom *et al.*, 1961). Van Reen (1959) reported reduced growth and mandibular exostoses to rats fed 50 mg Mo/kg diet (given as Na_2Mo0_4) plus a low sulfate supply. Long-term exposure to a dose of 10 mg/kg of molybdenum in drinking water increased sensitivity to thermal stress (Winston *et al.*, 1973; Winston and Trainor, 1979). Exposure to 80 mg/kg/day molybdenum resulted in mild renal failure in rats (Bompart *et al.*, 1990).

The mechanism of molybdenum toxicity is not yet understood. It is assumed that the primary factor is the formation of a copper-tetrathiomolybdate complex in the reducing medium of the gastrointestinal tract that reduces the biological utility of copper. This also explains the higher toxicity of molybdenum in ruminants compared with monogastric animals. In addition, tetrathiomolybdate reduces the activity of ceruloplasmin, a copper-containing enzyme (Kelleher and Mason, 1979; Winston, 1981).

7.2.2 Livestock

The effects of molybdenum intake in livestock depend on a number of factors and vary with species. Ruminants such as cows and sheep are sensitive to molybdenum levels as low as 2–30 mg/kg, whereas horses, pigs, and goats tolerate up to 1000 mg/kg (Rajagapolan, 1988).

Evidence of chronic molybdenum poisoning because of high molybdenum intake has been seen in cattle and is known as molybdenosis (or teart or peat scours). This effect occurs after exposure to a diet high in molybdenum and low in copper. Symptoms include anemia, gastrointestinal disturbances, bone disorders, growth retardation, and impaired reproduction. The molybdenum levels in typical teart pastures range from 20–100 mg/kg dry weight, compared with normal levels of 3–5 mg/kg (Ferguson *et al.*, 1943; Hogan *et al.*, 1971; Ward, 1978). The disease has been reproduced experimentally in cattle (Cook *et al.*, 1966; Ferguson *et al.*, 1943; Huber *et al.*, 1971). In lambs grazing on soil containing 2.5 times more molybdenum than copper, 15–39% had neonatal enzootic ataxia (swayback) (Mogilevskaya, 1963).

In Switzerland, beef cattle, raised in an environment with a high molybdenum content and with a calculated daily intake of 141.4 mg, developed the syndrome locally called Malmagliar or S-charl-Krankheit, which manifests itself in anorexia, diarrhea, and loss of weight (Hogl, 1975).

In areas with soil with a high molybdenum and low copper content, calves showed periodontal inflammatory changes in combination with the loosening and loss of teeth and edema of soft maxillary tissues. Addition of copper to the diet removed these symptoms entirely (Camargo *et al.*, 1982). In ruminants, upper limits of molybdenum intake have been reported to range from 6 mg/kg dry weight in the diet of cattle to 1000 mg/kg dry weight in goats and mule deer (Anke, 2004).

7.2.3 Humans

A high incidence of a goutlike disease was reported in an area of Armenia with levels of 77 mg Mo/kg and 39 mg Cu/kg in the soil (Koval'skiy and Yarovaya, 1966). On the basis of copper and molybdenum levels in different food products, the daily intake in the exposed area was calculated to be 10–15 mg Mo and 5–10 mg Cu compared with 1–2 mg Mo and 10–15 mg Cu in a control area. In this region of the U.S.S.R., more than 50% of the diet is based on locally grown products.

A medical survey of 400 subjects from two villages in the molybdenum-rich area revealed a prevalence of

symptoms similar to gout for 31% of the inhabitants in one of the villages and 18% in the other. The authors claimed that similar symptoms normally occurred in 1–4% of the general population of the U.S.S.R. The symptoms were characterized as arthralgia in the knee joints, hands, and feet. Joint deformities were also reported, as well as increased values of molybdenum and uric acid in blood and urine. There are difficulties in the interpretation of the data because of possible bias in the selection of groups to be examined, but it raises concern about regions where exposure to molybdenum through the diet is high. Molybdenum exposure could give rise to an increase in xanthine oxidase activity that in turn should give rise to an increase in uric acid formation (Gusev, 1969). However, another study found decreased uric acid in serum and urine with high molybdenum intakes (Chapell *et al*., 1979). Another found no change in uric acid, but increased urinary copper (Deosthale and Gopalan, 1974). No changes were observed in urinary copper in a controlled study with an intake of 1.5 mg/day of molybdenum (Turnlund and Keyes, 2000). There is one case report of molybdenum toxicity from molybdenum supplements of 300–800 µg/day (Momcilovic, 1999). Symptoms included severe headaches and psychosis, but a clear cause-and-effect relationship was not demonstrated, and results of a controlled study with intakes higher than this (Turnlund *et al*., 1995a) did not support that association.

Systemic effects from occupational exposure to molybdenum have been reported rarely. A single case report described the development of hyperuricemia and gout in a man who worked with molybdenum sheet metal for 10 years (Selden, *et al*., 2005). Symptoms were relieved when the exposure was eliminated. Exposure was reintroduced by reconstruction of conditions leading to symptoms. Exposure was accompanied by high urinary molybdenum, followed later by reoccurrence of gout. Occupational exposure led to high serum ceruloplasmin and uric acid in 25 workers in a roasting plant, in addition to other symptoms, as described in Section 7.1.2 (Walravens *et al*., 1979).

Studies of the effect of molybdenum on dental caries have given contradictory results. Epidemiological studies in Hungary (Adler and Straub, 1953), New Zealand (Ludwig *et al*., 1960; 1962), Columbia (Glass *et al*., 1973; Rothman *et al*., 1972), New Guinea (Adkins *et al*., 1974), and South Africa (Pienaar and Bartel, 1968) have demonstrated the positive effect of molybdenum on the incidence of caries in the teeth of humans. However, no such correlation was found in the population of the United States. (Curzon *et al*., 1971). There is no clear explanation of the mechanism of effect of molybdenum ions in reducing the incidence of dental caries. A complex action is assumed; the most important effect seems to be the direct chemical incorporation into dental tissues, especially into the hydroxyapatite lattice of the enamel (Bawden and Hammarstrom, 1976; Bertrand, 1972; Coulter and Russel, 1974; Curzon *et al*., 1971; Lener, 1978; Rahkamo and Tuompo, 1975).

No satisfactory data were available to establish safe levels of molybdenum intake in humans. Studies in rats provided a dose-response relationship for numerous adverse reproductive effects (Fungwe *et al*., 1990), suggesting a molybdenum LOAEL of 1.6 mg/kg/day and a NOAEL of 0.9 mg/kg/day. This was extrapolated to humans using a factor of 30, resulting in a NOAEL of 30 µg/kg/day. After multiplying by reference body weights and rounding, a UL of 2 mg/day was established (Institute of Medicine, 2002). The EPA set a Reference Dose (RfD) of 0.005 mg/kg/day on the basis of a higher LOAEL of 0.14 mg/kg/day (Goldhaber, 2003). However, the LOAEL was based on limited data and only gross physical effects, so the EPA has only medium confidence in the RfD.

7.3 Interaction with Copper and Sulfur

There is a complex relationship between molybdenum, copper, and sulfur. This topic has been reviewed in depth, but is still not completely understood (Allen and Gawthorne, 1986; Mason, 1986; Mills and Davis, 1987; Rajagopalan, 1998). Thiomolybdates, particularly trithiomolybdate and tetrathiomolybdate, have powerful effects on copper metabolism. These effects seem to be explained by alterations in the affinity for copper of competing ligands, such as albumin. As a result, changes in copper distribution lead to copper depletion. The formation of trithiomolybdate by ruminants seems to be primarily responsible for the biochemical pathogenesis leading to the symptoms of molybdenosis (Mason, 1986).

Species differences exist (e.g., sheep and cows are more susceptible to imbalances between these elements than pigs, horses, and rats). Copper generally has a beneficial effect on the symptoms caused by excessive molybdenum but the action of sulfur compounds, especially sulfate, is not yet clearly understood. Some of the symptoms of molybdenum toxicity resemble those of copper deficiency and treatment with copper may reverse them.

High dietary molybdenum results in redistribution of copper in sheep (Pott *et al*., 1999), and copper deficiency has been observed in ruminants grazing on pastures where the molybdenum content is high (Bremner, 1979). There is one report of a similar effect in humans

(Deosthale and Gopalan, 1974), but this effect was not observed in a controlled study with high dietary molybdenum (Turnlund and Keyes, 2000).

Copper toxicity has been treated with tetrahydromolybdate in humans and a number of other species. Wilson's disease, an autosomal recessive disorder of copper storage in humans, is associated with copper accumulation in the liver and brain resulting in neurological damage and cirrhosis. Penicillamine is often used to remove excess copper. An alternative treatment is the administration of tetrahydrothiomolybdate (Brewer and Yuzbasiyan-Gurkan, 1992; Turnlund, 2002). After administration of terathiomolybdate, plasma copper levels rise and copper is excreted in the urine. LEC rats have a disorder resulting in accumulation of copper in the liver similar to Wilson's disease. Administration of tetrathiomolybdate mobilizes copper from the liver complexed with tetrathiomolybdate. Molybdenum and copper are then excreted in equimolar amounts into the bile (Komatsu *et al*., 2000). Ammonium tetrahydromolybdate has been considered the treatment of choice for copper toxicity in sheep. Recent studies demonstrated that pituitary endocrinopathy and cessation of reproductive activity were observed with this treatment, raising concern about the practice (Haywood *et al*., 2004).

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CHAPTER

35

Nickel*

CATHERINE KLEIN AND MAX COSTA

ABSTRACT

IARC, the International Agency for Cancer Research, concluded in 1990 that nickel compounds were human carcinogens, and most animal data *in vivo* and genetic toxicology data *in vitro* before that time suggested that the insoluble particulate nickel species were the most carcinogenic; however, more recent human epidemiology and experimental data are pointing to the water-soluble nickel compounds as perhaps of equal hazard. Accumulating molecular and genetic toxicology data show that soluble $NiCl_2$ can, in fact, be mutagenic and genotoxic, especially when cells are chronically exposed for long durations up to several weeks to allow sufficient nickel uptake. On reconsideration of the previous experimental studies with shorter 24–48-hour acute exposures to the insoluble nickel compounds *in vitro*, it is likely that those actual nickel exposures were, in fact, of much longer duration than previously thought because of the unlikely removal of adherent nickel particles from the cell surfaces.

This chapter briefly reviews some of the most recent work on nickel uptake, metabolism, environmental and occupational exposures, and human disease epidemiology. Other resourceful reviews on nickel include Costa *et al*. (2001), Costa (2002), and Lu *et al*. (2005). The newest information on the molecular mechanisms of nickel's action in biological systems is briefly summarized herein, and the reader is specifically directed to Chapter 5 for additional details.

*Revised and updated from the chapter by Tor Norseth, 1986 edition of this Handbook

1 PHYSICAL AND CHEMICAL PROPERTIES

Nickel (Ni): atomic weight, 58.71; atomic number, 28; density, 8.9; melting point, 1453°C; boiling point, 2732°C; silver-white malleable metal; exists as oxidation states of 0, +1, +2, and +3. Predominate compounds of interest include nickel oxide, nickel hydroxide, nickel subsulfide, nickel sulfate, nickel chloride, and nickel carbonyl. Nickel carbonyl [$Ni(CO)_4$] is a volatile, colorless liquid (boiling point 43°C) that decomposes at temperatures >50°C. Nickel salts of strong acids and organic acids are soluble in water, whereas salts of weak inorganic acids are insoluble. Nickel is resistant to corrosion by air, water, and alkali. These desirable properties, along with its metallic shine and inexpensive costs, have made Ni metal alloys popular choices for use in body piercing jewelry (Vilaplana *et al*., 1991).

2 METHODS AND PROBLEMS OF ANALYSIS

Routine measurements of nickel and nickel complexes in a variety of matrices, including biological samples (e.g., urine, blood), workplace atmospheres, soil, water, and food crops can be obtained by analytical methods, including mass spectrometry (MS), nuclear magnetic resonance (NMR), infrared spectroscopy (IR), UV-Vis spectroscopy, and EPR (electron paramagnetic spin resonance) (reviewed in Chandra and Kumar, 2005). Some advanced mass spectrometry methods that have been applied to study nickel complexes include MALDI (matrix-assisted laser desorption/

ionization), LSIMS (liquid secondary ion mass spectrometry), APCI-MS (atmospheric pressure chemical ionization mass spectrometry), and ESI-MS (electron spray ionization mass spectrometry) carried out by means of Q-TOF MS (quadrupole time-of-flight mass spectrometry). ICP-MS (inductively coupled plasma-mass spectrometry) was used to quantify oil particulate matter (PM)–associated metals Ni and medium (v) in the olfactory mucosa, bulb, and brain of exposed animals (Calderon-Garciduenas *et al*., 2003). X-ray diffraction (XRF) analysis has been used to quantify nickel in biological samples such as archived lung from nickel workers, as well as from collected air samples from workplaces (Andersen and Svenes, 2003). Trevorite, an inert mineral where divalent iron is replaced by nickel, was commonly found by XRF in these archived lung samples. Both electrothermal atomic absorption spectrometry (ETAAS) and inductively coupled plasma optical emission spectrometry (ICP-OES) with ultrasonic nebulization techniques were found to be suitable and, in fact, better than flame atomic absorption spectrometry (FAAS) or ICP-MS for determination of nickel and other elemental metals in wines (Lara *et al*., 2005). A modified reverse-phase high-performance liquid chromatography (HPLC) method for the separation and determination of nickel(II) and other metals has high sensitivity, with the very low detection limits of 8.7 ng/mL for nickel(II) (Deng, 2005; Peng *et al*., 2005). Gas chromatography coupled with ICP-MS (GC-ICP-MS) is a useful method for detecting the highly toxic gaseous nickel carbonyl [$Ni(CO_4)$] (Feldman, 1999). XAS (X-ray absorption) has been useful to characterize structural nickel complexes with cellular proteins and histones (Carrington *et al*., 2002).

3 PRODUCTION AND USES

3.1 Production

Nickel exists as a natural earth element, primarily in sulfide or oxide ores that are mined underground or in open pits. It is the 24th most abundant element on earth, with the greatest deposits of nickel ores in Canada, Siberia, and New Caledonia, a French territory in the South Pacific. Methods for extraction of nickel matte from nickel sulfide ores include flotation, magnetic separation, roasting, and smelting. Raw nickel matte, composed of approximately 15% nickel and copper sulfides, metallic nickel, and metallic copper; 50% iron, and 25% sulfur, is processed to remove oxidized iron sulfide, leaving nickel matte that is approximately 50% nickel. Further refining by oxidation at high temperatures (780°C) and treatment with hot 12% sulfuric acid yields material that when reduced with hydrogen gas and reacted with carbon monoxide forms volatile nickel carbonyl. Pure nickel can be produced by heating nickel carbonyl until it decomposes to pure nickel and carbon monoxide. These refining processes can be repeated up to seven times to extract maximal amounts of valuable metallic elements from the matte (Draper *et al*., 1994a,b).

In 1997, worldwide production of nickel was approaching 1,000,000 tons per year *(http://www.chemlink.com.au/nickel.htm)*.

3.2 Uses

Industrial uses of nickel include steel and alloy production; electroplating; nickel-cadmium battery production (nickel hydroxide); chemical catalysis; the manufacture of electronic components such as vacuum tubes and transistors (nickel carbonate); and in the production of metal items such as armaments, factory tools, dental tools, and household utensils. Non-industrial uses of nickel alloys include coins; jewelry; watches; eyeglass frames; clothing buttons and studs; household and cooking utensils; orthodontic appliances such as braces, bridges, or wires; dental tools; orthopedic implants; and circulatory stents.

4 ENVIRONMENTAL EXPOSURES

4.1 General Environment

4.1.1 Air, Soil, and Water

Combustion of fossil fuel and pollution from nickel industries are the primary sources of environmental nickel exposure. More specifically, sources of nickel emissions into the air include coal and oil burning for power and heat, waste and sewage sludge incineration, mining and steel production industries, and electroplating (WHO, 1991). It has been estimated that approximately 180,000 metric tons of nickel per year are generated from fossil fuel–burning emissions and industrial processes (IARC, 1990). Atmospheric nickel concentrations from fossil fuel combustion and automobiles are approximately 120–170 ng/m^3 in industrialized regions and large cities (Norseth *et al*., 1979), and these are the major sources for nonoccupational nickel exposure by inhalation. Gaseous Ni carbonyl at sub ppb range can be detected by GC-ICP-MS in fermentation gases from municipal sewage treatment plants (Feldman, 1999).

In the soil, nickel has been found to vary widely in concentration, from 0.2–1000 mg/kg dry weight

(reviewed in Chang and Page, 1996). Unless complexed to organic matter, nickel in soil primarily exists as divalent cations. Near nickel refineries or in dried sludge, levels of nickel have been found to be 24,000–53,000 ppm Ni in soil, up from the nonindustrial average level of 500 ppm Ni (US EPA, 1990). For example, in the soil and lake sediments of the nickel mining region of northwestern Russia, it was estimated that during 60 years of mining/refining activity, 310 tons of Ni, plus another approximately 200 tons of other metals and mercury, have accumulated in the lake sediments, which are a secondary source of pollution (Dauvalter, 2003). During this century, the average concentrations of nickel in superficial sediments have increased by approximately 25 times. Metal, including nickel, contamination of garden soils may be widespread in urban areas because of past industrial activity and the use of fossil fuels; however, risk assessment models do not predict health risks from reuse of such soils for growing food crops in urban redevelopment areas (reviewed in Hough *et al*., 2004).

Nickel levels in natural waters have been found to range from 2–10 µg/L in fresh and tapwater and from 0.2–0.7 µg/L in marine water (reviewed in Rojas, *et al*., 1999). A survey of U. S. surface water indicated that the concentrations range from 5 µg–600 µg Ni/L in different regions of the country (US EPA, 1990). The maximum contaminant level (MCL) of nickel allowable in drinking water for lifetime consumption, as established by the US EPA (1993), is set at 0.1 mg Ni/L. Average total nickel concentrations in drinking water range from 3–7 µg Ni/L, with concentrations up to 35 µg Ni/L occasionally encountered (Andersen *et al*., 1983). In areas of nickel mining, however, up to 200 µg Ni/L drinking water have been recorded (McNeely *et al*., 1972). The US FDA limits nickel in bottled water to 0.1 mg Ni/L.

4.1.2 Food Intake

Ni ingestion in humans occurs through the consumption of plant and animal products. Vegetables such as legumes, spinach, lettuce, and nuts contain more nickel than other food items. In plants such as potatoes and corn, metals including nickel are primarily ligated to polysaccharides such as amylose and amylopectins (Ciesielski and Piotr, 2004). Other dietary sources of nickel exposure include baking powder, cocoa powder, and acid beverages, perhaps related to leaching during manufacturing from pipes and containers. In Western countries, nickel levels are generally <0.5 mg/kg fresh weight for produce, except for cacao and nuts with much higher levels in the 5–10 mg/kg range (reviewed in Rojas *et al*., 1999), and these levels are stable over time as measured from 1993 to 1997 (Larsen *et al*., 2002). Estimates of total dietary intake of nickel average approximately 200–300 µg/day, although oral nickel intake because of leaching from cooking ware, kitchen utensils, and water piping can perhaps be as high as 1 mg/day (Grandjean, 1984). It has been suggested that only 5% of ingested nickel is absorbed (Rojas, *et al*. 1999). Infants can ingest 5–15 µg Ni/day from breast milk. The mean concentration of nickel in breast milk was measured at 17 µg Ni/kg, exceeding the mother's serum nickel levels (Feeley *et al*., 1983).

4.1.3 Skin Absorption

Nickel absorption through skin contact is a major human exposure route, with a large portion of the population suffering from contact dermatitis and nickel allergy. Whereas nickel contact dermatitis was previously reported to occur in up to 10% of women but only 1% in males (WHO, 1991), recent estimates have nickel sensitivity approaching 30% of populations, again more prevalent in females (Brydl *et al*., 2004). An increasingly large variety of sources of nonoccupational nickel exposures include common objects such as nickel-plated tools and utensils, jewelry, coins, orthodontic braces and wires, and surgical joint prostheses. On the basis of increasing incidences of allergic contact dermatitis because of nickel and other metal exposures from household items, it has been recommended that such items contain no more than 1–5 ppm each of Ni, Cr, or Co (Basketter *et al*., 2003). In the European Union (EU) the "Nickel Directive" limits its nickel content and release from jewelry (e.g., < 0.05% in pierced-earring posts) and other routinely used metal items and utensils to minimize chronic nickel exposure (<0.2 µg/cm^2/week) and resulting sensitization and dermatitis (Liden and Norberg, 2005). Even the new 1- and 2-Euro coins that are composed of nickel alloys release nickel at levels that exceed the limits allowed by the European Union Nickel Directive. In a study of skin sensitivity to the Euro coins, eczematous skin reactions were observed, particularly in individuals with nickel sensitization (Seidenari *et al*., 2005). However, it was noted that daily contact with these coins would most likely be temporary, mitigating the hazardous effects except for occupational exposures or for individuals with nickel sensitivity.

4.1.4 Tobacco

Exposure to nickel can occur from cigarette smoking. The International Agency for Research on Cancer (IARC) lists 9 of the 44 "Group 1 human carcinogens" in mainstream cigarette smoke (Smith *et al*., 1997). These

agents include nickel, as well as other metals (arsenic, cadmium, chromium, and beryllium) and organic chemicals (benzene, 2-naphthyl-amine, vinyl chloride, and 4-aminobiphenyl). Metals, including nickel, can be accurately measured in tobacco by use of reverse-phase HPLC with detection limits as low as 8 ng Ni/L tobacco (Li *et al*., 2002). The nickel content in cigarettes and tobacco is reported to be high, 2.32–4.20 mg/kg and 2.20–4.91 mg/kg, respectively, regardless of the kind and the origin of tobacco (Stojanovic *et al*., 2004). Agricultural practices, soil characteristics such as pH, and rainfall can influence metal accumulation in tobacco and other plant leaves. In cigarette smoke, the amount of nickel varies from 0–0.51 µg/cigarette. However, atomic absorption analysis of nickel recovered on filters from mainstream cigarette smoke showed only 1.1% of the nickel was found in the smoke, whereas most of the tobacco nickel was recovered in the ash (Torjussen *et al*., 2003).

4.2 Working Environment

Occupational exposures to nickel occur in mines, refineries, smelters, factories and chemical plants, with an estimated 0.2% of the workforce being exposed to appreciable amounts of nickel (Grandjean, 1984). In addition, workers can be exposed to nickel by handling various nickel-plated tools, such as cutting tools. In mining operations, the miners can be exposed to high concentrations of respirable particles (<10 µm) at concentrations of 5 mg Ni/m^3, with time-weighted total nickel exposure from ambient air averaging 25 mg Ni/m^3 (Warner *et al*., 1984).

In the past, it was estimated that workers in the nickel refinery industry were exposed by inhalation to concentrations of approximately 1 mg/m^3 of mixtures of specific soluble and insoluble nickel compounds (Morgan and Rouge, 1984), leading to the bodily retention of perhaps up to 100 µg nickel/day (Grandjean, 1984). Actual levels of airborne nickel (stationary sampling) in a Finnish refinery were less, measured at 230–800 µg/m^3 from 1966–1988 and at 170–460 µg/m^3 since then (Kiilunen *et al*., 1997). Inside the protective masks used by some workers, the nickel concentrations were even lower, 0.9–2.4 µg/m^3. In tasks where masks were not used, the nickel concentrations in the breathing zone were 1.3–21 µg/m^3. In a recent evaluation of occupational exposures of Norwegian nickel refinery workers on the basis of personal monitoring from 1973 to 1994 (Grimsrud *et al*., 2000), the average concentration of nickel in breathing zones for all workers after 1978 was ≤0.7 mg/m^3. Before 1970, the exposure levels for smelter and roaster day workers were 2–6 mg/m^3, whereas electrolysis and electrolyte purification workers were exposed to concentrations in the range of 0.15–1.2 mg/m^3. In this factory, the levels of water-soluble nickel were about the same for workers in the smelting, roasting, and electrolyte purification departments. In a recent evaluation of changes over time in exposure to nickel aerosols in the nickel-producing and nickel-using industries, significantly negative linear trends for total nickel exposures in the mining (–7%/year), smelting (–9%/year), and refining (–7%/year) sectors, but not in total milling activities (+4%/year), were reported (Symanski *et al*., 2000).

Nickel fumes can also be released into industrial atmospheres by stainless steel welding and welding of high-nickel alloys. Metal inert gas (MIG) welding of stainless steel produces fumes with 3–6.5% Ni and particle sizes less than 0.1 µm, whereas manual metal arch (MMA) welding produces fumes with less nickel, 0.4– 1%, and larger particle sizes >0.1 µm (Sunderman *et al*., 1986). However, MMA welding methods can generate 3–4 times the volume of fumes than MIG methods.

The American Conference of Governmental and Industrial Hygienists (ACGIH) threshold limit value time-weighted average, TLV-TWA, for exposure to nickel compounds is 1.5 mg/m^3 for elemental nickel, 0.1 mg/m^3 for soluble inorganic nickel compounds and nickel subsulfide, 0.2 mg/m^3 for insoluble inorganic compounds, and 0.05 ppm for nickel carbonyl, although nickel carbonyl is listed "understated" by ACGIH as of January, 2005. The US Occupational Safety and Health Administration (OSHA) permissible exposure limit time-weighted averages, PEL-TWA, is set at 1 mg Ni/m^3 for soluble nickel compounds and 1 mg Ni/m^3 for metallic nickel and insoluble nickel compounds (NIOSH, 2005a). The NIOSH recommended exposure limit (REL) for elemental nickel and all nickel compounds excluding nickel carbonyl is much lower, 0.015 mg/m^3. For nickel carbonyl, the OSHA PEL-TWA and the NIOSH REL are both set at 0.001 ppm (0.007 mg/m^3) (NIOSH, 2005b). This occupational exposure limit for nickel carbonyl has been adopted by numerous countries worldwide, including, but not limited to, Sweden, Denmark, Finland, the Philippines, Russia, and Japan (NIOSH, 2005c). International exposure limits for other nickel compounds vary. For nickel carbonyl exposure to 2 ppm is considered by NIOSH to be "immediately dangerous to life and health." For all other nickel compounds exposure levels at 10 mg/m^3 are similarly hazardous.

5 METABOLISM

5.1 Essentiality

Nickel is thought to be essential to animals (reviewed in Anke *et al*., 1984) in that low nickel

reduces growth, especially intrauterine development, and severely interferes with lipid metabolism in rats (Stangl and Kirchgessner, 1996). But nickel's essentiality in humans has not been proven (National Research Council, 1986). Because the basis for the essentiality of nickel in humans has been difficult to define, the nutritional terminology "apparent beneficial intake (ABI)" has been deemed more appropriate for elements including nickel with beneficial effects as nutritional supplements on the basis of actions that can be extrapolated from animals (Nielsen, 1996). In bacteria, at least nine nickel-containing enzymes are known to date: urease, NiFe-hydrogenase, carbon monoxide dehydrogenase, acetyl-CoA decarboxylase/synthase, methyl coenzyme M reductase, certain superoxide dismutases, some glyoxalases, aci-reductone dioxygenase, and methylenediurease (Kanai *et al*., 2003; Lindhal, 2004; Mulrooney *et al*., 2003). Nickel deficiency results in histological and biochemical changes in cells, such as reduced iron resorption that leads to anemia in animals. The essentiality of nickel may be related to its ability to activate heme oxygenase (Sunderman *et al*., 1982) and its participation in the regulation of intestinal iron absorption through a mechanism that simulates hypoxic conditions in the tissues (Latunde-Dada *et al*., 2004). Nickel deficiency also reduces activities of dehydrogenases and transaminases, including α-amylase, thus affecting carbohydrate metabolism. Rat-feeding deprivation studies suggest that nickel and folate interact in metabolic processes, likely involving one-carbon metabolism and vitamin B_{12}–dependent pathways for methionine metabolism (Uthus and Poellot, 1996; 1997). The daily nickel nutritional supplementation of humans and animals is thought to be less than 500 µg/kg.

5.2 Absorption

Nickel absorption from food and water in humans occurs through intestinal absorption (Nielsen *et al*., 1999). Although in animal studies approximately 90% of ingested nickel was found excreted in the feces and only 10% or less was absorbed (NAS, 1975), in humans even less nickel (approximately 1%) is absorbed in the gastrointestinal tract (Sunderman, 1989). In a study of healthy human volunteers who ingested nickel sulfate in drinking water or food, Sunderman *et al*. (1989) found that absorbed nickel from the drinking water was approximately 40-fold greater than the nickel absorbed from the same nickel dose in food. In follow-up studies, Nielsen *et al*. (1999) confirmed the finding that nickel ingestion with a meal leads to substantially less absorbed nickel than from nickel-containing drinking water on an empty stomach, which can be an important consideration for nickel-sensitive individuals who can also consume low-nickel diets (Veien *et al*., 1993). Nielsen *et al*. (1999) suggest that food constituents, such as phosphate, phytate, fibers, and other metal-ion-binding components, may bind nickel and render it less available for absorption than nickel dissolved in water. It has been reported that approximately 20% of nickel from inhaled sources is absorbed by the respiratory tract, with approximately 30% inhaled nickel deposited in the lungs (Sunderman *et al*., 1988). Although nickel does not generally accumulate in tissues because of efficient excretion, with high levels of nickel exposure, the kidney is the primary target organ for nickel retention, followed by the lung, brain, and pancreas.

5.3 Transport

Until recently, nickel transport was primarily attributed to albumin and nickeloplasmin (reviewed in Sunderman *et al*., 1977). Nickel transport into and within cells has also been found to occur through transporters that are primarily functional in iron homeostasis. Transferrin, one of the major proteins involved in the cellular uptake of iron, also binds nickel, vanadium, and other metals (Boffi, 2003; Harris, 1986; Sun, 1999). Another iron transport protein, divalent metal transporter-1 (DMT1), transports nickel, manganese, cobalt, copper, and zinc as well (Chen, *et al*., 2005; Garrick, 2003). Thus, nickel can compete with iron for entry into cells, and adequate iron intake can limit nickel absorption as shown in a study of ^{63}Ni uptake into fully differentiated Caco-2 cells (Tallkvist *et al*., 2003). In addition to competition for iron uptake, nickel can also disrupt iron homeostasis by lowering cellular iron levels, thereby increasing the binding of the iron regulatory protein-1, IRP1, to its response element IRE, which will increase transferrin receptor mRNA translation but block the translation of ferritin mRNA (Chen *et al*., 2005).

5.4 Excretion

In animals and humans, nickel is primarily excreted in the urine, with salivary and sweat excretion being secondary (Onkelinx *et al*., 1973; WHO, 1991). Excretion of nickel in feces is composed of the unabsorbed dietary nickel intake, as well as biliary and tracheally cleared nickel. Metallothioneins (MT) are essential low-molecular weight, cysteine-rich proteins, with numerous thiol metal binding sites, and essential functions in metal detoxification processes (Waalkes *et al*., 1985; 1990). It was recently reported that MT overexpression

in mice did not protect these animals against nickel-induced tumors (Waalkes *et al*., 2004).

5.5 Biological Half-Time

In animals, the biological half-time for nickel clearance is on the order of 2–3 days (Onkelinx *et al*., 1973). In human studies that examined the absorption and retention of nickel added to drinking water, peak nickel concentrations in serum were found 1 hour after intake of nickel in drinking water, and median urinary nickel excretion half-times varied between 19.9 and 26.7 hours (Nielsen *et al*., 1999). This correlates well with previous reports of the biological half-time of nickel in human plasma and urine of approximately 20–34 hours and 17–39 hours, respectively (Tossavainen *et al*., 1980). Compared with the relatively efficient systemic clearance of soluble nickel salts, lung clearance of insoluble nickel particles such as nickel oxide can take several months.

6 BIOLOGICAL MONITORING

6.1 Levels in Human Tissues and Fluids

Both serum and urinary nickel measurements can be useful monitors of environmental or occupational exposures. Normal levels of nickel in unexposed adults are reported to be in the range of 0.05–0.1 μg Ni/L serum and 0.5–4.0 mg/g creatinine in urine (WHO, 1991). In a study of Finnish factory workers (Kiilunen *et al*., 1997), after-shift urinary concentrations of nickel were 0.1–2 μmol/L and were still elevated after a 2–4 week vacation. However, concentrations of nickel in urine in this study showed no correlation with nickel concentrations in the air. In a Norwegian study of potential nickel exposure from nearby Russian refineries, nickel levels in urine of residents living near the refineries were significantly lower than for urban residents living away from the refineries in areas of high vehicular traffic (Smith-Sivertsen *et al*., 1997). In flux-core-wire (FCW) stainless steel welders exposed to workplace air levels of nickel of 50.4 μg/m^3 (ranging from <2–416.7 μg/m^3), whole blood and plasma levels were found to be low at 0.84 μg Ni/L and 0.57 μg Ni/L, and urine levels were also very low at 2.5 μg/g creatinine (Stridsklev *et al*., 2004). These values were all near the current detection limit ranges for Ni in whole blood (0.48–1.5 μg Ni/L whole blood), plasma (0.3–0.9 μg Ni/L), and urine (0.3 μg/g creatinine). Although there were no associations between the Ni levels in air and in the biological fluids; statistically significant associations between Ni levels in whole blood and plasma before and after work were found. Compared with other welding methods such as manual metal arc (MMA) welding (Stridsklev *et al*., 1993) and tungsten inert gas welding (TIG) (Stridsklev *et al*., 1994), the levels of nickel in biological fluids were the same or slightly higher for the FCW welders than for the MMA welders and were lower for the FCW welders than TIG welders. When measuring blood, plasma, and serum levels of nickel, investigators must consider the use of nonstainless steel needles because of the potential for unintended Ni contamination of the samples.

The lungs have been found to contain the highest concentration of retained nickel in humans with no known occupational exposure. Reported levels of nickel in the lungs of autopsied U. S. subjects ranged from 1.8–2.1 μg/cm^2 of lung surface area (Edelman and Roggli, 1989). The pulmonary burden of nickel has been shown to increase with age (Kollmeier *et al*., 1987). Nickel measurements in smokers' blood ranges from 0.01–0.42 μg Ni/L, not much higher than in the nonsmokers' blood (0.01–0.26 μg Ni/L), whereas nickel in the urine of smokers (<0.01–8.20 μg/L, median) is significantly higher than in nonsmokers (<0.01–4.60 μg Ni/L) (Stojanovic *et al*., 2004).

In a study of nickel-allergic individuals with hand eczema compared with healthy controls, both groups had similar levels of urinary nickel, whereas nickel levels in serum were significantly lower in nickel-allergic individuals than controls, perhaps related to the lower dietary intake of nickel-rich foods by the sensitive individuals (Christensen *et al*., 1999).

6.2 Biomarkers of Exposure

To date, there are still no good, well-validated biomarkers for Ni exposure or nickel effects besides direct measures of nickel in serum or urine, although attempts have been made to identify informative biomarkers. In a Finnish nickel worker study, the frequency of micronucleated epithelial cells in the buccal mucosa was not significantly elevated, nor was any relationship observed between micronucleus frequencies and levels of nickel in air, urine, or blood (Kiilunen *et al*., 1997). However, in a recent study of dental workers exposed to airborne dusts of metal alloys (up to 30% Ni) during the production of dental appliances such as crowns and bridges, nickel levels in urine were found to be measurable at 7.65 ± 2.5 μg/g creatinine, with significant correlation between duration of exposure and micronucleus frequencies in lymphocytes ($r = 0.642$, $P < 0.01$), but not in nasal cells of technicians (Burgaz *et al*., 2002).

7 TOXICOLOGICAL EFFECTS

7.1 General Systemic Effects in Animals and Humans

Acute nickel poisoning by inhalation exposure or ingestion of nickel carbonyl or soluble nickel compounds can lead to headache, vertigo, nausea, vomiting, nephrotoxic effects, and pneumonia followed by pulmonary fibrosis. Chronic effects of nickel exposure by inhalation are reported among nickel refinery and plating workers to include rhinitis, sinusitis, nasal septum perforations, and asthma. Nickel is also hepatotoxic, and studies show that zinc can prevent nickel-induced toxicity and maintain normal levels and function of liver enzymes (Sidhu *et al*., 2004). Nickel, like other metals, can induce systemic lipid peroxidation and can deplete glutathione, most likely through oxidative stress mechanisms (reviewed in Kasprzak *et al*., 2003; Valko *et al*., 2005). Some of the specific responses of the respiratory tract, the skin, and reproductive effects are summarized in the following.

7.2 Inhalation Effects in Animals and Humans

Volatile nickel tetracarbonyl ($Ni[CO]_4$, CAS 13463-39-3) is extremely toxic, decomposing rapidly to nickel and carbon monoxide, thereby inducing systemic poisoning often leading to death. The lungs and brain are especially susceptible targets for nickel carbonyl poisoning (Kurta *et al*., 1993; Scott and Grier, 2002; Shi, 1994). Although initial respiratory symptoms may be mildly transient, in the immediate 2–3 days after nickel carbonyl exposure, progressive clinical symptoms include cough, shortness of breath (dyspnea), and worsening symptoms of pneumonia and acute respiratory distress. In the lungs, alveolar damage is maximal at 4–6 days after nickel carbonyl exposure. Other symptoms of severe nickel carbonyl poisoning include myalgias, fatigue, weakness, delirium, and convulsions. Fever is usually not present. After decomposition of nickel carbonyl, the nickel is oxidized and excreted by the kidneys. In the absence of chelation therapy, urine nickel levels peak at 24–48 hours after exposure and return to baseline within 1–2 weeks (Vuopala *et al*., 1970). Sunderman and Sunderman (1958) classified nickel carbonyl poisoning severity by urine nickel concentrations 18 hours after exposure: mild, 60–100 µg Ni/L; moderately severe, 100–500 µg Ni/L; and severe, >500 µg Ni/L.

Acute lung injury, invoked by nickel or any other respiratory toxin, is characterized by epithelial and endothelial cell perturbation, inflammatory alveolar macrophage influx, surfactant disruption, and pulmonary edema. Associated with these acute responses are numerous changes in cytokines, oxidants, and growth factors (reviewed in Barchowsky *et al*., 2002; Dick *et al*., 2003). After adjustment for tobacco habits, stainless steel welders showed a higher prevalence of irritant bronchial symptoms, cough and sputum production, compared with controls (Sobaszek *et al*., 1998). Pulmonary changes such as fibrosis and pneumoconiosis have been reported in workers inhaling nickel dust. Airway hypersensitivity and asthma has been reported for some workers in the nickel-plating industry (Block and Yeung, 1982; Dolovich *et al*., 1984), although asthma effects are rare. Genetic susceptibility studies to identify gene factors associated with acute lung injury after nickel exposure are being actively pursued in animals (Leikauf *et al*., 2002; Prows *et al*., 2003).

Other direct respiratory effects of the chronic inhalation of nickel include hypertrophic rhinitis and sinusitis, formation of nasal polyps, and perforations of the nasal septum (Boysen *et al*., 1982). Epithelial dysplasia of the nasal mucosa has been noted among nickel workers, especially those in smelting and roasting occupations (Torjussen and Andersen, 1979; Torjussen *et al*., 1979). Inhalation of nickel can also interfere with the sense of smell. In studies with rodents, olfactory epithelium atrophy has been observed after inhalation of $NiSO_4$ or Ni_3S_2 (Sunderman, 2001). After nasal instillation of nickel solutions, nickel can slowly (days to weeks) pass from the nasal lumen to the olfactory bulb through olfactory receptor neurons days (Henriksson *et al*., 1997) and can cross olfactory bulb synapses to migrate to distant nuclei of brain cells. Similar effects have been noted in fish (pike, *Esox lucius*) (Tallkvist *et al*., 1998). The molecular mechanisms responsible for metal translocation in olfactory neurons and deposition in the olfactory bulb are unclear, but complexation by metal-binding molecules such as carnosine (beta-alanyl-L-histidine) may be involved.

7.3 Skin Effects in Animals and Humans

Nickel is reported to be the most frequent cause of contact allergy (Liden *et al*., 1995). Nickel allergy is a very common disorder affecting up to 30% of the population with a rising incidence, especially in females who have previously had skin piercing (Brydl *et al*., 2004). Dermal metal contact activates immune responses and skin reactions such as urticaria, eczema, erythema, and pruritus. After body piercing, the most common delayed-type hypersensitivity reaction at piercing sites is eczematous, granulomatous reactions

being less common. It has recently been reported that 5% of a sensitized population react to as little as 0.44 μg Ni/cm^2 and 10% react to 1.04 μg Ni/cm^2 (Fisher *et al.*, 2005). Although nickel sensitivity is more pronounced in some individuals, and genetic susceptibilities are suspected (Fleming *et al.*, 1999), predictions of sensitivity on the basis of positive prick tests, patch tests, and IgE levels have not been informative (Uter *et al.*, 1995). Newer, more sensitive immune cytokine ELISA assays show some promise in discriminating between nickel-allergic and nonallergic individuals (Jakobson *et al.*, 2002).

The development of allergic reactions to nickel has been correlated with specific CD8+ T cells that induce apoptosis of nickel-loaded keratinocytes in conjunction with CD4+ T cells that contribute to the inflammatory reaction through release of IFN-γ, TNF-α, and IL-17, thus, regulating the immune responses to nickel in a cytokine-independent, and cell-contact–dependent manner (reviewed in Cavani, 2005). In nonallergic individuals, the circulating CD8+ T cells reactive to nickel are lacking. $NiSO_4$ also activates the NF-kB and AP-1 pathways and induces iNOS expression in skin dendritic cells (Cruz *et al.*, 2004). These and other studies show that nickel is proinflammatory and can bind to haptens to induce allergic reactions. Thus, it is not surprising that it is a major cause of contact dermatitis. Among Taiwanese electronics workers, hand dermatitis caused in part by nickel exposure is prevalent, with 35.5% of these workers diagnosed with irritant contact dermatitis (ICD) and 3.8% with full-blown allergic contact dermatitis (Shiao *et al.*, 2004). The topical use of tea tree oil, an anti-inflammatory and antibacterial agent, has been found to prevent nickel hapten–sensitized individuals from developing contact dermatitis (Pearce *et al.*, 2005). In a longitudinal epidemiological study to evaluate oral sensitization to nickel-containing orthodontic appliances, it was found that after 3 months, 20% of females and 10% of males exhibited allergic gingivitis that disappeared within 1 month after appliance removal and could be mitigated to some degree by using fluoride-free toothpaste and mouthwash (Ramadan, 2004).

7.4 Injection Site Effects in Animals

Many of the early animal studies of the carcinogenicity of nickel compounds focused on relatively insoluble compounds that were capable of inducing tumors at the site of injection (reviewed in WHO, 1991). The most frequently used exposure system was intramuscular injection with compounds such as crystalline nickel subsulfide or nickel oxides that induced rhabdomyosarcomas after intramuscular administration. In terms of human exposure, direct injection of nickel compounds bears no relevance; however, *in situ* exposure to nickel alloy replacement joints or to circulatory stents could present similar exposure scenarios (Lewis and Sunderman, 1996). The current orthopedic surgical use of metal-on-metal joint replacements in place of older metal-on-plastic joints results in less wear debris yielding less inflammation, but whether these joints further sensitize recipients who are already allergic to metals is still unclear (Gawkrodger, 2003; Hallab *et al.*, 2005). Furthermore, nickel and cobalt ions are released *in vitro* and *in vivo* from the metal alloys used for vascular stents, and these metal ions can activate ICAM1 expression in endothelial cells and keratinocytes (Messer *et al.*, 2005). However, the *in vivo* consequences of these surgical exposures are not yet known.

7.5 Teratogenicity

Nickel is toxic to reproductive systems, yielding aberrant sperm and deformed uteri in exposed animals. In rats and mice, dietary nickel (1 mg/kg) can damage sperm, reducing its normal motility and physiological functions (Yokoi *et al.*, 2003), perhaps because of oxidative damage (Doreswamy *et al.*, 2004; Kazprzak *et al.*, 2003). Nickel crosses the placenta in animals and humans, and nickel concentrations in fetuses are similar to those in adults; nickel levels can be measured in cord blood from full-term infants (McNeely *et al.*, 1971). Perinatal oxidative DNA damage has been demonstrated in the newborn offspring of rats fed nickel-supplemented diets containing 0.5–2.3 mg/kg Ni (Zhou *et al.*, 2001). Administration of nickel carbonyl during pregnancy of rats has been shown to induce offspring that are defective in one eye (Sunderman *et al.*, 1979). Although the mechanism for this effect has not been studied, it remains a very interesting example of a specific birth defect caused by this agent. Nickel chloride has also been reported to induce ocular lesions and retinal malformations in *Xenopus* embryos (Hauptman *et al.*, 1993). Experimental evidence in rats shows that transition metals in the maternal diet can affect perinatal oxidative DNA damage, presumably by means of a Fenton-type reaction (Zhou *et al.*, 2001). Evidence of teratogenic effects of nickel in humans is emerging. In a study of more than 300 Russian nickel-refinery workers compared with local construction workers, normal pregnancies were reduced in the nickel workers from the nonnickel worker average of 39% to 29%, whereas spontaneous abortions were increased from 9% to 16%,

and structural malformations in live births increased from 6 to 17% (Chashschin, *et al*., 1994). These findings are intriguing, despite the statistical limitations of the study design that did not allow comparison with a nonexposed population.

8 GENOTOXICITY AND CARCINOGENICITY

8.1 Genotoxicity and Mutagenicity

Various nickel compounds including insoluble nickel subsulfide, crystalline nickel monosulfide, and green (HT) and black (low-temperature, or LT) nickel oxides are phagocytized into mammalian cells, where nickel ions are released that can induce overt cytotoxicity and apoptosis, as well as chromosomal aberrations and morphological transformation in SHE cells (Costa *et al*., 1982; 1994; Heck and Costa, 1983; Pulido and Parrish, 2003) and 10T1/2 cells (Clemens and Landolph, 2003; Landolph, 1989; 1990; 1999; Landolph *et al*., 2002; Miura *et al*., 1989; Verma *et al*., 2004). X-chromosome–specific heterochromatin disturbances and chromosome deletions were found to predominate in mammalian cells exposed to various nickel compounds, leading to gene silencing of a senescence gene (Conway and Costa, 1989; Klein *et al*., 1991; Sen and Costa, 1985). More recently, nickel was found to induce genomic instability of the microsatellite nature in human lung cancer cells (Zienolddiny *et al*., 2000). In a recent study of a new nickel-free stainless steel, P558, there was no evidence of cytogenetic effects or chromosomal aberrations, nor was any mutagenicity in the Ames assay detected (Montanaro *et al*., 2005).

The production of redox active radicals and reactive molecules such as hydrogen peroxide and other reactive oxygen species (ROS) may be the effectors of nickel's genotoxicity (Huang *et al*., 1993; 1994; Seema *et al*., 2005). Ni-induced ROS played a role in oxidative base damage, measured by the formation of 7,8-dihydro-8-oxo-2'-deoxyguanosine (8-oxo-dG) (Huang *et al*., 1995). In Comet assays that used human colonic cells, nickel chloride at 2–250 µmol/L (1 hour) was found to induce DNA damage that could be lessened by the addition of the antioxidant quercitin (Blasiak *et al*., 2002). Ni(II) binding to chromatin proteins in somatic and sperm cells results in oxidative and structural damage to the proteins and DNA (Kazprzak *et al*., 2003). The formation of binary Ni(II) complexes with 2'-deoxyguanosine 5'-triphosphate (dGTP) and of ternary complexes with L-histidine were found to be stable at physiological pH of 7.4 and may generate formation of the 8-oxo-dGTP intermediates that could lead to mutagenesis and carcinogenesis (Kaczmarek *et al*., 2005). In terms of mutagenesis, however, nickel compounds have been shown to be inactive in bacterial assays. They are weak mutagens at best, even at highly toxic doses, in eukaryotic assays (reviewed in Klein and Costa, 1997), except in the *gpt* transgenic Chinese hamster G12 cells in which DNA methylation gene silencing rather than overt mutation was found to predominate (Lee *et al*., 1995). Numerous reviews of the nonmutagenic but genotoxic nature of nickel compounds have previously been presented, and this information will not be reproduced herein, in lieu of offering the reader the newest information.

Nickel dose and cell type are important considerations of much of the newer work, as efforts are being made to more closely mimic environmentally relevant human exposure scenarios. Therefore, many of the newer studies have used chronic (days–weeks) low-dose exposures of nickel in skin- or lung-derived cells to approximate occupational exposure situations or long-term wearing of nickel alloy jewelry. Short-term studies also have relevance related to intermittent exposure to nickel-containing metal utensils, coins, and the like.

Although much of the previous genetic toxicology data *in vitro* emphasized the effects of insoluble particulate nickel species, which were also thought to be the most carcinogenic, newer experimental data focus on the water-soluble nickel compounds. Recent molecular and genetic toxicology data show that soluble $NiCl_2$ (50–150 µmol/L, 3-week exposure, 60–100% survival) can, in fact, be genotoxic, similar to insoluble nickel, and can effectively silence the *gpt* transgene in G12 cells when cells are chronically exposed for long durations up to 3 weeks to allow sufficient nickel uptake (Ke, 2005). In fact, on reconsideration of previous mutagenesis studies with acute 24–48 hour exposures to the insoluble nickel compounds *in vitro* (Kargacin, *et al*., 1993; Klein *et al*., 1994; Lee *et al*., 1995), it is likely that the actual nickel exposures in those experiments were of much longer duration than previously thought, because it is unlikely that rinsing would have removed the adherent nickel particles from the cell surfaces at the end of the intended exposure period. Recently, it was shown that exposure of A549 lung cells to soluble nickel resulted in histone modifications consistent with gene silencing (Costa *et al*., 2005).

The inhibition of DNA repair processes has also been suggested to play a mechanistic role in the genotoxicity of metal compounds, including nickel. Specifically, nickel at nontoxic concentrations has been

reported to interfere with the recognition of incision and polymerization steps of base excision repair, possibly by interference with zinc finger proteins (Bal *et al*., 2004; Cavallo, *et al*., 2003; Hartwig *et al*., 1994a,b; 2002; Lynn *et al*., 1997). Both soluble and particulate nickel can inhibit repair of benzo[a]pyrene DNA adducts in human lung cells (Schwerdtle *et al*., 2002). Low doses of nickel chloride (1 µmol/L) inhibited repair of UV or MNNG-induced DNA damage as indicated by accumulating strand breaks, and 1–5 µm nickel chloride inhibited the formamidopyrimidine-DNA glycosylase (Fpg), 3-methyladenine-DNA glycosylase II (Alk A) and endonuclease III (Endo III) enzymes involved in DNA excision repair (Wozniak and Blaziak, 2004). Because Ni compounds, such as NiS, Ni_3S_2, NiO (black and green), and soluble $NiCl_2$, have been shown to be active inducers of reactive oxygen species (ROS) in Chinese hamster ovary (CHO) cells (Huang *et al*., 1994), the involvement of ROS has been implicated in the inhibition of DNA repair by nickel (Lynn, 1997). In addition, because soluble Ni compounds ($NiCl_2$, 1 mmol/L, 24 hour) actively stabilize HIF-1α by inhibiting the HIF-1a prolyl hydroxylase that targets the HIF transcription factor for degradation, the cell now responds to a state of hypoxia, which includes the down-regulation of several mismatch repair proteins (Davidson *et al*., 2003; 2004).

In cultured human diploid fibroblast cells, nickel compounds induced anchorage independence and cell transformation (Biedermann and Landolph, 1987). Insoluble nickel compounds generate oxygen radicals during the process of phagocytosis in mammalian cells that may contribute to induction of morphological transformation and the numerous changes in gene expression observed in nickel compound–transformed 10T1/2 cell lines (Landolph, 1999; Landolph *et al*., 2002). In recent cell transformation studies that used archived samples of nickel refinery dust from an INCO refinery in Clydach, South Wales, the older 1920 sample of green NiO dust was found to transform C3H10T1/2 mouse embryo cells, whereas a dust sample from 1929 did not (Clemens and Landolph, 2003). The primary difference between these samples was the presence of orcelite, nickel arsenide (Ni_5As_2), because of arsenic contamination of the sulfuric acid used in refining. Orcelite composed approximately 25% of the 1921 samples, but less than 3% of the 1929 samples.

8.2 Carcinogenicity in Animals and Humans

8.2.1 Animals

Animal studies indicate that crystalline nickel sulfide, nickel monoxides, and nickel hydroxides are potent carcinogens, whereas amorphous nickel sulfide, nickel alloys, and nickel salts have limited or no effects on tumor formation in animals (IARC, 1990). Rodent species are differentially sensitive to the carcinogenic effects of nickel compounds, with hamsters and mice being much less susceptible than rats. However, the bulk of the rodent data on nickel carcinogenicity conflicts with the most recent human epidemiology data, in that most of the previous rodent data do not support a role for soluble nickel compounds in inhalation carcinogenesis. In a 2-year inhalation study of nickel sulfide hexahydrate, for example, no tumors of respiratory tissues were found (Dunnick *et al*., 1995). However, in more recent studies in rodents, both soluble nickel subsulfide (Ni_3S_2) and nickel oxide (NiO) were found to be highly carcinogenic after injection or inhalation exposures (reviewed in Verma *et al*., 2004).

8.2.2. Humans

Historically, nasal sinus and respiratory cancers have been reported to predominate in nickel workers exposed in nickel refineries, cutlery factories, and alkaline battery manufacturing plants (reviewed in Sunderman, 2001). Occupational human exposures to nickel-containing metal dusts or aerosols by inhalation are reported to induce mucosal ulcers, perforated nasal septum, reduced olfactory acuity, and sinonasal cancers. In 1990, the International Agency for Research on Cancer (IARC) classified all nickel compounds as "Group 1" carcinogenic to humans on the basis of a sufficient amount of epidemiological data. Recent studies, however, question whether all nickel compounds should be classified equally as human carcinogens. The emphasis in the epidemiology studies of nickel workers in the last decade has been shifting away from the insoluble nickel compounds in favor of the soluble nickel compounds' being hazardous carcinogens. In addition, consideration of complex mixtures must be applied to studies of nickel refinery workers, because mixed exposures to arsenic, sulfuric acid mists, and cobalt must be considered, along with smoking status, in the exposed cohort. A brief summary of the human epidemiology data for nickel exposure is presented here, and the reader is referred to other comprehensive reviews (Sunderman *et al*., 2001; Grimsrud *et al*., 2002; Kasprzak *et al*., 2003).

Exposures to nickel subsulfide (Ni_3S_2) and insoluble nickel oxides in the nickel-refining process have been reported to be associated with the development of nasal and lung cancers among workers, and nickel subsulfide is an IARC Class A carcinogen with both sufficient human epidemiology data and supporting animal data to substantiate that classification (EPA IRIS

Database, 2004; Sanner and Dybling, 2005). However, current evidence supporting metallic nickel as a respiratory carcinogen is lacking. The International Committee on Nickel Carcinogenesis in Man (Doll *et al*., 1990) reported that "there was no evidence that metallic nickel was associated with increased lung and nasal cancer risks." Also in 1990, the International Agency for Research on Cancer concluded that there was "inadequate evidence in humans for the carcinogenicity of metallic nickel." This is supported by some of the most recent epidemiological studies in which lung cancer risks were evaluated in nickel alloy manufacture workers exposed to nickel oxide and metallic nickel or at least 5 years from 1953 to 1992 at the INCO nickel refinery in Clydach, South Wales (Sorahan, 2004). Despite numerous previous reports of excess (more than 350 cases) respiratory cancers among workers employed since the 1920s (Doll *et al*., 1977; Morgan, 1958; Peto *et al*., 1984), this recent study did not identify occupational cancer risk among the workers at this refinery. There were no nasal cancer deaths, and observed lung and other cancers were below expectations on the basis of national mortality rates. Clemens and Landolph (2003) suggest that this could have been partially because of changes in the refining processes at this plant in the later 1920s to exclude sulfuric acid–contaminating nickel arsenide (Ni_5As_2, orcelite), since unlike the 1920 samples of green NiO dust from the refinery that induced cellular transformation of C3H10T1/2 mouse embryo cells, the 1929 samples did not induce cellular transformation. Another study of 1649 male workers who were employed for at least 12 months during 1954–1978 at a nickel refinery and fertilizer plant in Alberta, Canada, showed no associations between exposure to nickel concentrate or metallic nickel and development of respiratory cancers involving the nasal cavity or paranasal sinuses (Egedahl *et al*., 2003). In that study, measurements taken in 1977 in nickel refinery work areas documented nickel dust concentrations averaging 95 mg/m^3 (ranging from 9–239 mg/m^3) in the concentration sheds, 4 mg/m^3 (0.3–49 mg/m^3) in the recovery areas, and 2 mg/m^3 (0.3–7 mg/m^3) in the mills and fabrication areas. A recent epidemiological study of workers in New Caledonia, where nickel mining and refining is the leading industry, also showed no significant association of lung cancer among workers (Menvielle *et al*., 2003). Likewise, a nested case-control study of the risk of lung cancer mortality of French workers involved in the production of stainless and alloyed steel from 1968 to 1992 (Moulin *et al*., 2000) also failed to detect any relationship between lung cancer and exposure to nickel, iron, or chromium compounds, in agreement with previous literature for stainless steel and nickel alloy manufacture (Cornell, 1984; Moulin *et al*., 1993). Even with highly toxic gas nickel carbonyl, excess risks of respiratory cancer in a cohort of 812 workers from a modern nickel carbonyl refinery showed only a nonsignificant excess of lung cancer and no other cancer risks (Sorahan and Williams, 2005).

Despite the carcinogenicity studies in rodents that showed no effect with soluble nickel sulfate hexahydrate (Dunnick *et al*., 1995), recent studies of nickel workers in Wales, Norway, and Finland indicate that water-soluble nickel could be the more important risk factor for excess respiratory cancer (see Andersen *et al*., 1996; Easton *et al*., 1992; Grimsrud *et al*., 2002). Actual measurements of soluble nickel in the 2002 Grimsrud *et al*. study indicated that 10–15% of the total nickel in the grinding, roasting, and smelting departments was of the soluble form, with nickel air concentrations ranging from 0.4–5.3 mg/m^3. In that study, the odds ratio for carcinogenic risk caused by water-soluble nickel was found to be maximal at 3.8 (95% confidence interval), compared with odds ratios of 2.8 for sulfidic nickel, 2.2 for nickel oxide, and 2.4 for metallic nickel. Further support for soluble nickel carcinogenesis was in the most recent case-control study of nickel refinery workers in Norway, in which lung cancer risk adjusted for smoking, showed a substantial association with cumulative exposure to water-soluble nickel (Grimsrud *et al*., 2005). However, it still cannot be determined in this study whether coexposure to tobacco smoke or other refinery exposures is a prerequisite for the carcinogenic effect of water-soluble nickel. Finally, it is likely that these workers had mixed exposures to both soluble and insoluble nickel compounds, making it difficult to tease out the form of nickel compound that was causing the cancer. Future research on mixtures of nickel plus other metals and on nickel plus organic carcinogens will be informative.

9 EFFECTS ON GENE EXPRESSION AND SIGNALING PATHWAYS

Nickel can activate several cellular stress response signaling pathways involving MAPKs, PI3K, HIF-1, NFAT, and NF-κB (reviewed in Harris and Shi, 2003; Lu *et al*., 2005). Although a thorough review of nickel and other metal induction of these signaling pathways can also be found in Chapter 5 of this Handbook (Davidson *et al*., 2006), a few particularly salient mechanisms related to nickel will be briefly summarized here. Nickel is now well known for its capacity to stabilize and activate the HIF-1α protein and to up-regulate a battery of hypoxia-inducible genes. Although some signaling pathways may involve nickel-induced ROS, the formation of ROS is not thought to be

involved in nickel's activation of HIF-1–dependent genes (Salnikow *et al*., 2002). When Affymetrix gene chips were used to study nickel treated (1 mmol/L $NiCl_2$, 24 hours) HIF-1α competent versus knockout cells, numerous genes were found to be up- or down-regulated in a HIF-dependent manner (Davidson *et al*., 2003; 2004; Salnikow *et al*., 2003a, b). This dose of nickel chloride is nontoxic, yielding approximately 90% survival of human A549 lung cells. Some examples of these nickel-induced genes include 1, 4-alpha-glucan branching enzyme 1 (GBE1) and Bcl-2-binding protein Nip3 (BNIP3). Serpina3g, a member of the mouse serpin family, was downregulated by nickel by means of HIF-1α dependent pathways, as was asparaginyl hydroxylase FIH-1 and acetyltransferase ARD-1 in A549 cells (Salnikow *et al*., 2003a). Although the exact mechanism for HIF-1 activation by Ni is not yet confirmed, several hypotheses have been proposed. These include the ability of nickel to stabilize HIF-1α protein by inhibiting the posttranslational modifications of HIF-1α; the impairment of prolyl hydroxylase activities by cellular iron depletion caused by nickel competition for the DMT-1 transporter, as well as by substitution of nickel for iron in the Hif-prolyl hydroxylase (Davidson *et al*., 2004).

Several other studies have identified genes whose expression is altered after nickel treatment of cells or organisms. By use of random, arbitrarily primed-polymerase chain reaction (RAP-PCR) mRNA differential display, Verma *et al*. (2004) identified several genes that were differentially expressed between nontransformed and insoluble nickel-transformed C3H10T1/2-derived mouse embryo cell lines. The calnexin gene (encoding a type I membrane protein/molecular chaperone), ect-2 proto-oncogene, and Wdr1 stress-inducible gene were up-regulated, whereas DRIP/TRAP-80 (vitamin D–interacting protein/thyroid hormone activating protein 80) gene, IGFR1 (insulin-like growth factor receptor 1), and SNAP (small nuclear activating protein C3) were down-regulated in the nickel-transformed cell lines.

Nickel activation of NF-κB activity and DNA binding have been reported in several studies (Aiba *et al*., 2003; Goebler *et al*., 1995), leading to the subsequent transcription of inflammatory cytokines (IL-6) and adhesion molecules such as ICAM-1, VCAM-1, and E-selectin. However high nickel doses (400 mmol/L $NaCl_2$) were often used in older studies (Goebeler *et al*., 1995). At relevant low doses, nickel chloride (0.25–0.5 mmol/L)–induced activation of HIF-1α also leads to the expression of the Cap43, a protein that may have utility as a potential biomarker of nickel exposure but has also been recently identified to be the same as NDRG1, a protein involved in a demyelinating neurodegenerative disease gene (Salnikow *et al*., 2003). Nickel induced NF-κB activity and adhesion molecule expression were found to be inhibited by the antioxidant pyrrolidine dithiocarbamate, indicating some involvement of redox-dependent mechanisms by high doses of nickel (Goebeler, 1995). Nickel subsulfide-induced ROS have also been implicated in activation of AP-1and IL-8 in human bronchial epithelial Beas-2B cells at relevant nickel doses of 1.17 μg/cm2 Ni_3S_2 (Barchowsky, 2002). At relevant nickel doses, the generation of hydrogen peroxide H_2O_2 by nickel (1 mmol/L $NiCl_2$ or 2 μg/cm2 Ni_3S_2, 6–48 hours) was also reported to be involved in NFAT activation (Huang *et al*., 2001). In contrast, pretreatment of cells with sodium formate (an OH radical scavenger) or superoxide dismutase (an $O_2^{\cdot-}$ radical scavenger) did not show any inhibitory effects (Huang *et al*., 2001). Related to inflammation associated with nickel-induced contact dermatitis, involvement of MAPK, JNK, and ERK pathways has been demonstrated in skin dendritic cells exposed to $NiSO_4$ (500 μmol/L, 24 hours) to simulate contact exposure (Boislève *et al*., 2005).

10 EPIGENETIC EFFECTS

10.1 Effects on DNA Methylation and Epigenetic Silencing

Because carcinogenic nickel compounds were not found to be mutagenic, alternative mechanisms for their carcinogenic effects were sought. In a seminal paper published in 1991 (Klein *et al*., 1991), carcinogenic nickel compounds were found to induce DNA methylation and silence tumor suppressor/senescence genes located on the mammalian X chromosome. More recent studies have suggested that the ETS transcription factor MEF may be the tumor suppressor gene that was epigenetically silenced by nickel on the X chromosome (Seki *et al*., 2002). Further studies showed that nickel-induced silencing exhibited positional effects both in mammalian cells and in yeast. For example, in yeast cells nickel silenced an Ura A gene when it was placed 1.7 kb from a telomere silencing element but not when it was 2.0 kb (Broday *et al*., 1999a). Similarly, in a mammalian transgenic system, nickel silenced the G12 transgenic cell line because of the position of the transgene on chromosome 1 near the telomere and near heterochromatin, whereas it could not silence the G10 cell line where an identical construct was placed on chromosome 6 but not near any heterochromatin (Broday *et al*., 1999b). A model was proposed for these effects involving the ability of nickel ions to bind to the phosphate

backbone of DNA in place of magnesium that caused more chromatin condensation. This occurred only in the G12 cells but not the G10 as revealed by DNAse 1 protection by nickel only in G12 cells. We hypothesized that this effect caused *de novo* methylation of DNA that neighbored heterochromatin regions that were susceptible to the effects of nickel substitution for magnesium.

10.2 Effects on Histone Acetylation

Recently, accumulating evidence shows that metals can have epigenetic effects by altering not only the methylation status of DNA but also by modifying histones by methylation, acetylation, phosphorylation, or ubiquitination. Several recent studies have shown that exposure of mammalian cells to nickel compounds causes a decrease in histone acetylation, (Broday *et al.*, 2000) as well as increases in histone H3-lysine 9 methylation, which work coordinately to condense nucleosomes and chromatin, thus silencing genes. The mechanisms underlying these histone effects are the subject of intense study, and are thoroughly reviewed in Chapter 5.

11 TREATMENT OF NICKEL CARBONYL POISONING

The chelating agent, sodium diethyldithiocarbamate is an effective drug against nickel carbonyl poisoning (Sunderman *et al*., 1981; 1990). In 1990, it was reported that 375 patients had been successfully treated with dithiocarb, with no reported deaths (Sunderman 1990). Other chelating agents are reported to be less effective than dithiocarb, or perhaps even aggravating (NAS, 1975). For example, the use of disulfiram (Antabuse), which is metabolized into two molecules of dithiocarb, is controversial as an alternative therapy when dithiocarb treatment is not available (Bradberry and Vale, 1999). Animal studies suggest that disulfiram increases the uptake of Ni by the brain (Baselt and Hanson, 1982). However, in one case study disulfiram was used for the first 2 days after nickel carbonyl exposure until dithiocarb could be obtained (Kurta *et al*., 1993). In another case study, the survival of patient exposed to inhaled nickel carbonyl was directly attributed to treatment with disulfiram when dithiocarb could not be obtained (Scott *et al*., 2002).

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CHAPTER

36

Palladium

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ABSTRACT

Palladium is one of the six platinum group metals and possesses strong catalytic activity. Demand for palladium is high for its uses in electrical equipment, dental materials, and automobile catalysts. Environmental levels of palladium in water, soil, and ambient air are not high, and environmental exposure and intake from food are not significant, but palladium concentrations are increasing in the general environment because of its increased use in automobile catalysts. Workers in palladium mines and refineries are possibly exposed to palladium, as are dental personnel during the processing of dental alloys containing palladium. The general population may be exposed to palladium through inhalation of ambient respirable particles from automobile catalytic converters that incorporate palladium, but relevant data are rare. The oral toxicity of palladium is believed to be low, although it does depend on the water solubility of the palladium compounds. Therefore, similar intravenous LD_{50} values have been reported for several palladium compounds: from 3–6.4 mg/kg body weight. From a 28-day toxicity study of tetraammine palladium hydrogen carbonate in rats performed through gavage, the no-observed-adverse-effect level (NOAEL) was 1.5 mg/kg body weight/day. Among the general population, skin exposure may occur through contact with jewelry containing palladium. Many case reports exist describing palladium sensitivity and recovery after removal of dental restorations. The symptoms that have been observed include swelling of the lips and cheeks, stomatitis, oral lichen planus, itching, dizziness, asthma, and chronic urticaria. Recently, ^{103}Pd has been used for radioactive sources implanted directly into a malignant tumor; no palladium-related complications have been reported. Although sensitization effects, such as bronchial asthma, can occur among workers exposed to palladium and its compounds, its incidence is extremely low. No data are available on the carcinogenicity, reproductive toxicity, or other effects in humans.

1 PHYSICAL AND CHEMICAL PROPERTIES

Palladium, Pd; atomic number, 46; relative atomic mass, 106.42 (abundance of major natural isotopes are ^{104}Pd [11.14%], ^{105}Pd [22.33%], ^{106}Pd [27.33%], ^{108}Pd [26.46%], and ^{110}Pd [11.72%]); density at 20°C (g/cm^3), 12.02; melting point, 1554°C; boiling point, 2940°C; steel-white metal; oxidation states: +2, +4.

Palladium is one of the six platinum group metals (the others being Pt, Rh, Ru, Ir, and Os). These metals commonly occur together in nature. Palladium, as well as Pt, and sometimes Ir, is considered to be a precious and noble metal. Palladium does not tarnish in air at room temperature. It has a strong catalytic activity for hydrogenation and oxidation reactions. Under an oxygen atmosphere, palladium powder may be a fire hazard.

2 METHODS AND PROBLEMS OF ANALYSIS

Current measurement techniques do not allow the separate chemical species of palladium to be differentiated when more than one form is present. Almost all measurements of palladium determine the total palladium content.

A solution of palladium(II) nitrate at high concentration (mg/L range) is frequently used as a chemical modifier in graphite furnace atomic absorption spectrometry (GF-AAS; Schlemmer and Welz, 1986; Taylor *et al*., 1998). Therefore, to avoid contamination, care must be taken in analytical laboratories that use palladium as a chemical modifier.

For biological materials, such as blood and urine, atomic absorption spectrometry (AAS) and inductively coupled plasma mass spectrometry (ICP-MS) are often applied. By using quadrupole ICP-MS, Schramel *et al*. (1997) reported a detection limit of palladium of 0.03 µg/L of urine after acidification with nitric acid. When using flameless AAS, decomposition with nitric acid gives much lower detection limits: 0.003 µg/L of urine and 0.01 µg/g of blood (Jones, 1976). More recently, Begerow *et al*. (1997a,b) reported a limit of 0.2 ng/L for whole blood and urine on cleaning of all materials (UV photolysis), resulting in a drastic reduction of blanks, when using sector field ICP-MS. Applying high-performance liquid chromatography, a detection limit of 10 ng/L for urine was reported after UV photolysis (Philippeit and Angerer, 2001).

For particulate matter in ambient air, X-ray fluorescence analysis has been performed at detection limits of 1 ng/m^3 (Lu *et al*., 1994) or 0.5 ng/m^3 (Gertler, 1994). For analysis of water, AAS and ICP-MS are often applied, with their limits of detection differing depending on the pretreatment procedure used.

Palladium is rarely found in significant concentrations in environmental and biological materials. Materials being investigated for very low levels of palladium must be sampled in large amounts. Therefore, homogenization, digestion, storage, and matrix effects become major problems. For biological materials, destructive methods are often required during the analytical procedures. For example, samples may be ashed to destroy organic materials and then treated with strong acids, causing information about the palladium species to be lost.

3 PRODUCTION AND USES

3.1. Production

The Republic of South Africa, Russia, Canada, and the United States are the four primary producers of palladium. In the year 2003, they produced 64.8, 74.0, 11.0, and 14.6 tons, respectively. Production from other countries was 7.0 tons, making the total 171.0 tons (USGS, 2005a). Because the production of palladium is relatively limited to a few production sites, supply shortages can cause substantial fluctuations in the market price. In 2000 and early 2001, the price rose and peaked at five times the usual market prices (Palladium, Metal of the 21st Century, 2005).

Because it is very expensive, a high proportion of palladium, and other platinum group metals, is recycled by either the users or the producers (e.g., catalysts) and does not appear on the market. In 2003, an estimated 8 tons of palladium was available in the United States for recycling from autocatalysts (USGS, 2005b).

3.2 Uses

Demand for palladium is high for its use in electrical equipment, dental materials, and automobile catalysts.

Palladium metal and silver-palladium powder pastes are important in the production of many electronic components. The pastes are used in active components such as diodes, transistors, integrated circuits, hybrid circuits, and semiconductor memories. They are also used for passive electronic components, such as small multilayer ceramic capacitors, and for thick film resistors and conductors. Palladium alloys are used in electrical contacts, electrical relays, and switching systems in telecommunications devices. The gold in the coatings of electronics, electrical connectors, and lead frames of semiconductors can sometimes be replaced with palladium (Kroschwitz, 1996).

Palladium is a component in some dental amalgams. Palladium alloys (gold-silver-copper-palladium) can be matched to any dental application such as inlays, full-cast crowns, long-span bridges, ceramic metal systems, and removable partial dentures (Stümke, 1992).

Catalysts are used to reduce levels of nitrogen oxides, carbon monoxide, and hydrocarbons in automobile exhausts. Recently developed catalysts use combinations of precious metals, such as platinum, palladium, and rhodium (Abthoff *et al*., 1994; Degussa, 1995; Kroschwitz, 1996). The concentrations of the precious metals in the catalysts vary depending on the specifications of the manufacturer (IPCS, 1991). Worldwide demand for palladium in automobile catalysts rose from 23.5 tons in 1993 to 139 tons in 1998. Approximately 60% of European gasoline-powered cars sold in 1997 were equipped with palladium-based catalysts; many Japanese cars are also equipped with palladium systems, but platinum-rich technology remains dominant elsewhere in Asia (Cowley, 1997). North American carmakers continue to use platinum-rich catalysts,

but there was increasing use of palladium catalysts to meet the hydrocarbon limits imposed by low-emission-vehicle legislation.

Palladium is used in jewelry and coinage. In the fabrication of jewelry in Japan, palladium is a subsidiary alloying component of the platinum alloys used (Coombes, 1990). From recent case reports (Bircher and Stern, 2001; Suhonen and Kanerva, 2001) the "titanium" spectacle frames that actually consist of palladium can cause contact allergies. Alloys are also used for bearings, springs, and balance wheels in watches and for mirrors in astronomical instruments.

Because palladium has a strong catalytic activity for hydrogenation, dehydrogenation, oxidation, and hydrogenolysis reactions, palladium compounds are used as catalysts for chemical processes in many industries.

The following list summarizes the uses of some important palladium compounds (IPCS, 2002a):

1. Ammine complexes of palladium: industrial separation of palladium, electroless plating, and bright palladium plating. Ammonium hexachloropalladate(IV) is important in separation technology.
2. Palladium(II) acetate: preparative chemistry.
3. Palladium(II) chloride: plating baths, photography, toning solutions, electroplating parts of clocks and watches, detecting carbon monoxide leaks in buried gas pipes, manufacture of indelible ink, the preparation of catalysts, and medical use.
4. Palladium(II) nitrate: a catalyst in organic syntheses, and for the separation of chlorine and iodine.
5. Palladium(II) oxide: a hydrogenation catalyst in the synthesis of organic compounds
6. Hydrogen tetrachloropalladate(II): palladium preparation.
7. Tetraammine palladium hydrogen carbonate: an intermediate in the production of automobile catalysts.

4 ENVIRONMENTAL LEVELS AND EXPOSURES

4.1 Water, Soil, and Ambient Air

Water samples collected from streams and ponds in and around the mining and ore processing facilities located in Sudbury, Ontario (Canada), were below the detection limit of 15 ng Pd/L (Johnson *et al.*, 1976). Although the detection limits vary, concentrations of palladium in the ng/L range in natural waters have been reported: 0.4 ± 0.1 ng Pd/L in Idaho (Shah and Wai, 1985); 1.0 ± 0.1 ng/L (Schwarzbach river) and 0.4 ± 0.1 ng/L (Rhine River) in Germany (Eller *et al.*, 1989); and 1–2 ng/L in the lakes of Canada (Hall and Pelchat, 1993).

For spring water samples, the values are somewhat or considerably higher: 3 ng/L in water samples from springs in Nevada (Stetzenbach *et al.*, 1994), 22 ng/L in Osaka, Japan (Chikuma *et al.*, 1991), and 0.1 µg/L in the People's Republic of China (Zhou and Liu, 1997).

The palladium concentrations in rain collected in Stuttgart, Germany, were at levels below the detection limit of 5 ng/L (Helmers *et al.*, 1998).

In seawater, palladium concentrations of 22 pg/L (depth, <10 m) and 60 pg/L (depth, 3000 m) were found in filtered samples of Pacific Ocean water (Goldberg, 1987). The palladium concentration in the water column of the northeast Pacific Ocean increased from 19 pg/kg at the surface to 70 pg/kg in deep waters (Lee, 1983).

It has been reported that the palladium concentration in the soil of an area with high traffic density in California was below the detection limit (0.7 µg/kg) of the AAS used (Johnson *et al.*, 1976). There have been two reports concerning the increased palladium concentrations along the autobahn near Frankfurt, Germany. Samples taken in 1990 and 1991 gave a mean value of 2 µg Pd/kg (Zereini *et al.*, 1993). More recent samples (1994) gave a mean value of 6 µg Pd/kg (Zereini *et al.*, 1997). It was suggested that the elevated level was due to the increased use of catalytic converters in automobiles.

The data on palladium concentrations in the surface soil near a palladium production site are extremely scarce. The palladium concentration in the area around a platinum-group metal mine (Sudbury, Ontario, Canada) was determined to be 2.0–4.5 µg/kg (Johnson *et al.*, 1976).

Although there are very few data on palladium concentrations in the atmosphere, these values are quite variable. Before the introduction of automobile catalytic converters, the palladium concentrations in an area in California were reported to be below the detection limit (0.06 pg Pd/m^3) despite the high traffic density (Johnson *et al.*, 1976). In the 1990s, the palladium concentrations in aerosols were determined in different places. In Imperial County, California, the level of PM10 (particulate matter having an aerodynamic diameter below 10 micrometers) was 52.2 µg/m^3, with the average palladium concentration <1 pg/m^3 (Lu *et al.*, 1994). At Caesarea, Israel, Gertler (1994) observed an averaged PM2.5 concentration of 25.5 µg/m^3 with a palladium average concentration of 3.3 pg/m^3. In contrast, in the city of Chernivtsi, Ukraine, the levels of

particulate matter and palladium were 144 µg/m^3 and 56.6 ng/m^3, respectively (Scheff *et al*., 1997).

The levels of platinum-group metals have been determined in ancient and recent samples of ice and snow in Greenland. The concentrations of these metals in snow from the mid-1990s are 40–120 times higher than those in ice from 7000 years ago (Barbante *et al*., 2001). This finding is considered to be due to air pollution caused by automobile catalysts.

4.2 Food and Daily Intake

In 1994, the United Kingdom's Ministry of Agriculture, Fisheries, and Food conducted a total diet study (MAFF, 1997, 1998; Ysart *et al*., 1999) in which they used ICP-MS to determine the levels of 30 elements (including palladium). Milk and poultry contained palladium concentrations below the limit of detection (0.3 µg/kg). Fish, offal, and bread contained 2 µg/kg, and nuts contained 3 µg/kg. Other groups, including miscellaneous cereals, carcass meat, meat products, oils and fats, eggs, sugars and preserves, green vegetables, potatoes, other vegetables, canned vegetables, fresh fruit, fruit products, beverages, and dairy produce, had mean values of 0.4–0.9 µg Pd/kg. Applying the dietary intake estimates based on the United Kingdom National Food Survey to the mean concentration of each food group provides an estimate of the daily palladium intake. The results are a mean of 1 µg Pd/day with the 97.5th percentile of 2 µg/day. Other data on the palladium contents of food and drinking water are scarce.

4.3 Working Environment

It is considered that workers involved in mining, processing, recycling, refining, and catalyst manufacture are exposed to palladium and/or its compounds. Dental personnel are also exposed to palladium during the processing of dental alloys containing palladium.

Air samples collected in and around the mining and ore processing facilities in Sudbury, Ontario, did not display measurable levels of palladium (detection limit, 0.003 µg Pd/m^3), except in the precious metals area, where the value was as high as 0.29 µg Pd/m^3 (Johnson *et al*., 1976). In a platinum and palladium refinery in New Jersey, air samples were reported to contain palladium at concentrations between 0.001 and 0.36 µg/m^3 (Johnson *et al*., 1976).

Average palladium concentrations of 3.5–5.5 µg/m^3 were reported in the breathing zone of dental technicians (Purt, 1991). Palladium consisted of approximately one third of the dust concentrations.

The urinary excretions of platinum, palladium, and gold have been analyzed in 27 dental technicians and 17 road construction workers, with 17 adolescents as the control (Begerow *et al*., 1999). The average urinary palladium concentration in dental technicians (135.4 ±; 183.5 ng/L) was significantly higher than that in the control group (31.0 ±; 10.8 ng/L); the road construction workers exhibited a level of 52.2 ± 35.3 ng/L. Although these results would indicate that the processing of palladium-containing alloys leads to occupational exposure, it is noteworthy that the dental technicians had a significantly larger number (246 for 27 technicians) of dental materials containing metals than did the other study groups (18 for 17 workers; 14 for 17 controls). More recently, however, no appreciable increases of palladium and other metals, such as titanium, mercury, platinum, and rhodium, were found in dental health care workers (Iavicoli *et al*. 2004).

4.4 Iatrogenic Exposure

A large number of palladium-containing dental alloys have been used. Many researchers have claimed that palladium is released from materials containing palladium alloy. The release rate of an alloy (Au52, Ni28, Ga13, Pd4, In4; atomic percentages) was calculated at 3 ng Pd/cm^2/day (Wataha *et al*., 1991; 1995), reaching a concentration of approximately 30 µg/L after a few days in a cell culture medium. This release rate is, however, much lower than those of either gallium (0.97 µg Ga/cm^2/day) or nickel (1.46 µg Ni/cm^2/day).

Palladium-based dental alloys containing copper or copper and tin released more palladium in the artificial saliva at 0.2–6 and 6–22.5 µg Pd/cm^2/day, respectively (Pfeiffer and Schwickerath, 1995).

The palladium content in saliva was higher in the group of persons having amalgam (mercury and silver) fillings (2.8 ± 2.7 µg/L) and significantly higher in the group of persons having amalgam fillings and metallic dental appliances (10.6 ± 7.4 µg/L) than was that in a control group of persons having intact teeth (1.5 ± 1.5 µg/L) (Wirz *et al*., 1993).

A palladium concentration of 1.4 mg/g was found in inflamed gingival tissue from a patient with allergic reactions (mainly to nickel, chromium, and jewelry) (Wirz *et al*., 1993).

Mechanical stimulation, such as continuous gum chewing, dramatically increased the palladium release rate from dental alloys in two patients, from 0.4 and 1.8 µg/L saliva to 204 and 472 µg/L saliva, respectively (Daunderer, 1993). Because the palladium-containing dental alloys exhibit complex release behavior, it is difficult to predict the release of palladium from their nominal composition. The results of limited clinical studies, however, suggest that a daily mean intake of 1.5–15 µg Pd/adult/day is to be expected, assuming a median value of 1–1.5 liters of ingested saliva (IPCS, 2002b).

5 METABOLISM

5.1 Absorption

Generally, the absorption of a metal seems to depend on its chemical form and its route of administration; its solubility in aqueous media is an important factor. It is noteworthy that palladium dust, which is nearly insoluble in distilled water, was found to dissolve appreciably in biological media, such as gastric juice and blood serum (Roshchin *et al*., 1984), or in aqueous solutions of biogenic compounds, such as peptides and amino acids, under an oxygen atmosphere (Freiesleben *et al*., 1993).

Quantitative data are scarce for the absorption of palladium. Palladium ions are absorbed less from the digestive tract in fasted adult rats (0.5% of the initial dose after 3 days) than in nonfasted suckling rats (approximately 5% at 4 days after dosing) (Moore *et al*., 1974; 1975). It has also been reported that intratracheal administration and inhalation of aqueous aerosols resulted in a higher absorption than did oral administration (Moore *et al*., 1974; 1975). Data on the absorption of palladium or palladium compounds in humans are not available.

5.2 Distribution

Limited data exist on the distribution of palladium in the tissues of rats, rabbits, and dogs after single oral, intravenous, or intratracheal doses of palladium compounds. After intratracheal exposure, the highest concentrations were found in the kidney, liver, lymph nodes, spleen, and lungs. Dietary administration to rats of high doses (approximately 700 mg/kg body weight/day) of palladium salts (palladium[II] chloride and palladium[II] sulfate) resulted in the following palladium tissue concentrations (in mg/kg wet weight): kidney (35 and 22, respectively) > liver (2 and 3) > spleen (0.7 and 0.9) > testis (0.24 and 0.26) > blood (<0.04 and 0.16) > brain (<0.01 and <0.01).

Placental transfer at considerably low concentration was found in the fetuses of rats given single intravenous doses of $^{103}PdCl_2$ (Moore *et al*., 1975). The content in fetal liver (as a radioactive count) was 1429, whereas that in maternal liver was 319,153 counts/g tissue. A small amount of ^{103}Pd was detected in the tissue of young rats whose dams had received single intravenous doses of $^{103}PdCl_2$ (25 µCi/rat) within 24 hours postpartum (Moore *et al*., 1974). Therefore, it is believed that transfer through the placenta or milk occurs only to a small degree.

5.3 Excretion

After rats had been given an oral dose of $PdCl_2$, most of it (>95%) was eliminated through the feces because of nonabsorption (Moore *et al*., 1974; 1975). Intravenous administration, however, resulted in similar (Moore *et al*., 1974; 1975) or greater (Durbin *et al*., 1957) contents in the urine than in the feces of rats. It is interesting that urinary excretion was much lower in tumor-bearing animals (Ando and Ando, 1994).

Taubler (1977) found increased palladium concentrations in the urine of guinea pigs and rabbits after their subcutaneous injections with palladium(II) sulfate and also in the urine of guinea pigs after their exposure to palladium-albumin complex. After dermal treatment with "chloropalladium," palladium was found in their urine (Roshchin *et al*., 1984).

Palladium was also found in human urine sampled from Germans who had not been subjected to occupational exposure. The values were <0.22 µg/L, with the minimum being 6 ng/L (Begerow *et al*., 1997b,c; 1998; 1999). A recent study (Violante *et al*., 2005) comparing the relationships between the concentration of palladium in ambient air and in biological materials, such as hair, blood, and urine, revealed that a strong correlation existed to the hair and urine; it was concluded that the palladium content in urine could be considered a satisfactory exposure biomarker for occupational monitoring.

6 LEVELS IN TISSUES AND BIOLOGICAL FLUIDS

In earlier studies, the palladium concentrations in blood, hair, feces, and urine were reported to be below the detection limit (Johnson *et al*., 1975). More recent studies in Germany gave levels of 0.03–0.2 (mean, 0.1) µg/L for 24–hour urine samples from 14 nonsmokers (Schramel *et al*., 1997), 0.02–0.08 (mean, 0.04) µg/L for 24-hour urine samples (Begerow *et al*., 1997c), 0.03–0.08 (mean, 0.05) µg/L for whole blood (Begerow *et al*., 1997a), 0.03–0.22 (mean, 0.14) µg/L for 24–hour urine samples (Begerow *et al*., 1997b), and slightly lower values of 0.013–0.048 (mean, 0.031) µg/L for morning urine. A comparison of the morning urine levels among groups living in areas having different automobile traffic densities did not display a definite difference; the overall range was 0.006–0.091 (mean, 0.033) µg/L, with mean values of 0.033, 0.035, and 0.032 µg/L in the low-, medium-, and high-traffic zones, respectively.

7 EFFECTS AND DOSE-RESPONSE RELATIONSHIPS

7.1 Animals

7.1.1 Single Exposure

Values of LD_{50} ranged from 3 mg/kg body weight (palladium[II] chloride; rat; intravenous; Moore *et al*., 1975) to >4.9 g/kg body weight (palladium[II] oxide; rat; oral; Holbrook *et al*., 1975), depending on the choice of animal, compound, and route of exposure. Compounds having lower absorption rates displayed lower toxicities after oral administration. In contrast, very similar values of LD_{50} were reported (Charles-River CD-1 rat; Moore *et al*., 1975) for intravenous exposure to palladium(II) chloride of 3 mg/kg body weight, potassium tetrachloropalladate(II) of 6.4 mg/kg body weight, and ammonium tetrachloropalladate(II) of 5.6 mg/kg body weight. Clinical signs of toxicity after such exposure included death, clonic and tonic convulsions, decreases in food and water uptake, emaciation, and cases of ataxia and tiptoe gait (Moore *et al*., 1975).

Six months after a single intratracheal application of 50 mg palladium dust, there were several signs of inflammatory responses in the lungs of rats, but no indications of interstitial fibrosis or carcinogenic changes (Augthun *et al*., 1991).

The lowest effective dose of 0.4 mg Pd^{2+}/kg body weight (intravenously) was reported for cardiovascular effects in rats (ventricular arrhythmia, ventricular fibrillation, and death at higher doses) (Wiester, 1975).

7.1.2 Repeated Exposure

A 28-day toxicity study (Johnson Matthey, 1997a) on tetraammine palladium hydrogen carbonate in rats through gavage at dose levels of 1.5, 15, and 150 mg/kg body weight established the NOAEL to be 1.5 mg/kg body weight/day, although an increase in the absolute brain and ovary weights occurred in females (15, 1.5). The effects observed at higher doses were reduced body weight gain, anemia, and increases in absolute and relative kidney weight (150); histopathological changes in the liver and kidney (150) and spleen and stomach (150, 15) were observed.

7.1.3 Chronic Exposure

Male mice given palladium(II) chloride (5 mg Pd/L) in drinking water from weaning until natural death displayed suppression of body weight gain and longer life spans. The mean age of the palladium-fed male mice was 555 days (vs 444 days in the controls). An increase in amyloidosis of several organs and malignant tumors [27.7% (n = 18 of 65) versus 13.8% in controls (n = 11 of 80)] was observed (Schroeder and Mitchener, 1971).

Chronic effects of palladium dust have also been investigated. Daily oral administration of 50 mg palladium powder/kg body weight to rats for 6 months resulted in suppressed body weight gain, shortening of the prothrombin clotting time, decreases in the urea and lipoprotein contents, and an increase in albumin (Roshchin *et al*., 1984).

7.1.4 Irritation and Sensitization

As expected from other metal compounds, palladium and its compounds have potential to cause of irritation and sensitization. Campbell *et al*. (1975) reported a skin irritation evaluation of eight palladium compounds, following the procedures and evaluation criteria of US National Institute of Occupational Safety and Health. Three compounds—$(NH_4)_2PdCl_6$, $(NH_4)_2PdCl_4$, and $(C_3H_5PdCl)_2$—caused erythema, edema, or eschar and were considered to be unsafe for skin contacts; K_2PdCl_6, K_2PdCl_4, and $PdCl_2$ were nonirritant to intact skin but caused erythema in abraded skin; $(NH_3)_2PdCl_2$ and PdO did not produce any adverse effects. The degree of irritation may correspond to the solubility of these compounds.

In guinea pig maximization tests, palladium(II) chloride (Wahlberg and Boman, 1990) and tetraammine palladium hydrogen carbonate (Johnson Matthey, 1997b) proved to be strong sensitizers. Significant primary immune responses were reported to palladium(II) chloride, sodium tetrachloropalladate(II), potassium tetrachloropalladate(II), and ammonium hexachloropalladate(IV) in the popliteal lymph node assay in BALB/c mice (Kulig *et al*., 1995; Schuppe *et al*., 1998). In one study, signs of respiratory sensitization were reported in cats after intravenous administration of several palladium salts (Tomilets and Zakharova, 1979).

Palladium(II) chloride is considered to be a stronger sensitizer than nickel sulfate, which is a well-known potent sensitizer. Animals sensitized with palladium(II) chloride showed cross-reactivity to nickel sulfate (Wahlberg and Boman, 1992), but the reverse situation did not hold.

7.1.5 DNA Interactions and Mutagenicity

Various palladium compounds have been found to interact with isolated DNA *in vitro* (Pillai and Nandi, 1977), and the interaction can induce conformational changes in DNA structures (Pillai and Nandi, 1977; Shishniashvili *et al*., 1971). Mutagenicity tests, such as the Ames test with *Salmonella typhimurium* (Bünger, 1997; Bünger *et al*., 1996; Suraikina *et al*., 1979; Uno and Morita, 1993), the SOS chromotest with *Escherichia coli*

(Gebel *et al*., 1997; Lantzsch and Gebel, 1997), and the micronucleus test with human lymphocytes (Gebel *et al*., 1997) gave negative results, however, with an exception of tetraammine palladium hydrogen carbonate, which induced a clastogenic response to human lymphocytes *in vitro* (Johnson Matthey, 1997c).

7.1.6 Carcinogenicity

Only limited information is available on the carcinogenicity of palladium.

As mentioned previously, Schroeder and Mitchener (1971) performed a long-term experiment with mice given 5 mg Pd/L in drinking water from their weaning until their natural death. An increased rate of malignant tumors was observed [27.7% (n = 18/65) versus 13.8% in the controls (n = 11/80)]. It was suggested, however, that the significantly enhanced longevity of the exposed group, relative to that of the controls, might have caused their higher rate of malignant tumors.

Subcutaneous implantation of a silver-palladium-gold alloy resulted in the formation of fibrosarcoma, myosarcoma, fibroma, and fibroadenoma at the implantation site in 7 of 14 rats after 504 days (Fujita, 1971). The author suggested that the observed carcinogenicity was due to the chronic physical stimulus of the imbedded alloy.

7.2 Humans

7.2.1 General Population Exposure

Humans may become exposed to palladium through inhalation of ambient respirable particles from automobile catalytic converters that incorporate palladium, but no data are available.

Among the general population, skin exposure may occur through contact with jewelry that contains palladium. Palladium's major role during jewelry fabrication in Japan is as a subsidiary alloying component of the platinum alloys used (Coombes, 1990). Body piercing is considered a risk factor to the development of sensitivity to palladium and other metals. It has been reported that individuals having more piercings had more positive reactions in patch tests involving palladium chloride, nickel sulfate, gold sodium thiosulfate, and cobalt chloride (Ehrlich *et al*. 2001).

7.2.2 Iatrogenic Exposure

Another source of exposure is dental restorations containing palladium. Many reports describe recovery from palladium sensitivity after removal of dental restorations. Symptoms observed included swelling of the lips and cheeks, stomatitis, oral lichen planus, itching, dizziness, asthma, and chronic urticaria (Adachi *et al*., 1997; Akiya *et al*., 1996; Castelain and Castelain, 1987; Downey, 1989; Fernandez-Redondo *et al*., 1998; Hackel *et al*., 1991; Hay and Ormerod, 1998; Koch and Baum, 1996; Kütting and Brehler, 1994; Mizoguchi *et al*., 1998; Richter, 1996; Stejskal *et al*., 1994; van Joost and Roesyanto-Mahadi, 1990; van Ketel and Niebber, 1981; Yoshida *et al*., 1999).

It is believed that the possible harmful effects of palladium in dental alloys may vary depending on the composition and preceding preparation of the alloy because of their different corrosive behavior and interactions with the other components. Drasch *et al*. (2000) reported the palladium concentrations found in saliva and other biological samples; an elevated concentration existed in the saliva of individuals having dental crowns and inlays. The concentration increased during chewing. The increasing amount of palladium in the feces with respect to the number of dental crowns and inlays in the mouth suggested a low absorption rate in the gastrointestinal tract.

Recently, ^{103}Pd has been used for brachytherapy, a process in which radioactive sources are implanted directly into a malignant tumor (Finger *et al*., 1999; Sharkey *et al*., 1998). There have been no palladium-related complications reported to date that might suggest the need to prohibit the use of ^{103}Pd needles in cancer radiotherapy.

7.2.3 Occupational Exposure

Although details were not given, Roshchin *et al*. (1984) reported (in a review article on the frequent occurrence of allergic diseases of the respiratory passages) that dermatosis and affections of the eyes occurred among Russian workers producing platinum group metals. In contrast, only one or two workers of 300 had positive skin-prick test reactions to solutions of palladium halide salts from a survey of South African platinum refinery workers who are known to be exposed to palladium (Murdoch and Pepys, 1987; Murdoch *et al*., 1986: Peschel *et al*., 1999).

A case study showed an isolated sensitization to palladium: a worker having rhinoconjunctivitis and asthma was examined through skin-prick tests and gave positive results for $Pd(NH_3)_4Cl_2$, but not for $Pd(NH_4)_2Cl_4$. The effects of corresponding platinum compounds were negative (Daenen *et al*., 1999).

Dental technicians were apparently exposed to respirable dust particles of palladium (Begerow *et al*., 1999; see Section 4.3), but the contribution of palladium (within a series of other substances generated during dental working processes) as a health hazard was unclear.

Because palladium is contained in automobile catalysts, workers in the automobile industry may be

exposed to it. Four of 130 workers displayed positive reactions to palladium(II) chloride after prick tests performed in 1990–1991 of workers in a German plant manufacturing automobile catalysts (Merget, 1991). They also reacted to hexachloroplatinic acid (H_2PtCl_6).

7.2.4 Carcinogenicity and Other Effects

No data are available on the carcinogenicity, reproductive toxicity, or other effects in humans.

7.3 Dose-Response Relationships

There are wide variations in the values of LD_{50} for oral administration in experimental animals. The intravenous injections of palladium compounds such as palladium(II) chloride, potassium tetrachloropalladate (II), and ammonium tetrachloropalladate(II) provided values of LD_{50} of 3–6.4 mg/kg body weight. The lowest effective dose—of 0.4 mg Pd^{2+}/kg body weight (intravenously)—was reported for cardiovascular effects (see Section 7.1).

From a 28-day toxicity study (Johnson Matthey, 1997a), it was determined that the NOAEL for oral administration in rats was 1.5 mg/kg body weight/day. Chronic exposure of males over a lifetime to palladium(II) chloride (5 mg Pd/L) in drinking water led to a suppression of body weight gain and a longer life span, with an increase in amyloidosis of several organs. The ingested amount of palladium was estimated to be 600 µg/day/mouse, assuming the drinking volume of 3 mL/day and a body weight of 25 g.

Given that sensitization is of major concern, it is difficult to establish dose-response relationships from the available data, including NOAEL, for sensitization in humans.

8 DIAGNOSIS, TREATMENT, PROGNOSIS, AND PREVENTION

Palladium and its compounds, if ingested, display very low to moderate acute toxicity. The toxicity depends mainly on their solubility. The intake of palladium from food or drinking water is low among the general population. From a survey conducted in the United Kingdom, the maximum daily intake of drinking water has been calculated to be 0.03 µg Pd/person/day (assuming the maximum palladium concentration of 15 ng/L × 2 L/day), with a total daily dietary intake of up to 2 µg/person/day. With dental alloys present, additional exposure to palladium occurs. It has been reported that the palladium concentration in saliva may reach as high as 10 µg/L and, thus, it contributes considerably (up to 15 µg/person/day) to the total palladium intake in subjects having dental alloys. Although there are few data available concerning the palladium concentration in ambient air, concentrations are usually found on the magnitude of pg/m^3. Therefore, it is considered that exposure to palladium is not considered to cause serious health problems among the general population. Skin or mucosal contact with palladium-containing jewelry and dental alloys seems, however, to be an important route of exposure. Some subgroups, such as individuals having pierced ears or other body parts, may be at special risk of developing sensitivity to palladium. Because palladium-containing dental alloys have been identified as a possible source of sensitization, the public should be protected from possible adverse effects by minimizing the use of palladium-containing alloys or the release of palladium from alloys.

It is evident that several palladium salts cause severe primary skin and eye irritations. Individuals with known palladium allergy should not work with palladium compounds. Workers with known nickel allergy should be advised that working with palladium salts might cause allergic reactions. It is advisable that a questionnaire for skin disease specifically for allergy to metals (nickel, cobalt, and palladium) be given during preemployment screening, although patch tests should be performed only to determine the cause of occupational dermatitis. Health checks—through questionnaires and examinations—for skin and respiratory disorders should be performed regularly during employment. Improvement of the working environment and protective equipment, if necessary, should be used to lower the exposure to palladium and its compounds. It is noteworthy that a protective cream containing diethylenetriaminepentaacetic acid did not protect against a contact allergic reaction to palladium chloride, even though it was effective to nickel sulfate, cobalt chloride, and copper sulfate (Wohrl *et al*. 2001).

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CHAPTER

37

Platinum*

MIRJA KIILUNEN AND ANTERO AITIO

ABSTRACT

Commercial sources of platinum are sulfide and arsenide minerals, and increasingly, recycled platinum. The main source of platinum in the environment is release from vehicle catalysts, and platinum concentrations near highways and urban areas have gradually increased. In occupational exposure, the form of platinum is mainly coordination complexes, most often with chlorine as a ligand, whereas the platinum released from automotive catalysts is metallic or oxidic.

The acute toxicity of platinum compounds depends mainly on their solubility, soluble platinum salts being more toxic than the compounds with lower solubility, such as oxides. Chloroplatinates are irritating to the eye and skin.

The main health effect of platinum compounds is sensitization; the symptoms of platinum salt sensitivity include irritation of the eyes and upper respiratory tract and asthma. Allergenic potency of platinum compounds seems to be limited to coordination complexes containing halogen ligands as leaving groups, hexa- and tetrachloroplatinates being the most potent sensitizers. Neutral coordination complexes such as diamminedichloroplatinum do not seem to be allergenic. Bromine and iodine analogs of platinates may cause sensitization but seem to be less potent. The mechanism of platinum salt allergy is likely to be type 1 (i.e., involves IgE). Atopic constitution is not associated with susceptibility to platinum sensitization. Smokers have a higher risk for platinum sensitization.

No health effects from environmental exposure to platinum have been reported.

With the exception of platinum-containing chemotherapeutic drugs such as cisplatin (*cis*-diamminedichloroplatinum), no relevant human or experimental data are available on the potential carcinogenicity or teratogenicity of platinum compounds. Cisplatin and other platinum-containing chemotherapeutic drugs are genotoxic in a variety of test systems, and even other platinum compounds have induced mutations *in vitro* (but not *in vivo*).

Platinum compounds used as anticancer drugs such as cisplatin and its analogs are not covered in detail in this chapter; they are discussed only when it is informative from the point of view of structure-activity relationships.

1 PHYSICAL AND CHEMICAL PROPERTIES

Platinum (Pt), CAS 7440-65-7: atomic mass, 195.078; atomic number, 78; density, 21.447 (calculated); melting point, 1773.5 ± 1°C; boiling point, approximately 3827°C; silver-grey, lustrous, malleable, and ductile metal, face-centered cubic; oxidation states chiefly +2, +4, also +1, +5, +6. Platinum is also prepared in the form of a black powder (platinum black) and spongy masses (platinum sponge). Six isotopes occur naturally, 190 (0.01%), 192 (0.8%), 194 (32.9%), 195 (33.8%), 196 (25.2%), and 198 (7.2%) The isotope 190 is radioactive with $t_{½}$ $6.9*10^{11}$ years. Artificial radioactive isotopes include 173–189, 191, 193, 197, 199–201.

*The views presented in this chapter are those of the authors and do not necessarily reflect the decisions or stated policies of the affiliated organizations

Platinum is unaffected by air at any temperature. It reacts with boiling *aqua regia* with formation of chloroplatinic acid and with molten alkali cyanides. Halogens, cyanides, sulfur, molten sulfur compounds, and hydroxides can affect platinum.

The most important platinum compounds are those with halogens. The binary compounds are chlorides (+2 and +4), the oxide, and also the sulfate and nitrate. Tetravalent platinum compounds are readily soluble in water except platinic oxide, which is soluble in dilute phosphoric acid, other concentrated acids, and easily soluble in dilute potassium hydroxide solutions. Divalent platinous chloride is insoluble in water, alcohol, and ether but soluble in hydrochloric acid. Platinum compounds are considered to be soluble in human body if they are soluble to 0.07 mol/L hydrochloric acid (HSE, 1996). Coordination complex compounds, hexachloroplatinic and tetrachloroplatinic acids, and their alkali salts, dominate in aqueous solutions.

Certain platinum coordination complexes with different ligands (cisplatin, carboplatin, oxaliplatin, lobaplatin, satraplatin) are used in cancer chemotherapy. Of the two stereoisomers of diammonium chloroplatinate (*cis*- and *trans*-diamminedichloroplatinum (II)), only *cis*-form is effective as chemotherapeutic.

2 METHODS AND PROBLEMS OF ANALYSIS

Several methods are available to determine platinum from biological materials. Earlier mainly electrothermal atomic absorption and adsorptive voltammetric methods were used; their detection limits are low enough for the studies of the body fluids of patients who were treated with drugs, but for environmental exposure more reliable methods are needed. Inductive coupled plasma with atomic emission spectrometry or with mass detector (ICP-MS), neutron activation analysis, and modern adsorptive voltammetry are sensitive enough and widely used techniques for the determination of platinum at nanogram and subnanogram levels in a wide variety of biological and environmental matrices. The most sensitive method is adsorptive voltammetry with a limit of quantification of few picograms per gram levels.

The limit of quantization 50pg/L for urine, 10pg/L for plasma, and 5pg/L for plasma were recently reported for electrothermal atomic absorption methods (Vouillamoz-Lorenz *et al*., 2001). In a comparison of sector field ICP-MS (SF-ICP-MS) with adsorptive cathodic stripping voltammetry at ultratrace levels of platinum in biological samples identical results were obtained with both methods, but voltammetry was 20 times more sensitive than ICP-MS (Renner *et al*., 2002; Zimmermann *et al*., 2001) and approximately 6000 times more sensitive than the standard flameless atomic absorption spectrophotometry (AAS) method (Gelevert *et al*., 2001) The lowest platinum concentrations measured in tissue samples were approximately 200 ng/kg analyzed with SF-ICP-MS (sector field). Also X-rays have been exploited in tissue analysis (Taylor *et al*., 2002). After UV irradiation of urine samples, platinum was determined by use of SF-ICP-MS on the level 1000 pg/L with the absolute detection of 30 pg/L (Caroli *et al*., 2001). An ultrasonic nebulizer connected with SF-ICP-MS has been used for plasma and whole blood samples (Morrison *et al*., 2000). For accurate determination of platinum by ICP-MS correction by use of interference equations is needed to prevent the molecular ion and doubly charged ion interferences induced by $CuAr^+$, HfO^+, SrO^+, YO^+ and Pb_2^+.

Because the need of detection is at nanogram level, preconcentration methods are required in many cases.

With improved analytical methods, part of the concentrations presented earlier in literature has been shown to be in error. There is a lack of useful control materials at required concentration levels and matrices; international quality assurance schemes are badly needed.

3 PRODUCTION AND USES

3.1 Production

Platinum is found mainly in sulfide and arsenide minerals as sperrylite $PtAs_2$, cooperite (Pt,Pd)S, and braggite (Pt,Pd,Ni)S; it usually occurs together with other platinum-groups metals (PGM). The main deposits are in the Republic of South Africa, Russia, Canada, and the United States. The world production of platinum-group metals in 2000 was 365 tons (Kelly and Hilliar, 2003). Demand for platinum in 2003 was 185 tons and is steadily increasing (Matthey, 2004b). In 2004, the demand was 223 tons, of which approximately 20 tons was recovered from recycling (Matthey, 2004a).

Sulfite and arsenide ores are first smelted to produce a matte of metal sulfide mixture. The other metals are precipitated from the mixture. Selenium, tellurium, and sulfide are removed from the concentrate of precious metals. Silver and gold are separated electrolytically, with liquid extraction or precipitation from the chlorinated solution. The platinum-group metals are precipitated and refined with different techniques (Renner *et al*., 2002).

3.2 Uses

The major industrial users of platinum are the automotive, electronic, chemical, jewelry, dental, and glass industries. Platinum has wide catalytic qualities. It is used in catalytic converters in car exhaust systems. The automotive industry accounts for approximately 80% of platinum use (Matthey, 2004a; Renner *et al.*, 2002). The platinum emission rate has been estimated to be 0.5–0.8 µg Pt/km from traffic (Pyrzynska, 2000). The second largest consumer is the chemical industry. Platinum is used as a catalyst in hydrogenation, dehydrogenation, and isomerization reactions; in the manufacturing of paints, acids, fertilizers, and explosives; and in oil refining. In the dental industry, platinum is used in the construction of crowns, bridges, pins, and other dental equipment, as well as fillings, which is expected to increase in the future. The jewelry industry uses platinum as "white gold," because its strength and luster makes it ideal setting for diamonds; the use in jewelry has decreased in the past decade. The electronics industry uses platinum in liquid crystal display lights, picture tubes, and fiberoptic cables. Platinum-based fuel technology is being promoted as the best option for electric cars (Matthey, 2004a; Renner *et al.*, 2002).

4 ENVIRONMENTAL LEVELS AND EXPOSURE

4.1 General Environment

Older studies indicate that the platinum concentration in the earth's crust is 1–5 µg/kg (IPCS, 1991); a more recent study reported an average concentration of 2.7 µg/kg (Tereshima *et al.*, 2002).

Quite limited data are available on platinum concentrations in the air in rural areas. Before the introduction of cars with catalytic converters, the values near freeways in the United States were below the detection limit of 0.05 pg/m^3 (Johnson *et al.*, 1975; 1976). During the 1980s in Japan, the values ranged from 0.014–0.184 µg/g of dust (Mukai *et al.*, 1990). The present reported platinum background concentrations are <4 pg/m^3 in Germany (Merget and Rosner, 2001). In urban areas, the reported concentrations of platinum in the air were between 0.9 and 147 pg/m^3 in the 2000s, and a trend toward continuous increase in ambient concentration of platinum was noticed. (Caroli *et al.*, 2001; Gómez *et al.*, 2001, 2003; Kanitsar *et al.*, 2003; Petrucci *et al.*, 2000; Probst *et al.*, 2001; Rauch *et al.*, 2001; Schierl, 2000; 2001; Zereini *et al.*, 2001a,b). Between 1988 and 1998, there was a 46-fold increase of platinum in ambient air and dust (Zereini *et al.*, 2001a); the concentrations depended on traffic density and driving speed (Gómez *et al.*, 2001).

The platinum concentration in ice from Greenland dating back 7000 years was 8 pg/kg, and snow from Greenland, Antarctica, and the Alps ranged from 8–2700 pg/kg (Barbante *et al.*, 1999). In water from the Pacific Ocean, platinum concentrations were 90–228 pg/L (Goldberg *et al.*, 1986) and in the Indian Ocean 33.2 pg/L (van der Berg and Jacinto, 1988). Similar concentrations have been reported from the coast of Australia (Adeloju *et al.*, 1990). In a single sample of tapwater in Liverpool, the platinum concentration was 60 pg/L (van der Berg and Jacinto, 1988) and in Victoria in Australia <100 pg/L (Adeloju *et al.*, 1990). In rainwater, platinum concentrations were <0.2 pg/kg in Germany (Alt *et al.*, 1997).

In sediments from the central Pacific Ocean, the platinum concentrations were on the average 8.6 µg/kg, near the Japan coast sediments were 2.3 µg/kg, and in lake sediments from Japan they were 3.2 µg/kg (Tereshima *et al.*, 2002).

Platinum usually occurs together with small quantities of iridium, osmium, palladium, ruthenium, and rhodium in alluvial deposits, where platinum concentrations of 10 µg/kg have been reported. The median content of platinum in peat bog was 5 µg/kg (Rauch *et al.*, 2004) and in garden soil, 0.15–3.9 µg/kg (Alt *et al.*, 1997; Hutchinson *et al.*, 2000).

A clear-cut increase in platinum concentration has been noticed in roadside dust and soil up to the order of 300 µg/kg (mean value), with the highest concentrations >2250 µg/kg (Ravindra *et al.*, 2004).

4.2 Working Environment

Occupational exposure to platinum occurs mainly through inhalation during mining, manufacturing, and processing different platinum compounds. The most common current occupational exposure to soluble platinum compounds is in platinum refining and catalyst manufacture (IPCS, 1991). Results of studies on occupational exposure, which are considered reliable, are presented in Table 1.

4.3 Food

In Germany in 1997, platinum concentrations were 0.31 µg/kg dry weight in peeled potatoes, ≤0.04 µg/kg in grain products, and 0.31–2.1 µg/kg in fruits and vegetables (Alt *et al.*, 1997). The total platinum intake with diet was reported to be 0.02 µg/day in the UK in 1994 (Ysart *et al.*, 1999).

TABLE 1 Platinum Exposure in Different Industries

Time of sampling	Exposure group[a]	No. and type of samples[b]	Parameter measured[c]	Result, average	Reference
	Refinery				
1977–1979	Residue	1; area	Total dust	0.5 µg/m^3	Baker *et al.*, 1990
	Recovery	9; area	Total dust	5.3 µg/m^3	
	Recovery, sampling	4; area	Total dust	2.7 µg/m^3	
	Refinery	3; area	Total dust	10.7 µg/m^3	
	Refinery, tray room	4; area	Total dust	27.1 µg/m^3	
	Analytical laboratories	3; area	Total dust	0.4 µg/m^3	
	Warehouse	8; area	Total dust	8.6 µg/m^3	
	Air conditioning unit	3; area	Total dust	0.6 µg/m^3	
1981	Refinery, recovery, warehouse area	75; area	Total dust	50–70 % ≥ 2 µg/m^3	Brooks *et al.*, 1990
1984	Platinum-processing department	2; area	Total dust	<0.2 µg/m^3 (detection limit)	Bolm-Audorff *et al.*, 1992
1986	Platinum-processing department	2; area	Total dust	0.08 µg/m^3, 0.1 µg/m^3	
	Filter press	2; personal		<0.05 µg/m^3 (detection limit)	
1986	Maintenance, incinerator	566	Total dust	27 % >2 µg/m^3	Calverley *et al.*, 1995
	security, canteen and cleaning, messengers,medical center, laboratories, boilerhouse, changehouse	45		all <2 µg/m^3	
1976–1995	PGM refinery	8573; area, personal	Total dust, soluble platinum	5.1% ≥ 2 µg/m^3 maximum 234 µg/m^3	Linnett and Hughes, 1999
1989–1991	Chemical process operators in PGM refinery	380; area, personal	Total dust; soluble platinum	2.4 % > 2 µg/m^3	
	Refining platinum	35	Respirable soluble platinum	*Long-term sampling* 0.663 µg/m^3, max 2.210 µg/m^3; *Short-term sampling* 2.53 µg/m^3, max 725.96 µg/m^3	Maynard *et al.*, 1997
	Manufacturing of platinum chemicals				
1976–1995	TPC production	511; area, personal	Total dust, soluble platinum	48.3 ≥2 µg/m^3, max 6500 µg/m^3	Linnett and Hughes,1999
1989–1991	Chemical process operators in TPC production	130; area, personal	Total dust; soluble platinum	28.5 % >2 µg/m^3	
	Catalyst production				
1992–1993		56 area 22, personal	Total dust, soluble platinum , Total dustsoluble platinum	*Low exposure area* 1992: 0.0066 µg/m^3, max 0.0086 µg/m^3 1993: 0.0004 µg/m^3, max 0.0015 µg/m^3 < 0.01 µg/m^3 for soluble salts, < 0.085 µg/m^3 for total Pt *High exposure area* 0.013–0.014 µg/m^3, max 3.7 µg/m^3 for soluble salts, insoluble salts 10-fold higher 4% >2 µg/m^3 0.12 µg/m^3	Merget *et al.*, 1999, 2000, 2002

1976–1995	Autocat	453; area, personal	Total dust, soluble platinum	28.7 % ≥2 µg/m^3, max 438 µg/m^3	Linnett and Hughes, 1999
1989–1991	Chemical process operators in Autocat	176; area, personal	Total dust; soluble platinum	8.5 % >2 µg/m^3	
	Platinum catalyst production	35	Respirable soluble platinum	*Long-term sampling* 0.216 µg/m^3, max 0.600 µg/m^3 *Short-term sampling* 0.975 µg/m^3, max 10.82 µg/m^3	Maynard *et al*., 1997
	Preparation of solutions for coating	3,2,2 area, PM10	Soluble PM10/ total/soluble platinum	0.02/0.08/0.02 µg/m^3	Petrucci *et al*., 2004, 2005
		8, 3,3 area, PM10		1.71/2.54/0.67 µg/m^3	
	Coating of supports for catalytic converters	4,2,2 area PM10		0.24/0.34/0.08 µg/m^3	
	Adsorptionon the carrier Recoveryof PGMs from exhaustedcatalytic converters	2,2,2, area, PM10		0.18/0.42/0.03 µg/m^3	
	Preparation of solutions for coating	3, personal	Total, soluble platinum	0.41 µg/m^3	Petrucci *et al*., 2005
		7, personal		2.70 µg/m^3	
	Coating of supports for catalytic converters	5, personal		0.05 µg/m^3	
	Adsorption on the carrier Recovery of PGMs from exhausted catalytic converters	2, personal		0.68 µg/m^3	
	Refinery and catalyst production				
		?, area		1.1 µg/m^3, max 3.4 µg/m^3	Schierl *et al*., 1998
		34, personal		2.5 µg/m^3, max 7.5 µg/m^3	
	Metal coating				
	Metal-coated electrodes	32	Respirable soluble platinum	*Long-term sampling* 0.017 µg/m^3, max 0.079 µg/m^3 *Short-term sampling* , <0.03 µg/m^3 max 0.99 µg/m^3	Maynard *et al*., 1997
	Metal-coated electrodes paint mixing	6	Respirable soluble platinum	*Short-term sampling* 0.36 µg/m^3,max 1.10 µg/m^3	Maynard *et al*., 1997

[a]PGM, Platinum group metals; TPC, Tetraammine platinum dichloride; Autocat, autocatalyst production.
[b]Median values taken from a logarithmic diagram.
[c]PM10, the average particle size below 10 µm.

5 KINETICS AND METABOLISM

5.1 Absorption, Distribution, and Excretion

After inhalation exposure of rats to metallic platinum, platinum oxide, platinum tetrachloride, and platinum sulfate (^{191}Pt) (aerodynamic droplet size nearly 1.0 µm), the bulk of the radioactivity was excreted in the feces within 2–3 days. Thereafter, there was a slow excretion to both feces and urine. The slow phase was fast for $Pt(SO_4)_2$, the pulmonary retention being 4.4% after 16 days, but slow for metallic Pt and PtO (17.9 and 28.0% retention) (Moore *et al*., 1975a). After intratracheal instillation of metallic platinum on aluminium oxide particles (a model aerosol for automobile exhaust, Al_2O_3 particles ≤5 µm coated with ≥ 4 nm platinum particles) (Artelt *et al*., 1998), approximately 97% of the dose was excreted in the feces within the first 2 days. After day 5, the urinary excretion accounted for approximately 5% of the total daily excretion.

Three days after an oral dose of ^{191}platinum(IV) chloride to rats, the whole body retention of radioactivity was <1% of the dose both in adult and suckling rats, indicating a very limited oral absorption (Moore *et al*., 1975b). A similar low absorption was observed after an oral dose of $Pt(SO_4)_2$ at dose levels corresponding to LD_5 and LD_{25} (Lown *et al*., 1980). No platinum could be detected in any organ after dietary administration of PtO_2 to rats, indicating that the absorption was <3% of that of $PtCl_4$ (Holbrook, 1977). After oral exposure to the model particulates for automotive exhaust gases (see preceding) containing metallic platinum, 0.11% of the platinum administered was absorbed (Artelt *et al*., 1999).

After intravenous administration of $PtCl_4$ or of metallic Pt by inhalation to rats, highest concentrations were observed in the kidney (Moore *et al*., 1975b,c). Similarly, after intratracheal instillation, inhalation, or oral administration of metallic platinum on aluminium oxide particles to rats (Artelt *et al*., 1998, 1999) and dietary administration of $Pt(SO_4)_2$ to mice (Lown *et al*., 1980), the highest concentrations were observed in the kidney. Concentrations similar to or higher than those in the blood were also observed in the adrenal glands, spleen, liver, and bone (Artelt *et al*., 1998; 1999; Lown *et al*., 1980; Moore *et al*., 1975b,c).

In plasma, 90% of platinum is bound to proteins (Artelt *et al*., 1999).

After intravenous administration of $K_2[PtCl_4]$ to rats, approximately 50% of the dose was excreted in the urine, 41% in the feces (Artelt *et al*., 1999).

In two human volunteers, exposed for 4 hours by inhalation to $[(NH_4)_2PtCl_4]$, the urinary excretion of platinum increased steeply (15–1000-fold compared with initial values) in the first urine samples after the exposure. Platinum excretion followed a two-phase exponential decay with a first $t_{1/2}$ of 50 hours (95% confidence interval [CI] 36–66 hours). The second half-time was 24 days (95% CI, 18–33 days). Still 167 days after the exposure, the urinary platinum concentration was above the concentration before the exposure, but within values observed for unexposed people (Schierl *et al*., 1998).

The absorption and elimination of the diamine complexes of platinum, such as cisplatin and its analogs, is quite different from those of inorganic platinum salts (Beauchamp Hewitt, 1997). The excretion of antineoplastic platinum drugs follows a biphasic pattern. The half-time ($t_{1/2}$ final) of ultrafiltrable platinum in plasma (131 ± 15 min) was much faster than that of total platinum (6.8 ± 4.3 days) after exposure to cisplatin and lobaplatin (Nygren and Lundgren, 1997; Welink *et al*., 1999). The rapid initial phase accounts for early high levels of platinum in the kidney and the prolonged second phase results in detectable urine platinum concentrations 30 days after a single dose (IPCS, 1991). After four cisplatin treatments, the mean half-time in serum was 7.5 days (Gamelin *et al*., 1995).

5.2 Reference Values in Tissues and Biological Fluids

As discussed previously, older studies tend to report high concentrations for platinum in biological fluids. In the following, only studies considered analytically reliable and comprising a reasonable number of study subjects have been reported.

Dental gold alloys were found to increase urinary and saliva platinum concentrations (mean age, 35.9 years, 326 urine samples, median 9.3 ng/g creatinine with dental gold inlays and 5.3 ng/g creatinine without dental inlays, $P < 0.001$ and 5–2770 pg/g saliva, median 160 pg/g and 4.8–26 pg/g saliva, median 8.1 pg/g saliva, $P < 0.001$) (Schierl, 2001). Also the German Environmental Survey (GerES III) from 1998 (Becker *et al*., 2003) found a positive relation between the platinum levels in urine and teeth with dental inlays, crowns, and bridge elements, whereas road traffic was related to urinary platinum concentrations. The geometric mean value for all occupationally unexposed persons (age, 18–69 years, $n = 1080$) was 2.18 ng/L (1–75 ng/g creatinine), and the 95% CI from the log-normal distribution was 2.01–2.36 ng/L; (1.60–1.90 ng/g creatinine). Those with no dental inlays ($n = 509$) had a geometric mean platinum concentration in urine 1.32 ng/L (0.99 ng/g creatinine) with 95% CI interval of 1.1–1.47 ng/L (0.89–1.11 ng/g creatinine).

In a population study (NHANES) in the United States, the 95th percentile of the urinary platinum concentrations was below the detection limit of the method, 40 ng/L ($n = 2465$, age, 6 years and older) (CDC, 2005).

In Austria, values <1 ng Pt/L urine are reported for unexposed people (number of subjects not given) (Adeloju *et al*., 1990). In the United Kingdom, office workers ($n = 5$) had 129 ng Pt/L in the blood and 113 ng Pt/g creatinine in the urine (Farago *et al*., 1998). Iavicoli *et al*. (2004a) reported a mean urinary platinum concentration 4.56 ng/L in 58 people from Italy. They found a statistical difference between female and males (4.09 *vs* 5.77 ng/L, $P = 0.004$). The subjects older than 40 years of age ($n = 28$) showed statistically significantly higher mean platinum concentrations in urine than those of younger colleagues ($n = 21$, $P = 0.03$). In Budapest and Vienna, platinum concentrations in the urine of adult inhabitants without occupational exposure were on average 10.1 ± 8.8 and 3.7 ± 4.3 ng/g creatinine ($n = 100$), respectively (Záray *et al*., 2004). In the urine of urban children in Italy, the platinum concentrations were 0.9 ± 1.1 ng/g creatinine, and they were not associated with traffic density in the area of residence (Caroli *et al*., 2001).

The serum platinum concentration in Germany in nonexposed person was 6.35 ng/L (median; range, <5.0–42.1 ng/L, number of subjects not given) (Merget *et al*., 1999). In Italy, the blood platinum level was 10 ± 2 ng/L, in urine 5 ± 0.1 ng/L in urine, and 50 ± 8 ng/k hair ($n = 25$) (Petrucci *et al*., 2005).

The liver platinum concentrations were 0.05–0.24 ng/g (Zeisler and Greenberg, 1982). The platinum concentration in human breast tissue was <0.05 ng/g (wet weight), but higher levels ranging from 25–90 ng/g were detected in fibrin layer and fat tissue of two patients with breast prostheses (Flassbeck *et al*., 2003).

5.3 Biological Monitoring

No validated methods are available for effect monitoring of platinum compounds. However, a significant correlation was observed between the number of neutrophils or epithelial cells and platinum concentration in the nasal lavage fluid of children (Schins *et al*., 2004).

Environmental exposure to platinum cannot be reliably assessed by biomonitoring, because leaching from dental devices is a much more important source of platinum than environmental exposure (Becker *et al*., 2003). For the biological monitoring of exposure to platinum in occupational settings, blood, serum, and urine specimens have been used. Information from studies in which the analytical methods used are reliable is given in Table 2. Although generally the concentrations of platinum seem to be higher when the exposure is high (Petrucci *et al*., 2005), no quantitative correlation between air platinum concentration and platinum concentration in biological fluids has been reported; thus, quantitative estimates of occupational exposure cannot be derived from biological monitoring—which thus is limited to the identification of populations exposed at work. In a small prospective study, concentrations of platinum in the plasma were not predictive of the development of platinum sensitivity (Merget *et al*., 2002).

The best separation of different exposure groups was achieved from the analysis of the urine of the workers at a catalyst production factory (Petrucci *et al*., 2005); thus, urine platinum measurements are likely to provide the best starting point for the development of validated biomonitoring of platinum and platinum compounds.

On the other hand, elevated serum platinum concentrations have been reported among patients treated with cisplatin (where the exposures are much higher): 20 years after the treatment, elevated serum platinum concentrations were still observed in treated patients (treated, $n = 61$ 64.9 ± 24.5 ng Pt/kg, controls, $n = 18$, <6 ng Pt /kg plasma) (Gietema *et al*., 2000). Large differences in urinary platinum concentrations have been reported among nurses in oncology departments. This range can be explained by differences in handling practices, protection, and use of safety hoods or alternate closed systems both during the preparation and administration of cytostatics.

6 EFFECTS IN ANIMALS AND HUMANS AND DOSE-RESPONSE RELATIONSHIPS

Platinum is not an essential element for microbes, plants, animals, or humans. Platinum readily complexes *in vitro* with O-, N-, and S-containing ligands such as ethers and thiocarbamide. Therefore, platinum ions would not be expected to occur free in living organisms.

6.1 Acute Toxicity

Metallic platinum has low acute toxicity. Fine metallic platinum dust orally administrated to rats caused slight necrotic changes in the gastrointestinal epithelium, granular dystrophy of hepatocytes, and swelling in the epithelium of the convoluted renal tubules, with no lethal effect (IPCS, 1991). After intrauterine application, platinum wire or foil only caused local effects, considered to be due to the physical presence of a foreign object in the uterus (Barlow and Sullivan, 1982).

TABLE 2 Platinum Concentrations in Blood/Urine in Different Industries

Time of sampling	Exposure group	No. and type of samples	Result, average	Result maximum or range	Reference
	Catalyst production				
1990–1995	Catalyst workers	5, serum		30–290 ng/L[a]	Merget *et al*., 2002
1994	Catalyst workers	?, serum	13.6 ng/L (median)	<5.0–263 ng/L	Merget *et al*., 1999
1994	Catalyst workers, high exposure[b, d]	10, serum	80 ng/L[a]	<500 ng/L[a]	Merget *et al*., 2000
	Catalyst workers, low exposure[b, d]	30, serum	20 ng/L[a]	<500 ng/L[a]	
	Preparation of solutions for coating	14, blood	70 ± 40 ng/L		Petrucci *et al*., 2005
		8, blood	140 ± 80 ng/L		
	Coating of supports for catalytic converters	26, blood	380 ± 310 ng/L		
	Adsorption on the carrier	14, blood	170 ± 170 ng/L		
	Sampling	14, blood	140 ± 110 ng/L		
	Recovery of PGEs from exhausted catalytic converters	8, blood	240 ± 170 ng/L		
	Preparation of solutions for	3, blood, serum	B: 110 ng/L, S: 80 ng/L		Petrucci *et al*., 2004
		3, urine	405 ng/L		
	Coating of supports for catalytic converters	3, blood, serum	B: 2900 ng/L, S: 1550 ng/L,		
		3, urine	2065 ng/L		
	Adsorption on the carrier	3, blood, serum	B: 110 ng/L S: 40 ng/L		
		3, urine	145 ng/L		
	Recovery of PGEs from exhausted catalytic converters	3, blood, serum	B: 210 ng/L, S: 150 ng/L		
		3, urine	130 ng/L		
	Platinum refinery and catalyst production[b]		1–12 ng/g creat		Schierl *et al*.,1998
	Current high exposure	15, urine	170–6270 ng/g creat.		
	Former high exposure (2–6 years after exposure)	4, urine	10–170 ng/ g creat.		
	Occasionally exposed to low levels	3, urine	16–230 ng/g creat.		
	Precious metal workers	7, blood	246 ± 91 ng/L	152–423 ng/L	Farago *et al*., 1998
		7, urine	470 ± 355 ng/ g creat.	210–1180 ng/ g creat.	
	Traffic workers				
	Motorway maintenance workers	10, blood			
		10, urine	145 ± 90 ng/L	126–158 ng/L	
			58 ± 37 ng/g creat	22–135 ng/g creat	
	Traffic police officers	94, urine	4.56 ± 2.84 ng/L	0.28–13.67 ng/L	Iavicoli *et al*., 2004a
	Dental workers				
	Dental health care workers	1, serum	2120 ng/L		Iavicoli *et al*.,2004b
		19, serum	<5 ng/L		
		20, urine	<10 ng/L		
	Dental technicians	27, urine	~30 ng/L	max 170 ng/L	Begerow *et al*., 1999
	Hospital workers				
	Nursing stuff, pharmacists	6, blood	470 ± 310 ng/L		Nygren and Lundgren, 1997
	graduate nurses staff nurses	13, blood	2200 ± 1700 ng/L		
		12, blood	3800 ± 4000 ng/L	max 13300 ng/L	
		31, urine	126 ± 92 ng/L		
	Nurses	3, urine		920–1300 ng/L	Turci *et al*., 2002
	Nurses and pharmacists	10, urine	10220 ± 7220 ng/L	600–23100 ng/L	Venitt *et al*., 1984
	Nurses	39, blood		<5 ng/L	Pilger *et al*., 2000
	Hospital personal	14, urine	12.6 ± 10.8 ng/L		Ensslin *et al*., 1994
		39, urine	(16.6 ± 15.5 ng/ g creat.) <1.8 ng/L		
	Nurses	13, urine	4.36 ± 5.61 ng/g creat		Ensslin *et al*., 1997

[a]Median and max values taken from a logarithmic diagram.
[b]See table 1.

The toxicity of orally dosed platinum compounds in rats decreased in the order:

$$PtCl_4 > Pt(SO_4)_2\ 4\ H_2O > PtCl_2 > PtO_2$$

Thus, water-soluble platinum compounds are more toxic than the insoluble (Holbrook *et al*., 1975).

Platinum coordination complexes with chloride ligands, administered orally, are more toxic than platinum salts (IPCS, 1991). After high doses of hexachloroplatinic acid, the kidney is the target organ, the cause of death being renal failure (Ward *et al*., 1976). Nephrotoxicity is the major side effect of cisplatin therapy (Bokemeyer *et al*., 1996; Ferrari *et al*., 2005; Markman 2003, Taguchi *et al*., 2005). Also, severe neurotoxicity has been observed when cisplatinum is used in chemotherapy (Sastry and Kellie, 2005).

Platinum coordination complexes, including $(NH_4)_2[PtCl_4]$, $Na_2[PtCl_6]$, but also $Na_2[Pt(OH)_6]$ $[PtNH_3]_4Cl_2$ and $[Pt(NO_2)_2(NH_3)_2]$, are strongly irritant or corrosive when applied to the eye and irritant or strong irritants to the skin; platinum(IV) chloride is an irritant to the skin, whereas platinum(II) chloride and platinum oxide are nonirritants (IPCS, 1991).

6.2 Sensitization

Hexa- and tetrachloroplatinates and cisplatin, but not $[Pt(NH_3)_4]Cl_2$, induced increased weight and cellularity in the popliteal lymph node after a single subcutaneous injection in C57 mice (Schuppe *et al*., 1992; 1997). An enhanced reaction was observed after preceding treatment with $[PtCl_6]^{2-}$, indicating a specific sensitization reaction. The reaction was considered to require T cells, because nude mice lacking T cells did not show the same reaction. Differences in the reaction between inbred mouse strains indicate genetic susceptibility to platinum sensitivity. Sodium hexachloroplatinate also produced a positive response in an auricular lymph node assay and mouse ear swelling test for contact hypersensitivity. However, the concentrations needed for positive reactions were high enough to also cause an irritant reaction (Schuppe *et al*., 1997). In an abstract, it was reported that PtO_2 and $PtCl_2$ did not elicit an immune response in the mouse popliteal lymph node assay (Schuppe *et al*., 1993).

Exposure to platinum causes a disease, which was originally called platinosis (Roberts, 1951), but which is now most often known as platinum salt sensitivity (IPCS, 1991). The symptoms include watering of the eyes, sneezing, tightness of the chest, wheezing, breathlessness, coughing, eczematous and urticarial skin lesions, and signs of mucous membrane inflammation. The respiratory symptoms subside usually approximately 1 hour after the cessation of exposure. The latency period from the first exposure to the first symptoms has usually varied between 3 months and 3 years but may, in some cases, be only a few weeks or several years. Patients show a positive reaction to challenge to platinum compounds in skin prick tests and a positive bronchial challenge test to platinum hexachloroplatinic acid (Calverley *et al*., 1999; IPCS, 1991; Merget *et al*., 1994; Pepys *et al*., 1972). Platinum-exposed workers showed an increased prevalence of bronchial reaction to cold air (Baker *et al*., 1990; Brooks *et al*., 1990), but bronchial hyperresponsiveness to methacholine was not consistently related to platinum sensitivity (Merget *et al*., 1991; 1994; 1996; 1999; 2000). In a follow-up study, it was shown that if the exposure of workers positive on prick testing continued unabated, 100% of the workers became symptomatic (Calverley *et al*., 1995). Of patients with a positive prick test, 2 of 23 also showed a positive reaction in patch test to $H_2[PtCl_6]$ (Cristaudo *et al*., 2005). The eczema described in the early studies (Roberts, 1951) apparently is not related to exposure to platinum but is rather caused by irritation by strong acids and alkalis used in the process. In contrast, urticaria may be related to platinum, notably to splashes of chloroplatinate solutions (Hughes, 1980).

In early studies (Roberts, 1951), the prevalence of "platinosis" was 60–100% of the exposed, indicating that platinum is a very potent sensitizer. In a more recent study (Niezborala and Garnier, 1996), >30% of the workforce developed platinum sensitivity within 3 years. In the South African registry of occupational respiratory diseases (Hnizdo *et al*., 2001), platinum was the third most frequent cause of occupational asthma (after latex and isocyanates) and represented 12% of all asthma cases. The risk of developing platinum salt sensitivity increases with increasing exposure (Baker *et al*., 1990; Bolm-Audorff *et al*., 1992; Calverley *et al*., 1995; 1999; Cristaudo *et al*., 2005; Merget *et al*., 1988; 1999; 2000), as well as with time of exposure (Cristaudo *et al*., 2005; Linnett and Hughes, 1999), and removal from exposure or decrease of exposure was linked to a decrease in the prevalence of respiratory symptoms (Merget *et al*., 1999; 2001).

Platinum dust has generally been considered to be biologically inert and nonallergenic. No adverse health effects have been reported from the exposure solely to platinum dust (Czerchak and Gromiec, 2001; Gómez *et al*., 2002). Surveillance of newly employed workers in a platinum company indicated that the proportion of sensitized workers was high among those exposed to chloroplatinates but nonexistent among those exposed to tetraamming platinum dichloride (i.e., a platinum coordination complex, in which there are no chloride ligands) (Linnett and Hughes, 1999). Among workers sensitized to platinum, the potency of different platinum complexes to induce positive skin prick tests decreased very constantly in the order:

$$(NH_4)_2[PtCl_6] \approx (NH_4)_2[PtCl_4] > Cs_2[PtNO_2Cl_3] > Cs_2[Pt(NO_2)_2Cl_2] > Cs_2[Pt(NO_2)_3Cl] > K_2[Pt(NO_2)_4]$$

the last-mentioned being inactive. Other complexes with strongly bound ligands, such as $[Pt(NH_3)_4]Cl_2$ and $[Pt(thiourea)_4]Cl_2$ were also inactive. Complexes containing amine (and chlorine) showed the same general pattern, with the important difference that the dichlorocomplexes (*cis*- and *trans*-$[Pt(NH_3)_2Cl_2]$), which are neutral, were completely inactive. When the chlorine ligand was replaced by bromine, the activity was decreased, but not abolished (Cleare *et al*., 1976). Platinum salt sensitivity has not been described among people treated with platinum chemotherapeutic drugs, but sensitivity reactions, including anaphylactic shock, are a well-known potential side effect of cisplatin and other platinum-containing chemotherapeutic agents (Basu *et al*., 2002; Lim *et al*., 2004; Shepherd, 2003; Sliesoraitis and Chikhale, 2005; Thomas *et al*., 2003; Watanabe *et al*., 2005; Windom *et al*., 1992; Zorzou *et al*., 2005; Zweizig *et al*., 1994).

The mechanism of platinum salt sensitivity is likely to be type 1, immunoglobulin IgE–mediated response. It is believed that platinum salts acting as haptens combine with serum proteins, probably through sulfhydryl and methionine groups, to form the complete antigen (Cromwell *et al*., 1979; Merget *et al*., 1994; Schultze-Werninghaus *et al*., 1978). Peripheral blood monocytes from symptomatic workers, positive in prick testing, showed an increased frequency of CD3 positive lymphocytes for Vα2a, Vß11, and Vß21.3-positive T cells and showed elevated proliferation responses to sodium hexachloroplatinate *in vitro* (Raulf-Heimsoth *et al*., 2000).

An increased level of total IgE has been demonstrated in the serum of patients diagnosed with platinum salt sensitivity (Baker *et al*., 1990; Brooks *et al*., 1990; Bolm-Audorff *et al*., 1992; Calverley *et al*., 1999; Cromwell *et al*., 1979; Merget *et al*., 1988; 1994; Murdoch *et al*., 1986). However, the increases in total IgE have generally been rather small, and there is a marked overlap with the values in nonsensitized referents (Baker *et al*., 1990; Brooks *et al*., 1990; Calverley *et al*., 1999; Cromwell *et al*., 1979; Merget *et al*., 1988; 1994; Murdoch *et al*., 1986). In some studies, workers with work-related symptoms of platinum sensitivity have had elevated concentrations of platinum-specific IgE in their blood (Bolm-Audorff *et al*., 1992; Brooks *et al*., 1990; Cromwell *et al*., 1979; Merget *et al*., 1988; 1999; Murdoch *et al*., 1986), but not all studies have reported this association (Calverley *et al*., 1999), and in some studies, there is an extensive overlap between the workers with positive and negative skin tests to platinum (Cromwell *et al*., 1979; Merget *et al*., 1988; Murdoch *et al*., 1986)..

Tobacco smoking is an important contributing factor in the generation of allergy to platinum salts (Baker *et al*., 1990; Calverley *et al*., 1995; Linnett and Hughes, 1999; Merget *et al*., 2000; Newman Taylor *et al*., 1999; Niezborala and Garnier, 1996; Venables *et al*., 1989), although this relationship was only observed in some subgroups of the exposed workers in a recent study (Cristaudo *et al*., 2005). Relative risks as high as 8.0 (CI, 2.6–25) for smokers to develop platinum sensitivity at work have been reported (Calverley *et al*., 1995). In a study, which was based on small numbers, it seemed that smoking is especially important as a predictor of platinum sensitization when the exposure is low: in a case-referent study (Newman Taylor *et al*., 1999) among workers exposed to ammonium hexachloroplatinate (usually at concentrations less than $2\,\mu g/m^3$), the odds ratio for platinum sensitization was 9.1 for the group in whom the exposure was estimated to be "low," and 3.1 in the "high" exposure group.

Although an early study reported an association between atopy and platinum sensitization (Hughes, 1980), and in two studies a statistically nonsignificant excess relative risk was reported for atopic persons to develop platinum sensitivity (Cristaudo *et al*., 2005; Venables *et al*., 1989), the weight of evidence seems to be that atopic constitution is not related to the predisposition to platinum sensitivity (Baker *et al*., 1990; Bolm-Audorff *et al*., 1992; Calverley *et al*., 1999; Cristaudo *et al*., 2005; Merget *et al*., 2000; Niezborala and Garnier, 1996).

HLA-type DR3 was more common and DR6 less common among workers sensitized to platinum than among matched nonsensitized referents (Newman Taylor *et al*., 1999). The difference was more marked in the group whose exposure was considered low than among the highly exposed. Because, in addition, the prevalence of sensitization in the old studies—reflecting high exposures—were very high, up to 100%, it is likely that at high exposure, sensitization will take place independent of the HLA phenotype. The odds ratio for the presence of DR3 was rather low (1.6 for the high exposure and 2.3 for the low exposure group), and thus the attributable fraction for HLA DR3 is low. In contrast, the odds ratio for the presence of DR6 was 0.6 for high exposure and 0.1 for low exposure—denoting a high attributable fraction at low exposures. It should be noted, however, that the point estimates have very large confidence intervals (0.02–1.1 for HLA DR6, low exposure).

There is at present no evidence of adverse health effects from platinum in the general environment. In a series of 749 patients with eczema and 51 with urticaria

from the Rome area (exposed to environmental platinum), no positive patch or prick test toward platinum was observed (Santucci *et al*., 2000).

6.3 Carcinogenicity, Mutagenicity, and Reproductive Effects

There are no data available on the carcinogenicity of metallic platinum or its halogenated complexes in humans or experimental animals, except for cisplatin. IARC has concluded, on the basis of *sufficient evidence* of carcinogenicity in experimental animals and *inadequate evidence* in humans that *cisplatin* is *probably carcinogenic to humans* (Group 2A) (IARC, 1981; 1987) and further that etoposide, in combination with cisplatin and bleomycin, is *carcinogenic to humans* (Group 1) (IARC, 2000).

Cisplatin was mutagenic in a variety of different test systems (IARC, 1981; 1987; IPCS, 1991; Lantzsch and Gebel, 1997; Nersesyan *et al*., 2003; Overbeck *et al*., 1996; Silva *et al*., 2005; Uno and Morita, 1993) and similarly, other platinum cytostatic drugs have been shown to be genotoxic (IPCS, 1991; Lantzsch and Gebel, 1997; Nersesyan *et al*., 2003; Overbeck *et al*., 1996; Silva *et al*., 2005).

Also platinum complexes, not sharing the planar structure, have been shown to induce mutations in *S. typhimurium*, *E. coli*, *B. subtilis*, or in L 5178 mouse lymphoma cells, or chromosomal damage in CHO cells, V79 cells, or human lymphocytes *in vitro* (Bunger *et al*., 1996; Gebel *et al*., 1997; IPCS, 1991; Kanematsu *et al*., 1990; Lantzsch and Gebel, 1997). No genotoxicity has been reported in *in vivo* studies (IPCS, 1991).

In HGPRT assay in CHO cells, the approximate relative mutagenic activity of *cis*-$[Pt(NH_3)_2Cl_2]$, $K[Pt(NH_3)Cl_3]$ and $[Pt(NH_3)_3]Cl]Cl$ was 100:9:0.3; whereas the mutagenicity of $K_2[PtCl_4]$ and *trans*-$[Pt(NH_3)_2Cl_2]$ was close to the spontaneous mutation frequency (but increased in a concentration-dependent fashion). No mutagenic activity was observed for $[Pt(NH_3)_4Cl_2$ (IPCS, 1991).

Cisplatin binds to metallothionein, and trapping to MT may contribute to cisplatin resistance (Hagrman *et al*., 2003). Transfection of the gene encoding for metallothionein increases cisplatin resistance in an osteosarcoma cell system (Kasahara *et al*., 1991; Vandier *et al*., 2000)

Platinum crosses the rat placenta, but only in small amounts: after a single oral dose of ^{191}platinum in hydrogen chloride to pregnant rats on the 18th day of gestation, the radioactivity in the fetal liver was 0.77% of that in the liver of the dam. Most of the platinum was retained by the placenta and did not reach the fetus: the concentration in the placenta was 65 times the average concentration in the fetus (Moore *et al*., 1975b). No information is available on the reproductive toxicity of platinum compounds other than cisplatin; doses on the order of LD_{50} of cisplatin (13 mg/kg) caused a high mortality of the pups, but survivors did not show teratogenicity (IARC, 1981).

After an intravenous dose of ^{191}platinum chloride to rats, the radioactivity in the testis was 18–33% of that in the blood 1–14 days after the administration (Moore *et al*., 1975b). Intraperitoneal administration of platinum chloride (9–18 mg/kg, approximately ¼–½ of the LD_{50}) to male rats resulted in a decrease in testicular DNA synthesis; this effect, however, was more pronounced in the spleen, liver, and kidneys (Fisher *et al*., 1975).

Hydrogen hexachloroplatinate at concentrations up to 1000 µmol/L did not affect sperm motility, membrane integrity, or viability after 3 hours (Kohn *et al*., 1995). Sperm mobility was markedly reduced when spermatozoa were treated with 1000 µmol/L of sodium hexachloroplatinate or tetraamineplatinium(II) chloride solutions. Acrosome reaction was observed at concentrations affecting sperm motility or lower (Eberl *et al*., 2000; Kohn *et al*., 1995). Metallic platinum did not inhibit the motility or oxygen consumption in human spermatozoa (Holland and White, 1980).

7 RISK ASSESSMENT

The main health effect of platinum compounds is sensitization. Allergenic potency of platinum compounds seems to be limited to coordination complexes containing halogen ligands as leaving groups, hexa- and tetrachloroplatinates being the most potent sensitizers. Neutral coordination complexes such as diamminedichloroplatinum do not seem to be allergenic. The incidence of platinum sensitivity has been very high among exposed worker groups but has been shown to decrease with decreasing exposure. Smokers have a higher risk for platinum sensitization.

No adverse effects have been reported from environmental exposure to platinum, but concentrations of platinum show a constant increase, mainly from the use of platinum as automotive catalyzators.

Uptake from platinum-containing noble metal dental alloy restorations is an important source of platinum; health effects from this exposure have not been reported.

At pharmacological doses, cisplatin causes nephrotoxicity. Cisplatin is mutagenic in several test systems and is considered probably carcinogenic to humans. Although clearly the major population at risk for the carcinogenicity is the treated patients, oncological nurses may also be exposed to cisplatin unless appropriate work practices are adhered to.

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CHAPTER

38

Selenium

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ABSTRACT

Selenium is an essential trace element and has been shown to be a natural component in the enzymes glutathione peroxidases (GSH-px 1-5), iodothyrodine deiodinases, thioredoxin reductases, selenoprotein P, and other proteins, altogether 25 proteins. Most selenium inorganic and low molecular compounds are water soluble and can efficiently be taken up in the intestine. Soluble, as well as nonsoluble, compounds can be taken up by the lungs. Selenium occurs as cysteine in selenoproteins, whereas selenomethionine can unspecifically substitute for methionine in proteins. The selenium requirement to compensate minimal losses in man is 50–70 μg/day.

When given in excess, selenium compounds are rapidly distributed to major organs of the body. In the liver, many selenium compounds are biotransformed to excretable metabolites. Identified metabolites include selenosugars in urine and dimethylselenide in breath. Biotransformation seems to be a major mechanism by which selenium homeostasis is maintained during excessive exposure, and there is a rapid phase and a slow phase of elimination. The half-life of the rapid phase is 1–3 days, depending on the compound ingested, and for the slow phase it is 30–110 days.

Blood levels are approximately 0.027 μg/mL in geographical areas with low selenium intake, and an intake of about 90 μg/day would correspond to approximately 0.11 μg/mL. Toxic effects have been seen at blood levels ranging from 0.179–7.5 μg/mL. In most parts of the world, normal urine levels are 0.03 mg/L. Occupationally exposed workers usually excrete < 0.1 μg/mL.

The LD_{50} ranges between 1.5 and 6 mg/kg body weight (bw) for many selenium compounds and animal species. The central nervous system (CNS) seems to be the target organ at these dose levels, but the liver, heart, and lungs may also be affected. Cases of acute selenium poisoning in humans have been described, a few with fatal outcomes. These cases occurred either after consumption of selenium or after exposure through inhalation. Gastrointestinal and neurological symptoms predominated.

Chronic poisoning caused by long-term exposure has been reported in livestock and humans from geographical areas where soil contains high levels of selenium. In rodents, liver cirrhosis is a common effect, whereas typical effects in domestic animals are emaciation, deformation of hooves, loss of hair, and joint erosions. In humans consuming 1–5 mg Se/day, hair and nail problems have been reported and are very common at 5 mg Se/day. An intake of approximately 1200 μg/day is the LOAEL for clinical selenosis, and 850 μg/day can be taken as a NOAEL for clinical selenosis. However, a clinically insignificant rise of serum ALAT has been reported at slightly lower doses. Other adverse effects among few individuals in larger study cohorts have also been reported at lower doses. Skin lesions and depigmentation are also common signs of intoxication. In more severe cases, neurological and gastrointestinal symptoms predominate. Death, albeit rarely, has been associated with chronic selenium poisoning.

Although several selenium compounds have been tested for possible carcinogenic potential, only selenium sulfide in large oral doses has shown convincing carcinogenic effect. On the contrary, some of these compounds have been shown to prevent the development of cancer.

Some selenium compounds can cause DNA damage, probably by inducing oxidative stress. Teratogenicity has been observed particularly in avian species and fish. Selenite on hamsters and mice and selenomethionine in macaques were essentially negative.

Selenium may prevent or alleviate toxic effects of arsenic, cadmium, mercury, platinum, and silver. Conversely, some of these metals protect against selenium toxicity.

1 PHYSICAL AND CHEMICAL PROPERTIES

Selenium belongs to subgroup VIa of the periodic system and has both metallic and nonmetallic properties. Subgroup VIa also contains oxygen, sulfur, and tellurium, and the similarities between the two latter elements and selenium are often pointed out. The atomic weight is 78.96. Elemental selenium is insoluble in water and has several allotropes; it may be grey or "metallic," a red amorphous powder, or it may have a vitreous form. The boiling point is 685°C and on boiling in air, selenium dioxide is formed. Selenium dioxide (the formal oxidation state +4) is a crystalline-white powder at room temperature. In contact with water it forms selenous acid (H_2SeO_3), which is a strong acid. Selenium trioxide (oxidation state +6) is a yellowish white powder, which forms selenic acid (H_2SeO_4) in water. The salts of these acids, selenites and selenates, respectively, are usually soluble in water.

Another oxide of commercial and toxicological interest is selenium oxychloride ($SeOCl_2$). The reduced form of selenium (selenide) may be formed from water-soluble selenium compounds under acid conditions or in biological systems. The formal oxidation state is –2, and of biological interest are hydrogen selenide, dimethylselenide and trimethylselenonium ions, and selenosugars. The reduced form may also be found in the place of sulfur in amino acids (e.g., selenomethionine and selenocysteine). A large number of low molecular selenium-containing compounds are present in plants and other organisms used for food. Selenide may form heavy metal complexes, which are practically water insoluble.

2 METHODS AND PROBLEMS OF ANALYSIS

The low concentrations of selenium (ng/g) usually occurring in biological and environmental samples require sensitive and accurate analytical techniques. Care should also be taken to avoid sources of systematic error including loss of volatile selenium compounds in all the steps from sampling to the final measurement (Varo and Koivistoinen, 1981). Apart from instrumental neutron activation and X-ray fluorescence analysis, most analytical methods are based on acid digestion/separation before determination of the total selenium content. A variety of analytical techniques have been used, such as spectrophotometry, atomic absorption spectrometry (AAS), atomic emission spectrometry (AES), atomic fluorescence spectrometry, mass spectrometry (MS), inductively coupled plasma (ICP)/AES, ICP/MS, gas chromatography (GC), and activation analysis using neutrons or protons (ATSDR, 2003; Bem, 1981; Thomassen, 1994). The spectrofluorometric methods are based on extraction of Se(IV) with 2,3-diaminonaphthalene from the digested sample. Chelated selenium can be measured by GC/MS. Neutron activation is based on the direct measurement of 77mSe (half-time, 17.5 seconds) or 75Se (half-time, 120.4 days). The detection limit is below the nanogram per gram range (Raptis, 1983). Isotope dilution mass spectrometry is a highly accurate method for determination of the selenium.

AAS and ICP-MS are the most widely used analytical methods for biological samples (ATSDR, 2003; Thomassen, 1994; Verlinden, 1981). AAS with conventional flame atomization does not have adequate sensitivity for biological samples. The hydride technique, which entails generating SeH_2 from the digested sample, requires large samples (Raptis, 1983). Graphite furnace (GF)-AAS is a highly sensitive method, but the volatility of selenium requires stabilization during ashing to avoid loss of selenium. Furthermore, chemical and spectral interferences from biological matrices (e.g., Fe, PO_2^{-4}) are serious problems. Whereas a number of metal reagents prevent volatilization of inorganic selenium, only nickel, palladium, and silver work in thermal stabilization of biological forms of selenium (Alexander and Aaseth, 1980; Johannesson *et al*., 1993; Thomassen, Lewis *et al*., 1994). The spectral interferences are eliminated by use of Zeeman-based background correction. Determination of selenium by ICP-MS suffers from spectral interference with Ar_2 for example. This can be solved by use of reaction collision cell or high-resolution MS. ICP-HRMS is a highly sensitive method.

During the past decade, great advances have been made in the speciation of selenium compounds in biological matrices such as serum and urine, plants, and food (Block *et al*., 2004; Francesconi and Pannier, 2004; Kotrebai *et al*., 2000; Rosenberg, 2003; Uden, 2002; 2004; Wrobel *et al*., 2003). The most used methods are various hyphenated methods based on a coupling of an electrophoretic or chromatographic separation with an atomic spectrometric or selenium-specific measurement. It can be off-line or on-line

(e.g., HPLC coupled with ICP-MS has been the most used, but recently HPLC-electrospray injection [ESI]-MS or MS-MS was introduced and also time-of-flight MS detection) (Rosenberg, 2003). This has helped in addition to the use of NMR to structurally determine low molecular selenium compound in urine, yeast, garlic, marine algae, soybean, and fish. Also selenium-containing proteins have been analyzed.

3 PRODUCTION AND USES

3.1 Production

Selenium is a by-product of sulfide ores, and the chief world suppliers are copper refining industries. The estimated world production in 2002 was approximately 1480 tons (Porter, 2004; *http://minerals.usgs.gov/minerals/pubs/of01-006/selenium.pdf*). Commercially produced compounds include selenium dioxide, sodium selenite, sodium selenate, and selenium oxychloride. Complex organic compounds are also produced for use in medicine and research.

3.2 Uses

Selenium is used in several industrial processes. The greatest amount is used in low-voltage rectifiers. Selenium is also used in the glass industry as a decolorizing agent and in the rubber industry as a vulcanizing agent. Other known uses are in insecticides, in photoelectric cells and other electrical equipment, in toning baths used in photography, and in xerography. Selenium oxychloride has been used as a solvent, and selenium hexafluoride ($SeFe_6$) is used for electrical insulation. Medical uses include selenium sulfide in shampoos as an antidandruff agent. Previously, ^{75}Se-selenomethionine was used as a scanning agent for pancreas in roentgen diagnostics. In some countries, selenium (as selenate or selenite) is added to the food chain foliar spraying or as additive to artificial fertilizers. The only country to add sodium selenate to all creak and forge fertilizer is Finland. The practice started in 1985 and is continuing (Aro *et al*., 1995).

4 ENVIRONMENTAL LEVELS AND EXPOSURE

4.1 General Environment

4.1.1 Food and Daily Intake

A wide variation of selenium concentrations is found in food products. The level depends on the class of food and on the concentration and availability of selenium in the soil where the plants are grown. Vegetables and fruits are mostly low in selenium content (<0.01 µg/g). However, certain products such as garlic and mushrooms contain 0.25 and 0.12 µg/g, respectively. Typical values found in foods worldwide range from (wet weight basis) 0.4–1.5 µg/g in liver kidney and seafood, 0.1–0.4 µg/g in muscle meat, <0.1–0.3 or more in cereals, and <0.1–0.3 µg/g in dairy products. Generally, foodstuffs from seleniferous areas of Venezuela contain much more selenium than the same types of products from Scandinavia or New Zealand where selenium levels are low (WHO, 1987).

Foodstuffs constitute the main source of selenium in the general population. Because of geochemical differences, the estimated daily intakes of selenium for adults ranged from 11 µg with Keshan disease in the Keshan area, 25 µg in New Zealand to 750 µg, without selenosis or 4990 µg, with selenosis in China (Levander, 1976; Robinson, 1976; WHO, 1987; Yang *et al*., 1983). Selenium may be lost from cereals during dry heating, but generally, little is lost during cooking (Higgs *et al*., 1972). Approximately 80% of selenium is absorbed from food. Numerous new organic selenium species have been identified in plant foods such as garlic and yeast (Block *et al*., 2004; Kotrebai *et al*., 2000; Uden, 2004). Knowledge to what extent they are absorbed and used is incomplete. Cereals, meat, and fish products are the main dietary sources of selenium. However, the importance of cereals varies considerably among regions. The dietary reference intake (DRI) for selenium was set to 55 µg/day for adult men and women (NAS, 2000). The Nordic recommended intake levels of selenium are 50 and 40 µg/day, respectively (4th edition of the Nordic Nutrition Recommendations, 2005). The EU Scientific Committee on Food established a tolerable upper intake level of 300 µg/day (EUSCF, 2000).

4.1.2 Ambient Air

The atmospheric level of selenium in most urban regions ranges from 0.1–10 ng/m^3, but values up to 42 ng/m^3 have been measured in the UK. The estimated average is well below 10 ng/m^3 and in remote areas below 1 ng/m^3 (ATSDR, 2003). A greater part of the atmospheric selenium is bound to fly ash and to suspended particles that contain from 1.4–11 and from 1–110 µg/g, respectively. Up to 90% of the selenium content in ambient air is emitted during the burning of fossil fuels (ATSDR, 2003; Kut, 1981). Selenium dioxide, which is formed during combustion of fossil fuel, is solid at ambient temperatures and is less readily transported in the atmosphere than sulfur dioxide. Point source emissions of selenium caused by burning of

fossil fuels or industrial processes (glass manufacture, metal-processing plants) may contribute to local air levels. Estimates of yearly releases to the atmosphere in the United States are approximately 1500 tons from fossil fuel combustion and about the same amount from industry and municipal waste (ATSDR, 2003).

4.1.3 Water

Surface water receives selenium from atmospheric deposition, surface runoff, and subsurface drainage. Selenium levels in seawater range from 0.06–0.12 pg/L (Schutz, 1965; Sugimura, 1976). The levels vary widely in ground and surface water, from 0.06–≈400 µg/L (Morette and Divin, 1965; WHO, 1987), and in some areas the ground water may reach concentrations of up to 6000 µg/L (Cannon, 1964). The selenium content of surface water is greatly influenced by pH; it is high in acidic surface water (pH < 3.0), and in alkaline water (pH > 7.5). If iron is present, selenium precipitates as soluble basic ferric selenite at pH 6.3–6.7. At approximately pH 8, selenite may be oxidized to soluble selenates, thereby increasing the water concentration up to 10–400 µg/L. Levels of selenium in tapwater samples from public water supplies in the United States, Canada, the F.R.G., and Australia only exceptionally exceeded 10 µg/L (ATSDR, 2003; WHO, 1987).

4.1.4 Rocks and Soil

Selenium occurs in the earth's crust in concentrations from 0.05–0.09 µg/g. Selenium mainly occurs as selenites and selenides in association with sulfide minerals (ATSDR, 2003; Shamberger, 1981; WHO, 1987). High selenium concentrations may be found in volcanic rock (120 µg/g) but also in sedimentary rocks such as in shales, sandstone uranium deposits (1000 µg/g), and in some carbonate rocks (30 µg/g). The selenium content of coal and oil may vary from 0.47–8.1 µg/g and from 2.4–7.5 µg/g, respectively (Raptis, 1983), but coal levels of selenium up to 84 mg/g (average, 0.3 mg/g) have been found in China and was identified as the source of soil contamination in that area (Levander, 1983; WHO, 1987).

The selenium content of soils varies greatly from 0.005–1200 µg/g, and most commonly between 0.1 and 10 µg/g. In selenium-deficient areas, the soil content may range from 0.005–2 µg/g. The selenium in soil, which varies in availability, may occur in many forms (e.g., as selenites, selenates, elemental selenium, and as selenium in association with minerals). Both the total selenium content and the chemical forms (including availability) are critical for production of vegetation with low, high, or even toxic levels of selenium. In alkaline soils (pH > 7.5), selenium is present as water-soluble selenate and available to plants, whereas in acidic soils it is usually bound as basic ferric selenite of very low solubility (Fishbein, 1983; Johnsson *et al*., 1997; Raptis, 1983; WHO, 1987).

4.1.5 Plants

Plants can be divided into different groups based on their ability to accumulate selenium when grown in soils with high selenium content (Rosenfeld, 1964). "Primary selenium indicators"(such as several species of *Astragalus* and some species of *Machaeranthera*, *Haplopappus*, and *Stanleya*) grow well on soil containing high levels of selenium and normally accumulate selenium at very high levels (100–10,000 mg/kg). "Secondary selenium absorbers" (e.g., Aster, Atriplex, Castelleja, Grindelia, Gutierrezia, Machaeranthera, and Menzelia) can concentrate selenium in the range of 50–500 mg/kg. All these plants add very little to the selenium content of feed because they normally grow in dry nonagricultural areas, but when consumed by livestock, they cause disease symptoms. Other plants including grasses, grains, and weeds do not accumulate selenium in excess of 50 mg/kg when grown on seleniferous soil. Plants also vary widely in their ability to accumulate selenium from low-content selenium soils. Extensive experimentation with plant uptake has been performed with selenium fertilization of selenium-poor soil in Finland and Denmark (ATSDR, 2003; Johnsson *et al*., 1997).

4.1.6 Tobacco

Pipe and cigarette tobaccos contain selenium in concentrations ranging from 0.01–0.4 mg/kg and cigar tobacco from 0.33–1.01 mg/kg, depending on where the plants are grown. Selenium levels in cigarette paper are usually low (0.05 mg/kg) (Bogden *et al*., 1981).

4.2 Work Environment

Human occupational exposure to selenium occurs mainly through the air. Grinding of selenium compounds or mechanical processing during mining of ores containing high levels of selenium can lead to contamination of the work atmosphere with selenium-containing dusts. Release of selenium into the air may also be a result of heating selenium-containing materials, because sublimation of selenium can take place from 170–180°C and formation of selenium dioxide from 230–240°C. Little reliable information is, however, available on the levels of selenium in the work environment. Air concentrations of selenium between 40 and 400 µg/m^3 were reported

from a selenium-rectifier production plant, whereas higher values were observed in a few samples from grinding workshops (up to 3600 µg/m^3) (Glover, 1967). Selenium fumes may contain selenium dioxide in concentrations as high as 50% (Glover, 1970) and are often described as the main selenium problem in the work environment. Air levels in a plant melting selenium-containing alumina used in electronic industries varied from 100–1000 µg/m^3 (Thomassen, 1985, personal communication).

5 BIOLOGICAL FUNCTION AND METABOLISM

5.1 Biological Functions

Selenium is an essential trace element in many species including man. Selenium is an integral part of five glutathione peroxidase (GSH-px1-5) enzymes and several other proteins. The human selenoproteome consists of 25 selenoproteins (Kryukov *et al*., 2003). All of them contain SeCys, and about half of them have so far been ascribed a biological function (Schomburg *et al*., 2004). Bacterial selenoproteins are primarily involved in catabolic processes, whereas mammalian selenoproteins, as far as is known, participate in antioxidant, anabolic processes (Hatfield and Gladyshev, 2002). Viral selenoproteins may compete for selenium with selenoprotein synthesis in host animals (Zhao *et al*., 2000). Selenium-dependent GSH-px enzymes protect the organism against oxidative damage by reducing lipoperoxides and hydrogen peroxide. It has recently been shown that homocysteine, a risk factor of cardiovascular disease, down-regulates GSH-px1 (Handy *et al*., 2005). Interestingly, GSH-px4 may also be converted to structural protein during sperm maturation (Ursini *et al*., 1999). Thioredoxin reductases are other selenoproteins that catalyze reduction of oxidized cellular proteins such as thioredoxin and may also play a role in redox regulation and resistance to oxidative stress and apoptosis (Smart *et al*., 2004). A third group is iodothyrodine deiodinases, which controls the level of active triiodothyronine and thereby thyroid hormone signaling.

Disruption of the gene for selenocysteine (SeCys) tRNA is embryonically lethal (Bosl *et al*., 1997), as well as disruption of the GSH-px 4 gene in mouse embryos (Yant *et al*., 2003). A high reactivity of SeCys in selenoproteins has been related to a lower pKa for the selenol group than that for corresponding sulfhydryl group of Cys. Sulfur-containing analogs of selenoenzymes can be orders of magnitude less efficient (Schomburg *et al*., 2004).

5.2 Selenium Deficiency and Diseases Related to Selenium Status

In animals, a number of well-defined selenium deficiency diseases have been described, and several diseases in man have been associated with relative selenium deficiency (Rayman, 2000). A hypothesis that the side effects of statins, cholesterol-lowering drugs that inhibit mevalonate synthesis, may be due to induction of selenium deficiency by reducing the isopentenylation of selenocysteine-tRNA has been discussed (Moosmann and Behl, 2004). Biochemical effects of experimentally induced selenium deficiency will be discussed in Section 5.3.3.

5.2.1 Animals

Deficiency diseases have been described in many farm animals and laboratory animals and include dysfunction of several organs and growth retardation. Reproductive failures are seen in rodents, dogs, pigs, monkeys, cows, and ewes. Myopathies are also seen in a number of species, especially in ruminants. Pigs are often affected by cardiomyopathy. Liver necrosis, hemolysis, kidney degeneration, exudative diathesis, and pancreatic fibrosis are other important signs of deficiency, the latter two especially in poultry (Diplock, 1976; WHO, 1987). Experiments that used genetically manipulated mice to obtain selenium deficiency targeted to the liver show that this organ is severely damaged within a few months (Carlson *et al*., 2004).

5.2.2 Selenium and Cardiovascular Diseases

Keshan disease is an endemic cardiomyopathy in children and pregnant women who live in the Keshan region of China, where selenium levels in food are extremely low. The incidence of the disease was dramatically reduced when the population was supplemented with sodium selenite. However, a cardiotoxic coxsackievirus or another unknown environmental factor has also been implicated as part of the etiology (Beck *et al*., 2004; Chen, 1980). Cardiomyopathy has also been reported in selenium-deficient patients receiving total parenteral nutrition; and lower serum selenium levels have been found in patients with cardiomyopathy (Johnson *et al*., 1981; Oster *et al*., 1983). Patients with phenylketonuria and on a diet have very low selenium levels, but show no signs of cardiomyopathy. Salonen *et al*. (1982) showed a significant inverse correlation between serum selenium levels and cardiovascular death in a matched-pair longitudinal study.

5.2.3 Selenium and Cancer

A large number of experiments, probably hundreds, have been carried out to assess the effect of selenium

compounds in various animal tumor models (Combs and Gray, 1998; El-Bayoumy, 2001; Whanger, 2004). In general, most show some inhibitory effect on a variety of chemically induced cancers in skin, liver, colon, and breast, as well as spontaneous mammary and intestinal tumors. Most experiments have used inorganic forms of selenium or organic selenium from yeast or garlic (e.g., methylselenocysteine), but also various synthetic forms (e.g., ebselen, benzyl selenocyanate) have been shown to be effective. It seems that the various compounds differ with respect both to anticarcinogenic potency and ability to support synthesis of selenoenzymes. It is well recognized that the most effective doses of selenium for anticarcinogenesis are above nutritional or at pharmacological levels. Apparently, selenium works best in early phases of carcinogenesis, and manifest tumors respond poorly. The mechanisms for the anticarcinogenic effects of selenium compounds are not known. Suggested mechanisms include effects on programmed cell death, effects on DNA repair, the role of selenoenzymes (e.g., antioxidative effect of GSH-Px), effects on carcinogen metabolism (e.g., selenocysteine and other derivatives inhibiting CYP enzymes) (Venhorst *et al*., 2003), immune system effects, antiangiogenic effects, and specific inhibition of tumor growth.

A number of epidemiological studies with different designs have been carried out. These include the early ecological studies, which showed that geographical areas with high selenium levels (as measured in blood or food) had lower incidence of breast, digestive, and lung cancer, as well as lymphomas (Combs and Gray, 1998; Ferguson *et al*., 2004; Shamberger *et al*., 1973; Whanger, 2004). Several studies have shown relationships between selenium status and cancer risk. In diseased smelter workers, the workers dying of malignancies had a higher arsenic/selenium quotient in lung tissue than workers dying of other diseases (Gerhardsson *et al*., 1985; Wester *et al*., 1981). Several case-control studies have been carried out. The different studies show approximately 5% lower plasma selenium in cases versus controls, the absolute levels being quite different (Fex *et al*., 1987; Salonen *et al*., 1984; Willett *et al*., 1983). However, some studies did not show an association. Altogether these studies show an inverse association between selenium status and cancer risk in several sites, gastrointestinal, prostate, bladder, thyroid, lung, and breast (Combs and Gray, 1998; Whanger, 2004).

Five sets of intervention trials from China have given support to the efficacy of selenium against liver cancer risk and esophageal and stomach cancer and lung cancer (Combs and Gray, 1998; Whanger, 2004).

In a randomized double-blind placebo controlled trial in the United States, older patients with a history of basal and/or squamous cell carcinoma were given 200 μg Se/day as selenium-enriched yeast or placebo (Clark *et al*., 1996). The study started in 1983 and was analyzed in 1993. Selenium treatment did not affect recurrent skin cancers but reduced nonskin cancer, including lung cancer, colorectal cancer, and prostate cancer. In a recent report following the patients to 1996, selenium supplementation was associated with an increased risk of squamous cell carcinomas and total nonmelanoma skin cancer (Duffield-Lillico *et al*., 2003). It was noted by the authors that farming could be a confounder, because farmers could have been exposed to arsenic-containing pesticides. The effect of antioxidant supplementation, including selenium, was evaluated for gastrointestinal cancers in a systematic review and meta-analysis (Bjelakovic *et al*., 2004). In four trials of which three had unclear or inadequate method, selenium had significant beneficial effect on the incidence of gastrointestinal cancer. Currently there are more trials ongoing or planned (Lippman *et al*., 2005; Rayman, 2000; Whanger, 2004).

5.2.4. Infectious Diseases

Selenium deficiency has been implicated to play a role particularly in viral diseases (Beck *et al*., 2003; 2004; Whanger, 2004). In Keshan disease, which occurred in an area with extremely low intake of selenium, an infectious cofactor was apparently required in addition to selenium deficiency. Enteroviruses, and in particular coxsackieviruses, were isolated from Keshan disease victims (Su *et al*., 1979, cited in Beck *et al*., 2003), and this was later confirmed with RT-PCR techniques on archived heart tissues (Li *et al*., 1995 cited in Beck *et al*., 2003). Selenium-deficient mice became ill on inoculation with coxsackievirus B4 isolated from Keshan disease victims (Bai *et al*., 1980 cited in Beck *et al*., 2003). In other experiments, an amyocarditic strain of coxsackievirus B3, CVB3/0 converted to virulence when inoculated into selenium-deficient mice. The virus showed genetic conversion to a pattern more like the virulent CVB3 strains. This also happened in selenium-replete GSHpx-1 knockout mice, but not in normal selenium-replete mice. A mild strain of influenza virus, influenza A/Bangkok/1/79, also showed increased virulence and multiple changes in the genome when given to selenium-deficient mice.

In studied patients with pulmonary tuberculosis with and without HIV coinfection from Malawi, selenium status was extremely poor among those infected with HIV, and the extent of selenium deficiency was associated with higher plasma HIV load (van Lettow *et al*., 2004; 2005). HIV progression was decreased on selenium supplementation in a double-blind placebo-controlled clinical trial (Burbano *et al*., 2002).

5.2.5 Other Diseases

Several diseases are associated with low levels of selenium in serum or blood. Patients with kwashiorkor had low selenium levels and responded better to feeding when administered selenium simultaneously (Burk *et al*., 1967). Patients with liver cirrhosis, especially alcoholic cirrhosis, had very low levels of selenium in both serum and liver (Aaseth *et al*., 1980; Czuczejko *et al*., 2003; Dworkin and Rosenthal, 1984; Johansson *et al*., 1986; Valimaki *et al*., 1983). Low serum selenium has also been seen in patients with celiac disease, rheumatoid arthritis, cystic fibrosis, and other degenerative diseases (Aaseth *et al*., 1978; Hinks *et al*., 1984). Some improvement regarding morning stiffness was observed in a double-blind study in patients with rheumatoid arthritis supplemented with selenium and vitamin E (Aaseth *et al*., 1998). Neurological seizures in infants have been related to low blood Se levels and were treated with Se supplementation (Ramaekers *et al*., 1994; Weber *et al*., 1991). Combined Se and iodine deficiency may result in severe myxedematous cretinism (Zimmermann and Kohrle, 2002).

5.3 Kinetics

5.3.1 Absorption

Most water-soluble selenium compounds are effectively absorbed in the gastrointestinal tract, and neither size of the dose nor nutritional state of the animal has been shown to influence the absorption. Thus, more than 90% of orally administered selenite or more than 80% of orally administered selenomethionine or selenocysteine is absorbed by rats (Bopp *et al*., 1982). Selenium incorporated into animal tissues (Thomson *et al*., 1975) or corn (Cary *et al*., 1973) is absorbed by rats to a high extent, whereas elemental selenium (cf. Medinsky *et al*., 1981) or selenium sulfide (Cummins and Kimura, 1971) is poorly absorbed. Selenite is readily absorbed in mice and dogs, whereas monkeys seem to have a slower absorption rate (Furchner *et al*., 1975). In sheep and cows, values as low as 30–35% have been obtained; this low rate of absorption might be due to selenite reduction to elemental selenium by bacteria in the gastrointestinal tract of ruminants. Both selenite and selenomethionine are mainly absorbed in the duodenum (Whanger *et al*., 1976). Active uptake mechanisms for selenomethionine (McConnell and Cho, 1965) and selenite (Anundi *et al*., 1984) have been indicated in *in vitro* studies. Studies on man indicate a less efficient uptake of selenite than documented for rats. Forty to eighty percent of a perorally given dose of selenite may be absorbed. On the other hand, higher values (75–97%) have been obtained for selenomethionine in humans (Bopp *et al*., 1982). A recent study indicates that selenomethionine is twice as bioavailable as selenite (Xia *et al*., 2005).

Inhalation studies on rats show that 94% of selenious acid deposited in the lungs was absorbed within 4 hours. The corresponding value for elemental selenium was 57% (Medinsky *et al*., 1981). Skin absorption was also noted in this study. A model based on these rat studies predicted that organ concentrations in man caused by inhalation of selenious acid or elemental selenium in urban atmospheres will not contribute significantly to human body burdens (Medinsky *et al*., 1981).

5.3.2 Distribution

In laboratory animals, there is a rapid distribution of water-soluble selenium compounds to most organs. In later phases, the distribution is influenced by the nutritional state of the animal and the dose of selenium. Rats raised on a low selenium diet accumulate and retain selenium, given as a tracer of selenite, in reproductive organs, brain, and thymus. In blood, muscle, liver, spleen, kidney, and lung, accumulation was more transient, and the concentrations decreased after the first day. The degree of whole-body retention is inversely related to the dietary level of selenium (Burk *et al*., 1972). After lung absorption, elemental selenium exhibited the same type of distribution as selenious acid (Weissman *et al*., 1983).

Results from human studies tend to suggest that disposition of selenium in man is similar to that in rats and other laboratory animals (Bopp *et al*., 1982). However, there are indications that selenium distribution among blood components is different. Thus, only 10–15% of erythrocyte selenium in man was associated with GSH-px activity, whereas the corresponding value in rats was 75–85% (Beilstein *et al*., 1984). Comparative long-term studies on the bioavailability of selenite and selenomethionine in man (Thomson *et al*., 1982) and of selenate and selenium-rich wheat or yeast in rats (Levander *et al*., 1983) suggest that organically bound selenium maintained platelet GSH-px activity better than selenite or selenate.

Rats were injected intraperitoneally with ^{75}Se sodium selenite (5 mg/kg), and it was concluded that selenium in the form of selenite accumulated in the anterior pituitary gland. The maximum selenium content was observed after 2 hours, at which time the anterior pituitary gland contained 2.9 µg/g wet weight. The selenium content in pituitary glands from untreated rats was 0.48 µg/g wet weight (Thorlacius-Ussing and Jensen, 1988).

Selenoprotein P, which seems to be the only selenoprotein that contains more than one SeCys, may have

specific transport functions. When selenium-deficient rats were injected with ^{75}Se-selenoprotein P, five times as much tracer accumulated in the brain as in controls. ^{75}Se-selenite given to Se-deficient rats was rapidly cleared from the plasma and accumulated in the liver. The tracer peaked at 1–2 hours. It then declined in the liver and started to accumulate in the brain. The accumulation of Se in the brain was preceded or coincided with the appearance of the tracer in plasma selenoprotein P (Burk *et al*., 2003). This series of events suggest that the liver, but not the brain, is able to take up small-molecule forms of Se, that selenoprotein P has a transport function, and indicates a mechanism by which the brain can maintain a high selenium level at the expense of other organs in a state of selenium deficiency. The presumed transport function of selenoprotein P is supported by studies that used gene knock out models (Hill *et al*., 2003; Schomburg *et al*., 2003). In earlier studies Sandholm (1973) found that a tracer dose of selenite accumulated in red blood cells, translocated to plasma proteins, and then to the liver. Selenoprotein P seems to be involved in the transport of Se from the liver to other tissues, even though other transporters may exist and different organs may have different preferences for selenium sources.

The placental transfer was investigated in mice. Na2^{75}SeO3 (Se^{+4}) or Na2^{75}SeO4 (Se^{+6}) was injected intravenously. The doses were 45 µg Se/kg. The embryonic and fetal uptake after administration of Se^{+4} and Se^{+6} was similar. The placental transfer of Se increased with time after dosing and with progression from embryonic through fetal stages. The highest uptake in the embryo was observed in the neuroepithelium, whereas the eye, liver, and skeleton dominated the distribution pattern in the fetal period. (Danielsson *et al*., 1990). The transfer of selenite was studied in human placenta. An equilibration across the placenta was obtained within 4 hours. No adverse effects were noted at concentrations of up to 40 nmol/mL (Eisenmann and Miller, 1994).

Pregnant cynomolgus macaques were given L-selenomethionine orally (0, 25, 150, or 300 µg Se/kg bw) daily from gestational day 20 to 50; 7–8 pregnancies per group were terminated at day 100, whereas 2–3 pregnancies proceeded to term. The area under maternal plasma selenium concentration time curves, the maximum maternal plasma selenium concentrations, and the maternal urinary selenium excretion rates were proportional to dose. Se concentrations in all fetal and neonatal tissues were also proportional to dose. The selenium concentration in fetal plasma was on average 33% of that in maternal plasma. Se concentration in milk was doubled by the highest dose. Intrauterine selenium accumulation accounted for most of the neonatal selenium body burden (Hawkes *et al*., 1994).

5.3.3 Biotransformation

Selenium compounds are biotransformed to excretable metabolites, and when given in high doses, a major fraction is excreted within a few days. Dimethylselenide formation has been indicated after selenate and selenite administration (Nakamuro *et al*., 1977). The biotransformation of selenite to dimethylselenide has been characterized in *in vitro* systems. It was shown that GSH was an essential cofactor and that the stable reaction product, GSSeSG, could be further metabolized by GSH reductase by means of GSSeH to hydrogen selenide, and that subsequent methylations were catalyzed by methyltransferases (Hsieh and Ganther, 1977). More recent data show that thioredoxin reductase or reduced thioredoxin is an alternative pathway for GSSeSG reduction to hydrogen selenide (Bjornstedt *et al*., 1992). The reduction of selenite may proceed in oxygenated cultures of isolated hepatocytes (Stahl *et al*., 1984); however, oxygen is consumed and intracellular hypoxia may support the metabolism (Garberg and Hogberg, 1987). In case oxygen is available, GSH-dependent selenite reduction may result in redox cycling of metabolites and high consumption of NADPH (Anundi *et al*., 1984; Bjornstedt *et al*., 1992).

The incorporation of inorganic selenium into amino acids and selenoproteins has been characterized more recently. Hydrogen selenide, which is the crucial intermediate, is bound to selenophosphate synthetase 2, a selenonoprotein, and selenophosphate is formed (Guimaraes *et al*., 1996; Tamura *et al*., 2004). Selenocysteine (SeCys or Sec) synthesis then occurs on its tRNA. So formed SeCys is incorporated into polypeptides guided by the UGA codon in all selenoprotein mRNAs. UGA may also serve as a stop codon, but selenoprotein mRNAs have a SeCys insertion (SECIS) element that recruits specific proteins necessary for the incorporation of SeCys. SeCys may also be formed nonspecifically or from proteolysis of selenoproteins. SeCys can be degraded by a lyase enzyme (Esaki *et al*., 1982). As already mentioned (Section 5.1), all 25 selenoproteins contain SeCys. However, other than these selenoproteins may unspecifically incorporate dietary selenomethionine (SeMet), in which Se has replaced sulfur (Schomburg *et al*., 2004).

Studies on experimentally induced selenium deficiency indicate hierarchies for selenium incorporation into selenoproteins. Thus, Se deficiency reduces GSHpx1 activity down to 1% in rat liver, whereas GSHpx4 activity was reduced to 25% and was not affected in testes. It has also been noted that the total amount of Se is substantially reduced in the liver and kidney but not in the brain and testes. The sensitivity of GSHpx1 may relate to an increased degradation

of GSHpx1 mRNA. Of further interest is that SeCys tRNA exists in two isoforms, with or without a methyl group that may regulate the mammalian SeCys insertion machinery (Hatfield and Gladyshev, 2002). In addition to the Se status, GSHpx4 is also regulated by several other factors (Sneddon *et al*., 2003).

Recent data indicate that GSH selenopersulfide (GSSeH) is further metabolized to selenosugars in the liver (Kobayashi *et al*., 2002).

5.3.4 Excretion

Under most conditions, urine is the major excretory pathway, but the fraction excreted depends on the nutritional status of the animal and the amount administered. However, fecal excretion may dominate in cases of deficiency states and with tracer doses (Burk *et al*., 1972). With an adequate supplementation of the diet (1.0 mg/kg.), 67% of a tracer dose of selenite was excreted in the urine, whereas in a state of deficiency only 6% of the same dose was excreted (Burk *et al*., 1972). It has been reported by several authors that trimethylselenonium is the dominating urinary metabolite at high doses, particularly in experimental animal studies (1.5 mg/kg), but the true nature of this high dose metabolite has been questioned (Francesconi and Pannier, 2004).

At high or toxic dose levels, excretion through expired air becomes important and even predominant. In one study on rats, it was shown that only 0.2% was exhaled when selenite was given as a small dose (0.005 mg/kg bw), whereas as much as 50–60% was exhaled at lethal doses (McConnell and Roth, 1966). The only metabolite identified in the breath is dimethylselenide. Results available on selenite biotransformation and excretion are thus compatible with the idea that biotransformation to methylated selenides mainly serves to facilitate excretion of high or potentially toxic amounts of ingested selenium.

Other selenium compounds that have been studied seem to have excretion patterns similar to those of selenite. Available data also suggest that man excretes selenium compounds in a way similar to that shown for rats (Bopp *et al*., 1982).

In a recent critical review, selenium metabolites found in urine have been listed (Francesconi and Pannier, 2004). It was mentioned that previously held views that methylselenol and trimethylselenoium ions are major human urinary metabolites seems unjustified. Instead, a selenosuger (methyl 2-acetamido-2-deoxy-1-seleno-β-D-galactopyranoside) has been found to be a major metabolite in rat and human urine, and low and moderate doses of selenium are excreted in this way (Kobayashi *et al*., 2002). However, in a recent study, both the selenosuger metabolite and the trimethylselenoium ion were recovered in rat urine after selenite administration in drinking water. In young rats, the trimethylselenoium ion concentration increased with dose, but not in adult rats (Suzuki *et al*., 2005). Unmetabolized selenite has also been recovered in urine (Francesconi and Pannier, 2004).

The fate of selenate at a dose of 0.3 mg Se kg^{-1} body weight administered intravenously to rats was studied. The results suggest that in contrast to selenite, which is taken up by and reduced in RBCs, and then transferred to the liver, approximately 20% of the selenate administered to rats was excreted into the urine without any change in its chemical form, and the major portion of selenate was taken up by the liver, reduced, and then used for the synthesis of selenoproteins or excreted into the urine after being methylated (Shiobara *et al*., 1999).

Forty pregnant long-tailed macaques were treated daily for 30 days with 0, 25, 150, or 300 µg selenium as L-selenomethionine/kg body weight. Hair selenium was the most sensitive indicator of L-selenomethionine dose, but urinary excretion, plasma, erythrocyte, and fecal selenium also responded. Erythrocyte and plasma glutathione peroxidase specific activities increased 154 and 69% over controls, respectively. Toxicity was associated with erythrocyte selenium >2.3 µg/mL, plasma selenium >2.8 µg/mL, and hair selenium >27 µg/g (Hawkes *et al*., 1992).

5.3.5 Biological Half-Time

Several studies indicate at least biphasic elimination of whole-body selenium in rats (Ewan *et al*., 1967) and dogs (Weissman *et al*., 1983). The amount excreted during this initial phase has been shown to be dose dependent in rats (Burk *et al*., 1972). A second phase, which does not seem to be influenced by selenium dose, has a half-time of approximately 30–70 days in most species (Weissman *et al*., 1983).

In studies on humans, three phases of elimination can usually be identified after selenite administration. The half-time of the first phase is approximately 1 day, the second 8–20 days, and the third 65–116 days (Bopp *et al*., 1982). In some studies (cf. Griffiths *et al*., 1976), the half-time of the terminal phase for selenium elimination was longer when selenomethionine was used instead of selenite. This finding is in agreement with results discussed in Section 5.3.2.

6 BIOLOGICAL MONITORING

6.1 Levels in Tissues and Biological Fluids

Levels in organs have been compiled from different publications (Thomassen and Aaseth, 1986), and

the range per dry weights was found to be as follows: brain, 0.46–1.59 µg/g; lung, 0.45–2.16 µg/g; liver, 0.58–52 µg/g; kidney, 1.18 – 8.0 µg/g; muscle, 0.29–1.7 µg/g; and bone, 0.48 µg/g.

6.2 Biomarkers of Exposure

The reference urinary level should not exceed 0.03 µg/mL (Robberecht and Deelstra, 1984). It is recommended that selenium in urine is measured in 24-hour samples or is related to the creatinine level. Urinary levels in workers exposed to 0.2–0.4 mg Se/m^3 was usually <0.1 mg/L. At air concentrations as high as 3.6 mg/m^3 times the urinary level averaged 0.25–0.45 mg/L. Garlic odor of the breath usually accompanied these higher levels (Glover, 1967).

Toxic effects of selenium are reflected by increased blood, urine, and hair levels. In China, people consuming selenium-rich food and exhibiting signs of selenosis had mean levels in blood, urine, and hair of 3.2 µg/mL, 2.7 µg/mL, and 32.2 µg/g, respectively. The corresponding values in people from a high selenium area without selenosis were 0.44 µg/mL, 0.14 µg/mL, and 3.7 µg/g in blood, urine, and hair, respectively. In selenium-adequate areas, the levels were 0.095 µg/mL, 0.026 µg/mL, and 0.36 µg/g in blood, urine, and hair, respectively (Yang *et al*., 1983).

Supplementation of the diet with yeast or wheat, so that 200 µg Se/day was consumed, resulted in an increased plasma level from 0.07–0.17 µg/mL within 11 weeks (Levander *et al*., 1983). Supplementation with selenate was less efficient. On a low-selenium diet (33 µg/day), the level in breast milk was 5.8 µg/L. At a higher dietary level (50 µg/day), the milk level was 10.0 µg/L (Kumpulainen *et al*., 1984). Many supplementation studies have been carried out since then. The highest levels are often achieved on supplementation with selenomethionine, a common form in plants and which is nonspecifically incorporated into proteins. The efficiency of selenium compounds to raise plasma selenium levels also depends on the selenium status (Alexander and Meltzer, 1995; Johnsson *et al*., 1997; Rayman, 2004) (see also Chapter 8 for blood levels in humans).

In deficiency, selenium in blood or plasma is decreased.

6.3 Biomarkers of Effect

Whereas selenium-dependent enzymes can be used as biomarkers of selenium status at replete and low levels, there does not seem to be any specific biomarker of deficiency in humans. Platelet GSH-px activity has been suggested as a valuable indicator of the selenium status in humans. In a study on rats, platelet GSH-px was shown to respond quickly to decreased selenium administration (Levander *et al*., 1983; Rayman, 2004). In humans, studies of platelet GSH-Px responded within weeks to selenium administration (Levander *et al*., 1983; Thomson *et al*., 1985; Xia *et al*., 2005). Recently, selenoprotein P was suggested as a better indicator of selenium status, because this selenoprotein seems to require a greater selenium intake to reach full expression (Xia *et al*., 2005).

There are no specific biomarkers for excess selenium. Garlic breath indicates high acute exposure; however, other metals are also methylated, which may result in garlic odor. Nonspecific clinical signs of chronic overexposure are those of selenosis described in Section 7.2.3.2 (i.e., nail and hair changes). Also clinical chemical signs are nonspecific such as signs of liver toxicity (i.e., raised serum levels of liver aspartate aminotransferase [ASAT] and alanine aminotransferase [ALAT] and increased prothrombin time).

7 EFFECTS AND DOSE-RESPONSE RELATIONSHIPS

7.1 Acute Toxicity

7.1.1 Laboratory Animals

Many selenium compounds are very toxic and kill laboratory animals in single doses as small as 1.5–6 mg/kg bw. Thus, selenite killed 75% of injected (intraperitoneally) rats within 2 days at doses of 3.25–3.5 mg Se/kg; the corresponding lethal doses for selenate were 5.5–5.75 mg Se/kg, and for selenocysteine 4 mg Se/kg (Wilber, 1980). However, some other compounds are less toxic, such as selenium sulfide, with an oral LD_{50} in the rat of 138 mg/kg (Cummins and Kimura, 1971) or dimethylselenide with an LD_{50} (intraperitoneally) in the rat of 1600 mg/kg (Wilber, 1980). Animals given lethal doses acquire a garlic odor of the breath, show signs of nervousness and fear, followed by somnolence. Respiration becomes dyspneic and then opisthotonic; tetanic spasms and ultimately clonic spasms develop before the animal finally dies. The critical organ under these conditions seems to be the CNS, whereas morphological changes are dominated by liver fatty degeneration or necrosis.

Acute poisoning after inhalational exposure to "selenium dust" at a concentration of 30 mg/m^3 has also been described (Hall *et al*., 1951). Ten percent of the rats died; the major pathological change was interstitial pneumonitis. At levels of 150–600 mg/m^3, selenium dioxide caused rapid death in all rats tested (Filatova, 1951). Selenium oxychloride is a strong vesicant, and

the minimal lethal dose when applied to the skin of rabbits is 7 mg/kg (Dudley, 1938).

Biochemical aspects of selenite toxicity have been studied in different *in vitro* systems. In general, selenite concentrations of approximately 20–100 µmol/L (1.6–7.9 mg Se/L) damage or lyse most cell types. In isolated hepatocytes, it was demonstrated that redox changes are of major importance for the cellular lysis. The severe redox changes can be explained by redox cycling of autooxidizable selenium metabolites (Anundi *et al*., 1984). Other toxic mechanisms discussed in the literature are GSH depletion, protein synthesis inhibition (Vernie *et al*., 1974), depletion of S-adenosylmethionine (Hoffman, 1977), and a general replacement of sulfur by selenium in cellular metabolism (Stadtman, 1974).

Methylselenol selectively inactivates protein kinase C, and the selenoprotein thioredoxin reductase reverses this effect, suggesting that selenoproteins may serve as a safeguard against the toxicity induced by selenometabolites (Gopalakrishna and Gundimeda, 2002).

The importance of the prooxidative effects for three Se compounds, selenite, selenocystamine, and selenomethionine, and apoptosis was studied in keratinocytes. Selenite and selenocystamine generated 8-hydroxydeoxyguanosine DNA adducts and induced apoptosis, whereas selenomethionine did not. The data indicate that apoptosis was initiated by oxidative free radicals (Stewart *et al*., 1999).

Sodium selenite was administered subcutaneously to adult male mice; 10–60 µmol/kg resulted in a transient and dose-dependent hypothermia and reduced oxygen consumption. An involvement of neural pathways was suggested (Watanabe and Suzuki, 1986).

Cataracts of the lens can be produced by high doses of selenite (1.5 mg/kg body weight) in young rats (5–15 days) (Ostadalova *et al*., 1979). A biochemical characterization indicates redox changes in the lens (Bunce and Hess, 1981), similar to those described in isolated hepatocytes (Anundi *et al*., 1984). Cataract was induced in rat pups by a single subcutaneous injection of sodium selenite (30 µmol/kg body weight). A disruption of protein tyrosine phosphorylation was observed within 2–3 days during a precataract stage (Chandrasekher and Sailaja, 2004).

Weanling male rats were fed a Se-deficient diet supplemented with 0, 0.15, or 2.0 mg/kg Se as sodium selenite for 10 weeks. High levels of a liver carcinogen and high levels of selenite each increased concentration of 8-hydroxy-2′-deoxyguanosine in liver DNA. These findings suggest that high dietary intake of inorganic Se may promote *in vivo* DNA oxidation (Wycherly *et al*., 2004).

Male mice received repeated oral administration of selenocystine (10 or 20 mg/kg) for 10 days. The animals exposed only to the high dose showed a significant rise of ASAT and ALAT activities in plasma. Liver toxicity induced by selenocystine was enhanced by inhibition of selenium methylation (Hasegawa *et al*., 1996).

A total of 60 adult male rats was exposed to dimethylselenide (DMSe) vapor at 1607, 4499, or 8034 ppm for 1 hour (20 rats/group). The animals were sacrificed either 1 or 7 days after inhalation. Lung, liver, kidney, spleen, thymus, lymph nodes, pancreas, and adrenal gland were examined. The data indicate that inhalation of DMSe for 1 hour has relatively low toxicity in rats even at high concentrations (al-Bayati *et al*., 1992).

7.1.2 Humans

Five men ingested turkey-feed supplement containing large amounts of selenite. All five developed nausea and vomited and had diarrhea, abdominal pain, chills, and tremor shortly after ingestion. Serum levels ranged from 0.41–0.81 µg/mL, but returned to normal within 4 days. The 24-hour urinary excretion was 1175–4365 µg. All symptoms resolved within 24 hours (Sioris, 1980).

A 35-year-old woman ate approximately 10 g of a breakfast cereal to which selenium (35 mg/g) in some unknown form had been added. One year later when the analysis was carried out, 12 mg/g was in the form of selenite and the rest probably in the form of elemental selenium. She vomited, had diarrhea, cramps, and felt numbness in her arms 30 minutes after intake. She recovered within days, but later experienced irregular menstrual bleeding and a marked hair loss. Hair analysis (neutron activation) was performed 13 months later. The subject had long hair and it was cut in 10-mm sections before analysis. In most sections of the hair, the level was approximately 0.50 µg/g, but in the section 12–15 cm from the root, the level was 1.20 µg/g. Because her hair grew 1.2 cm per month, this high level probably reflected the time of intoxication (Plantin and Högberg, unpublished data).

Another case was reported as a criminal poisoning. A 46-year-old man was admitted to the hospital on several occasions. He complained of sudden onset of profuse watery diarrhea and vomiting. He lost scalp hair, and a strong odor of garlic was noted. There was also a purple-red discoloration of his nails. Later criminal investigation revealed that his girlfriend had bought large quantities of gun blue containing selenious acid (Ruta and Haider, 1989).

After ingestion of an unknown amount of a gun blueing compound containing selenious acid (11 mL from the bottle fluid were missing, equivalent to 2.9 g Se) a 2-year-old girl had continuous hypersalivation, vomiting, diarrhea, restlessness, and muscle spasm.

Blood pressure and pulse rate were increased. Symptomatic treatment was performed by parenteral fluid administration. The plasma Se concentration was increased to 20 times normal 5 hours after ingestion. Erythrocyte Se exceeded plasma Se 24 hours after intoxication. Urinary Se excretion decreased parallel to the plasma Se concentration. Ten weeks later, the Se content in her hair had risen to 10 times normal. The child fully recovered (Lombeck *et al*., 1987).

A patient died 8 days after ingesting selenious acid in the form of gun blueing. The patient's clinical course demonstrated hypotension, respiratory distress, severe myopathy that contributed to respiratory failure, and a garlicky odor to the breath. Four days after ingestion, the serum selenium concentration was 20 times normal and urinary excretion 70 times normal. Tissue selenium concentrations were up to 40 times normal (Pentel *et al*., 1985).

One patient ingested a mouthful of selenic acid (30 g/L); he only suffered mild gastrointestinal disturbances; 3 hours after ingestion, the plasma concentration was the highest (931 µg/L). A second patient ingested 1.7 g of sodium selenite. He had severe gastroenteritis, transient electrocardiographic changes, and developed a slight elevation of serum bilirubin; 3 hours after ingestion, the selenium level was 2.716 µg/L (Gasmi *et al*., 1997).

A 44-year-old worker neutralized 450 liters of selenic acid with caustic soda. Boiling occurred, and the mixture was spread all over the room. He suffered skin burns and died 90 minutes later with pulmonary edema, unstable blood pressure, and garlic breath (Schellmann *et al*., 1986).

A 15-year-old girl drank a selenate solution that contained 1160 mg selenium (Civil and McDonald, 1978). She was forced to vomit after 20 minutes and was treated with vitamin C and dimercaprol. She developed only slight symptoms (minor aches, irritability, and garlic breath). Nevertheless, the initial serum level was 3.1 µg/mL. After 2 days, the serum level was 0.21 µg/mL. There were also minor ECG changes, and tests showed slightly abnormal liver function.

A 3-year-old boy swallowed an unknown amount of a liquid containing 1.8% selenious acid and other poisons. He rapidly became comatose with unrecordable blood pressure and peripheral pulse and died after 1.5 hours. There was garlic odor of the breath (Carter, 1966).

There are also reports on acute intoxication after exposure through inhalation. A group of workers briefly and accidentally exposed to high concentrations of "selenium fume" complained of intense irritation of the eyes, nose, and throat, followed by headache (Clinton, 1947). In another incident, 37 men were exposed to "selenium oxide" during a fire in a rectifier plant. Bronchial spasm was followed by chills, nausea and vomiting, headache, fever, and bronchitis. Chemical pneumonia also developed in some of the cases (Wilson, 1962).

Hydrogen selenide inhalation may also cause acute intoxication at concentrations as low as 0.7 mg/m^3 (Buchan, 1947). Signs of toxicity include irritation of the respiratory tract, pulmonary edema, severe bronchitis, and bronchial pneumonia (Glover, 1970).

A well-documented case of subacute selenite intoxication has been reported. A 57-year-old woman took daily doses of tablets that accidentally contained approximately 31 mg selenium (probably the major amount in the form of selenite). After approximately 11 days, she noted marked hair loss that eventually developed into alopecia. Two weeks later, she noted white horizontal streaking on her fingernails, tenderness and swelling of the fingertips, and purulent discharge. Over a 3-week period, all fingernails were affected, and one fingernail was lost. She had periodic episodes of nausea and vomiting, a sour-milk breath odor, and felt increasing fatigue. Altogether, she consumed 77 tablets. Four days after the last tablet, her serum selenium level was 0.528 µg/mL. Excessive simultaneous intake of vitamin C might have reduced selenite to elemental selenium and thus alleviated the toxic reaction (Jensen, 1984).

A 48-year-old woman took 2000 mg of selenium dioxide. She presented with mildly altered consciousness and hematemesis and mucosal damage throughout the oral cavity, esophagus, and stomach. After intubation and gastric lavage, hemodialysis was performed. The patient was discharged uneventfully on the 16th day (Kise *et al*., 2004).

In another case report, a woman used selenium sulfide–containing shampoo two or three times weekly for 8 months to cure excoriations of the scalp. One hour after the last wash, she developed tremor, abdominal pain, and occasionally vomited. She had garlic odor on her breath, and the urinary level of selenium 5 days later was 32 µg/mL (Ransone *et al*., 1961).

7.2 Chronic Toxicity

7.2.1 Laboratory Animals

There are several early reports on the adverse effects of diets containing 5 mg Se/kg, usually given as selenite, in several laboratory animal species (Moxon, 1943). In rats, a 5 mg Se/kg diet may result in growth reduction (Ip, 1981). At a dietary level of 6.4 mg Se/kg (given as selenite), there were liver changes and splenomegaly. At 8 mg Se/kg, anemia, pancreatic enlargement, and

increased mortality were also observed (Halverson, 1966). The composition of the diet is of importance for the chronic toxicity of selenium. In early experiments it was realized that selenium was less toxic with high protein content in the diet (Moxon, 1943). Methionine might be an essential factor for this effect. A combination of methionine and tocopherol (or other antioxidants) alleviated liver damage caused by 10 mg Se/kg, given as selenate for 8 weeks (Levander and Morris, 1970). Other factors of importance are certain glycosides, isolated from linseed oil, which have protected against growth depression caused by 9 mg Se/kg diet given as selenite (Palmer *et al*., 1980).

Selenium is known to accumulate in the anterior pituitary and in selenium-treated animals (15 mg sodium selenite/L drinking water) the growth hormone and somatomedin C secretion on stimulation was decreased, indicating that growth retardation in selenium-treated rats could be mediated by reduced GH and somatomedin C production (Thorlacius-Ussing *et al*., 1987).

Twenty female long-tailed macaques received nasogastric intubation of 0–600 µg/kg/day L-selenomethionine for up to 30 consecutive days. Two animals given 600 µg/kg/day died. The incidence of anorexia, gastrointestinal distress, mucocutaneous toxicity, and frequency of reduced body temperature increased with time and dose. At higher doses, disturbances in menstruation occurred. Also reproductive toxicity was noted. The maximum tolerated dose was 150 µg/kg/day (Cukierski *et al*., 1989).

7.2.2 Domestic Animals

A chronic syndrome described in livestock and horses is "alkali disease." This disease has been associated with the consumption of grains or plants containing 5–25 mg Se/kg for periods of less than a month (Moxon, 1943). The disease is characterized by lack of vitality, loss of appetite, emaciation, deformation, and shedding of hooves, loss of long hair, and erosions of joints of the long bones. In more advanced cases, liver cirrhosis may develop.

The syndrome "blind staggers" in livestock has also been associated with the consumption of selenium in accumulator plants. Characteristics of the syndrome are impaired vision, depressed appetite, and a tendency to "wander" in circles. This latter stage is characterized by various degrees of paralysis, and death results from respiratory failure (Moxon, 1943). However, the classification of blind staggers has been questioned. Seventeen yearling steers were fed 0.15, 0.28, and 0.8 mg Se/kg bw as selenomethionine or selenite for 120 days; 0.28 and 0.8 mg selenomethionine/kg and 0.8 mg selenite/kg caused, in some cattle, mild to severe forms of alkali disease. No significant neurological, renal, or hepatic lesions developed. According to the authors, this supports the contention that blind staggers is caused by factors other than excessive dietary selenium (O'Toole and Raisbeck, 1995).

In male buffalo calves after repeated oral administration of 0.3 mg selenourea/kg (providing 0.19 mg/Se kg) for 75 days, selenosis was induced and selenium increased from 0.70 µg/mL blood on day 0 to 3.12 µg/mL on day 75. Signs occurred when Se levels were about 2.0 µg/mL and were prominent above 2.5–2.6 µg/mL (Deore *et al*., 2002).

In crossbred cow calves given sodium selenite (0.25 mg/kg bw) for 16 weeks the treatment resulted in characteristic signs of subchronic selenosis (i.e., alopecia, cracking, and enlargement of hooves, interdigital lesions, ring formation on the coronet region, and gangrene at tip of the tail). The treatment resulted in a significant rise of blood selenium levels and concurrent increase in erythrocytic glutathione peroxidase activity. Blood glutathione levels were lowered. Selenosis caused oxidative stress as evidenced by increased lipid peroxidation. Glutathione-S-transferase, glutathione reductase, superoxide dismutase, and catalase activities were significantly increased (Kaur *et al*., 2003).

Pigs were fed sodium selenite or Se-enriched yeast, added at 0, 5, 10, 15, or 20 mg/kg Se to corn-soybean meal diets for 12 weeks. The results suggest that dietary Se from inorganic or organic sources was toxic at 5 mg/kg Se, but subsequent selenosis were more severe and occurred sooner with sodium selenite. Decreased weight gain, hair loss (alopecia), and separation of the hoofs, increased plasma GOT activity, and other effects were reported (Kim and Mahan, 2001).

In controlled feeding trials comparing seleno-DL-methionine with *Astragalus bisulcatus* or sodium selenate, growing swine developed signs of Se toxicity including paralysis. Signs were more severe and occurred sooner in the *A. bisulcatus* group (Panter *et al*., 1996).

7.2.3 Humans

7.2.3.1 Exposure to Supplements

Two individuals took selenium-containing yeast at doses of 200 and 400 µg selenium daily for 18 months. Together with dietary intake, they received approximately 350 and 600 µg/day. Marginal hematological changes and a borderline increase in serum ALAT were seen (Schrauzer and White, 1978).

A small group of patients with rheumatoid arthritis receiving 250 µg Se as organic selenium in addition to selenium from food for 6 months had decreased levels

of somatomedin C in serum compared with a group receiving a placebo (Thorlacius-Ussing *et al*., 1989). A similar effect was not observed when graded doses of 100, 200, and 300 µg selenium as selenium wheat was given to healthy, Norwegian volunteers for a 6-week period (Meltzer *et al*., 1993), nor was the effect observed in North Americans with a natural selenium intake range of 68–724 µg/day (Salbe *et al*., 1993).

In a study by van Dokkum *et al*. (1992), two groups of six male volunteers were given eight slices of bread per day for 6 weeks. The bread was made with selenium-rich and selenium-poor wheat. In the treatment group, the bread provided 200 µg selenium/day per subject. In a study by Longnecker *et al*. (1993) groups of four healthy male volunteers were fed bread containing 32.4, 206, or 388 µg selenium/day. Before the study, the intake was 80 µg/day. In both studies, no adverse effects were reported, although such information was not specifically sought.

In a supplementation study where 400 µg/day of selenium as selenite or selenomethionine (total dose 450–500 µg/day) were given for 3 months to 32 healthy women, half of them experienced symptoms of depression and extreme tiredness during the month after the termination of the study (Meltzer and Haug, 1995).

In a randomized, double-blind, placebo-controlled study, the effect of selenium supplementation on prevention of skin cancer was investigated (Clark *et al*., 1996). A total of 1312 patients (mean age, 63; range, 18–80) with a history of basal cell or squamous cell carcinoma were treated with 200 µg selenium/day in the form of high-selenium brewer's yeast tablet (Nutrition 21, La Jolla, Calif.) or placebo for up to 10 years (mean, 4.5 years). The percentage of males in the control and treatment groups was 75.6 and 73.8, respectively. Mean plasma selenium concentration at the start of the study was 114 µg/L (1.44 µmol/L), which was in the lower end of the range of normal plasma levels reported in the United States. (In most European countries, however, the mean serum levels are lower [Alexander and Meltzer, 1995; Johnsson *et al*., 1997]). Plasma selenium levels remained constant throughout the study in the placebo group, whereas plasma selenium rose to 190 µg/L (2.4 µmol/L) in the treatment group within 6–9 months from the beginning. The safety endpoints investigated included known signs of frank selenosis (see later) including garlic breath, pathological nail changes, and brittle hair. Patients were assessed every 6 months, and the authors observed no dermatological or other signs of selenium toxicity. A total of 35 patients, 21 in the selenium group and 14 in the control group, complained about adverse effects, mostly gastrointestinal, that resulted in withdrawal from the study. Although it is difficult to assess the intake on the basis of serum values because these might vary according to the source of selenium, an estimate can be that a mean intake of approximately 90 µg selenium/day would correspond to a serum value of 114 µg/L (1.44 µmol/L) (Alexander and Meltzer, 1995; Johnsson *et al*., 1997). Hence, the total intake after supplementation would be approximately 290 µg selenium/day.

Twenty-four men with biopsy-proven prostate cancer were randomly assigned to either 1600 ($n = 8$) or 3200 µg/day (n = 16) of selenized yeast and treated for an average of almost 12 months. Symptoms of selenium toxicity were assessed by patient interview with specific questions regarding breath, hair, and nail changes. Several liver and kidney function tests and hematology were measured at 6-month intervals. The mean plasma selenium levels achieved with supplementation were 4492.2 ± 188.3 ng/mL and 639.7 ± 490.7 ng/mL for the 1600 and 3200 µg/day doses, respectively. Blood chemistry and hematology results were all within normal limits for both treatment groups. More subjects on 3200 µg/day reported symptoms of selenium toxicity; however, these reports did not correspond to peaks in plasma selenium levels. No obvious selenium-related serious toxicities were observed. The authors conclude that additional information on selenium species, sequestration of selenium in specific organs, excretion, and toxicities is needed (Reid *et al*., 2004).

7.2.3.2 Epidemiological Studies

Public health consequences of high dietary intakes of selenium have been investigated in selenium-rich areas of South Dakota (Smith, 1937). The most common symptoms were gastrointestinal disturbances, icteroid discoloration of the skin, and decayed teeth. Less frequent symptoms were recurrent jaundice, dermatitis, and pathological nails. It is difficult to evaluate the exposure levels and validity of these early findings (WHO, 1987). Children living in a seleniferous area in Venezuela have been compared with children living in Caracas (Jaffe, 1976). The level of selenium in blood averaged 813 µg/L (10.3 µmol/L) in the seleniferous area, and in one child reached 1800 µg/L (22.8 µmol/L). Using the Chinese data on blood/intake relationships (Yang *et al*., 1989), a level of 813 µg/L (10.3 µmol/L) corresponds to a daily intake of approximately 10 µg Se/kg bw. It was found that pathological nail changes, loss of hair, and dermatitis were more common in the seleniferous area. However, the reason whether these differences were due to selenium toxicity was not entirely clear, because the groups differed in several other nutritional aspects.

Clinical symptoms associated with selenium poisoning such as those described previously are usually

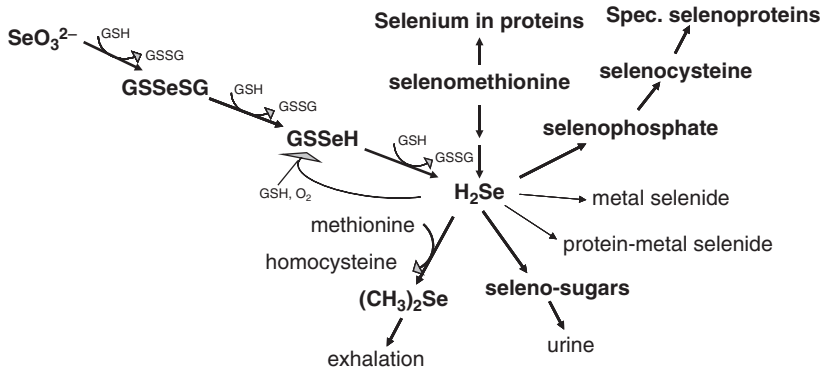

FIGURE 1 Biotransformation of selenite and selenomethionine to selenide and excretable metabolites. Specific and unspecific incorporation of selenium into proteins and interactions of selenide with molecular oxygen and metals are also shown.

referred to as selenosis. A more detailed description of symptoms is given in the following.

In China, endemic selenium intoxications caused by high selenium in soil have been studied by Yang and colleagues (1983). Morbidity was 49% among 248 inhabitants of five villages with a daily intake of approximately 5000 µg selenium. The main symptoms were brittle hair with intact follicles, new hair with no pigment, and thickened nails, as well as brittle nails with spots and longitudinal streaks on the surface. In more severe cases, fluid effused from around the nailbed. Another common finding was lesions of the skin, mainly on the backs of hands and feet, the outer side of the legs, the forearms, and the neck. Affected skin became red and swollen, followed by the appearance of blisters and the occurrence of eruptions. Symptoms of neurological disturbances were observed in 18 of the 22 inhabitants of one heavily affected village alone. Patients complained of peripheral anesthesia, acroparesthesia, pain, and hyperreflexia. Later numbness, convulsions, paralysis, and motor disturbances developed. The daily intake among those with clinical signs of selenosis was estimated to range from 3200–6690 µg with an average of 4990 µg selenium. The mean blood level was 3200 µg/L (40.5 µmol/L), and the mean urine level was 2.68 mg/L (33.9 µmol/L). Livestock were also affected in these areas. The residents recovered as soon as the diets were changed. In high-selenium areas without occurrence of selenosis, the daily intake of selenium was calculated to range from 240–1510 µg, with a mean intake of 750 µg. The corresponding blood levels were 440 (350–580) µg/L and 5.6 (4.4–7.3) µmol/L) (mean and SD). The chemical forms of selenium were determined in Chinese rice and maize, and the major form was selenomethionine (Beilstein *et al.*, 1991).

In a follow-up to their earlier study, Yang *et al*. (1989; 1989) studied a population of approximately 400 individuals that was evaluated for clinical and biochemical signs of selenium toxicity. A detailed study of Se intake through various food items as well as measurements of Se in tissues (i.e., whole blood, urine, hair, and fingernails and toenails) allowed more accurate estimation of the dose-response relationships observed for selenium toxicity.

The average daily intakes based on lifetime exposures were 70, 195, and 1438 µg and 62, 198, and 1288 µg for adult male and females, respectively, in the low-, medium- and high-selenium areas. Clinical signs of selenosis (i.e., hair or nail loss, nail abnormalities, mottled teeth, skin lesions, and changes in peripheral nerves) were examined among 349 adult residents and were observed among subjects in the high-selenium area. Subjects with clinical signs of selenosis were classified as ++ or + (mainly finger-nail disease/changes alone and with severe hair loss/skin changes). No clinical signs were observed among those with a blood selenium concentration <1000 µg/L (12.7 µmol/L) (intake according to regression equation (Figure 1; Yang *et al.*, 1989; 853 µg Se/day). The prevalence of subjects with selenosis ++ varied between 3 and 7% in the groups with blood concentrations 1000–1250, 1250–1500, and 1500–2000 µg/L (12.7–15.8, 15.8–19.0, 19.0–25.3 µmol/L). The prevalence of subjects with selenosis + varied from 10–35% in the same groups. No dose-response relationships were seen. The prevalence of subjects with selenosis + was 45% in subjects with a blood concentration >2000 µg/L (25.3 µmol/L) (Yang *et al.*, 1989). Blood selenium concentrations in five subjects with long persistent clinical signs ranged from 1054–1854 µg/L (13.3 to 23.5 µmol/L), with a mean of 1346 µg/L (17.0 µmol/L) corresponding to a daily

intake of 1260 μg Se (range, 913–1907 μg Se) (intake calculated from the regression equation). Prolonged bleeding time was observed clinically on blood collection in the high-selenium areas. Prolonged prothrombin time (>14 seconds) was observed among 1 of 20 subjects with a blood selenium level of 200–990 μg/L (2.53–12.5 μmol/L) and among 45% of those subjects with a blood selenium level >1000 μg/L (12.7 μmol/L) (corresponding to an intake of approximately 850 μg). The mean prothrombin time only increased marginally, but ranges were not given in the publication. A strong reduction in the plasma Se/red cell Se was seen at blood concentrations exceeding 900 μg Se/l (11.4 μmol/L), corresponding to an intake of 750 μg/day. A decreased concentration of glutathione in blood was observed at dietary intakes exceeding 850 μg/day. The prevalence of mottled enamel teeth of school children of 7–14 years of age were 0, 49, and 95% in groups with low (130 ± 20 μg/L [1.65 ± 0.25 μmol/L]), medium (370 ± 320 μg/L [4.68 ± 4.05 μmol/L]), and high (1570 ± 440 μg/L [19.9 ± 5.57 μmol/L]) blood selenium concentration, respectively.

The five patients showing overt signs of selenosis were followed up in a later study (Yang and Zhou, 1994). After a change in the diet, the symptoms disappeared. Their blood levels decreased from 1346 ± 366–968 ± 115 μg Se/L (17.0 ± 4.63–12.3 ± 1.46 μmol/L), corresponding to an in intake of 1270 ± 450–819 ± 126 μg Se/day (calculated from the regression equation) (819 μg corresponds to about 15 μg/kg bw according to the authors). The latter mean value had a lower 95% confidence limit of 567 μg/day. The range was 654–952 μg Se/day.

In an American study (Longnecker *et al*., 1991) 142 subjects from geographical areas with high dietary selenium intakes in the United States were followed over a 2-year period with respect to adverse health effects. The daily dietary intake was assessed by several 48-hour duplicate-plate food collections for selenium determination from one person in the household and by diet questionnaires. The subjects were followed for 1 year, completed health questionnaires, underwent physical examinations, and clinical chemistry tests. Selenium in whole blood, serum, urine, and toenails was also determined. The average selenium intake among adults in South Dakota and Wyoming was 239 μg/day, varying from 68–724 μg/day (half of them had an intake >200 μg/day and 12 individuals >400 μg/day). There was no variation of prothrombin time with selenium intake. However, an association of selenium intake with ALAT in serum was observed. The values were within the reference range and considered clinically insignificant. Increased prevalence of lethargy was also seen with increased selenium values. Nail abnormalities were not related to selenium intake, neither were any other symptoms or physical findings. In contrast to a study from Denmark (Thorlacius-Ussing *et al*., 1989) (see preceding), no relationship between plasma somatomedin C and any of the selenium indices was observed in this study (Longnecker *et al*., 1991).

Brätter and Negreti de Brätter (1996) studied the influence of high dietary selenium intake on the thyroid hormone levels in serum of 125 lactating mothers 20–24 days postpartum from three regions with different selenium intake in Venezuela. The serum concentration of FT_3 (free, unbound T_3) (T_3 is formed from T_4 in the liver) was lower in the group having the highest mean intake, 552 (range, 250–980) μg/day, but all values were found to be within the reference range. The two other regions had mean intakes of 274 (range, 170–500) and 205 (range, 90–350) μg Se/day, respectively. None of the investigated individuals had signs of selenosis.

7.2.3.3 Dose-Response Assessment for Chronic Toxicity in Humans

The Chinese studies of endemic selenium toxicity in humans (Yang *et al*., 1983; 1989; 1994) and the 1991 American study (Longnecker *et al*., 1991) are the only reliable studies on chronic selenium toxicity in humans.

On the basis of the Chinese studies (Yang *et al*., 1989; 1994) the minimum daily dietary intake sufficient to cause symptoms of selenosis (i.e., hair or nail loss, nail abnormalities, mottled teeth, skin lesions, and changes in peripheral nerves) is approximately 1200 μg Se (range, 913–1907 μg Se). The LOAEL for clinical symptoms of selenosis is approximately 900–1000 μg Se/day. No clinical signs of selenosis were recorded in individuals with blood selenium <1000 μg/L, corresponding to an intake of approximately 850 μg/day, which could be taken as a NOAEL for clinical selenosis. Symptoms were also observed in a man taking 913 μg Se/day as selenite (Yang *et al*., 1983). In a follow-up study (Yang and Zhou, 1994), 5 patients (from the study of 349 individuals) recovered from selenosis when their mean intake was reduced to 819 μg Se/day. The 95% lower confidence limit of the mean intake was 567 μg/day.

Symptoms from the liver, which is also affected in animal studies, manifested in increased prothrombin time and a reduction in glutathione in blood cells caused by impaired synthesis of coagulation factors in the liver, became statistically significant at dietary intakes ≥850 μg Se/day (Yang *et al*., 1989). These effects were not considered to be clinically significant.

In the American study (Longnecker *et al*., 1991), no signs of toxicity were seen in a population consuming on average 239 μg Se/day from food. The liver enzyme ALAT in serum, although within the reference range, showed a correlation with selenium intake (68–724 μg/

day), but this was not considered to be clinically significant. No effect on prothrombin time was seen in the latter study. However, the American population studied covered a lower range of selenium intake than the Chinese study, and considering mean body weights it is also likely that the intake per kilogram of body weight was greater in the Chinese study in comparison with the American one.

7.3 Other Diseases Related to Selenium Overexposure

Airborne selenium dioxide may cause conjunctivitis and allergic reactions in the eyes. Occupational long-term exposure may cause a condition known as "pink eye," in which the mucous membrane of the eyelids becomes discolored and puffy (Glover, 1970). Selenium dioxide applied to the skin may produce burns or dermatitis and occasionally urticaria (Glover, 1967). A case of chronic lung granulomatosus has also been reported (Diskin *et al*., 1979).

7.4 Mutagenic Effects

A moderate genotoxic activity of selenium compounds (i.e., selenite, selenate, selenide, selenocysteine, and selenosulfide) has been found in several *in vitro* systems (ATSDR, 2003; Kramer and Ames, 1988; Lofroth and Ames, 1978; Noda *et al*., 1979; Ray, 1984; Ray and Altenburg, 1978; Tennant *et al*., 1987; Whiting *et al*., 1980). There is one *in vivo* study showing chromosomal aberrations and increased SCE in hamster bone marrow cells after selenite treatment (Norppa *et al*., 1980). This occurred only at doses of 3, 4, and 6 mg Se/kg bw intraperitoneally that were associated with severe systemic toxicity including lethality some hours after dosing. The numbers of aberrations in these groups were 13–55% compared with 0.9–1% in the controls. Doses of 0.3, 0.6, 1, and 2 mg Se/kg bw did not cause any clastogenic effects. Mice fed high doses (7–28 mg/kg bw) of selenite or selenate showed dose-dependent chromosome aberrations and affection of spindle structure (Biswas *et al*., 1999). Itoh and Shimada (1996) had similar findings. A nonpregnant macaque dosed with 600 µg selenomethionine for 15 days (lethal dose) showed, in comparison with the control animal, a sevenfold increase in bone marrow micronuclei (Choy *et al*., 1989). In pregnant macaques receiving 0, 150, or 300 µg selenomethionine/kg bw and showing signs of selenosis, fetal bone marrow smears did not show any increase in the number of micronuclei (Choy *et al*., 1993).

Studies *in vitro* indicate that the mutagenic effects of selenium salts are associated with production of reactive oxygen radicals and that glutathione promotes these reactions (Kramer and Ames, 1988). It is well known that autooxidizing selenium metabolites, such as hydrogen selenide, can undergo redox cycling, producing oxygen radicals and cause DNA strand breaks (Anundi *et al*., 1984; Garberg *et al*., 1988; Nuttall, 1987) (see also Section 7.1.1). *In vivo* only toxic amounts were shown to be active, keeping in mind the central role of hydrogen selenide in the metabolism of most selenium compound it is likely that overproduction of this and other autoxidizing selenium metabolites could promote the formation of DNA reactive oxygen radicals. It follows, given such a mechanism, that expression of selenium-dependent genotoxic activity is likely to be concentration and threshold dependent, but this remains to be shown.

7.5 Carcinogenic Effects

7.5.1 Animals

Several early studies showed tumors after selenium exposure (IARC, 1975; 1987; Innes *et al*., 1969; Nelson *et al*., 1943; Schrauzer and Ishmael, 1974; Schroeder and Mitchener, 1971; 1972). These studies have been evaluated on several occasions, and, in general, the data have been considered inconclusive because of many problems with the studies (Diplock, 1984). Nelson gave low-protein diets supplemented with seleniferous wheat or 10 mg selenium salts/kg bw. A number of rats died before 18 months, but none with tumors. In surviving rats, hepatic tumors were found in animals with liver cirrhosis. It has also been questioned whether identified tumors actually were regeneration nodules. The study by Schroeder and Mitchener (1971) lacked adequate controls, and the colony suffered from severe infections.

Synthetic selenium compounds that have shown effects indicative of carcinogenicity are as follows. Selenium diethyldithiocarbamate given to mice (10 mg/kg by gavage daily for 3 weeks) was found to increase the incidence of hepatomas, lymphomas and pulmonary tumors (Innes *et al*., 1969). Seifter *et al*. (1946) gave 0.05% of bis-amino-phenyl selenium dihydroxide in the diet to rats and found an increased incidence of adenomatous hepatic hyperplasia and thyroid adenomas. Selenium sulfide in large oral doses (3 and 15 mg/kg bw/day to rats and 20 and 100 mg/kg bw/day to mice) was found to be carcinogenic to rats and mice (NCI, 1980). In a separate study in mice, selenium sulfide was applied to the skin, and there was no increased incidence of tumors attributable to selenium treatment (NCI, 1980). Under most conditions, the systemic uptake of topically selenium sulfide might be insignificant (Cummins and Kimura, 1971).

Carcinogenicity of selenium compounds seems primarily to be associated with the nature of the compound than with the element itself. The selenium compounds mentioned previously are not relevant regarding the use as sources of selenium in food and or as nutrients. Inhibiting effects of selenium on experimental carcinogenicity are discussed in Section 5.2.3.

7.5.2 Humans

Possible carcinogenesis during occupational exposure has been studied in a group working with selenium in rectifier processing for 26 years. The number of total deaths ($n=17$) and deaths caused by cancer ($n=6$) in this group did not differ from the national incidences (Glover, 1970). Cancer chemoprevention by selenium is discussed in Section 5.2.3.

7.6 Reproductive and Developmental Effects

There are no human studies on adverse effects of selenium on human reproduction (ATSDR, 2003). Animal studies show that excessive selenium exposure has adverse effects on sperm production, formation of abnormal sperm, and testosterone levels. Decreased sperm counts were observed in rats given selenite (0.17 mg/kg bw) or selenate (0.29 mg/kg bw/day) in drinking water for 13 weeks. Similar effects were not observed in mice (NTP, 1994). In female rats exposed to selenate in drinking water (0.418 mg/kg bw/day), a reduced number of corpus lutea and implants of litters and shorter estrous cycles were observed (NTP, 1996). Decreased conception rate and increased fetal absorption were observed in cattle, sheep, and horses fed organic selenium in a dose of approximately 0.5–1.5 mg/kg bw/day (ATSDR, 2003).

It is well established that several selenium compounds such as selenate, selenite, selenocysteine, and, in particular, selenomethionine are teratogens in avian species and in fish (Birge *et al*., 1976; Halverson *et al*., 1965; Heinz *et al*., 1988; Hoffman and Heinz, 1988; Moxon, 1943; Pyron and Beitinger, 1989; Woock *et al*., 1987). Both inorganic and organic forms of selenium pass the placenta in humans and experimental animals (Willhite *et al*., 1990). Terata have also been produced in sheep (Holmberg and Ferm, 1969) and pigs (Wahlström and Olson, 1959). Effects of selenium compounds on reproduction and offspring in rodents have usually been associated with overt maternal poisoning and nutritional deprivation (Berschneider *et al*., 1977; Ferm *et al*., 1990; Nobunaga *et al*., 1979; Schroeder and Mitchener, 1971). Recent studies on macaques fed selenomethionine (25, 150, and 300 µg/kg/day) during organogenesis showed no signs of terata (Hawkes *et al*., 1994; Tarantal *et al*., 1991). Elevated selenium concentrations in fetal tissues, neonatal blood, and milk correlating with selenium intake of the dam were observed. A dose-dependent maternal toxicity was observed in this study. Whereas no signs of treatment-related toxicity in the dams were observed at the dose of 25 µg/kg bw/day (NOAEL), maternal toxicity as indicated by poor appetite and emesis was observed in the mid- and high-dose groups. No treatment-related changes in the teeth, skin, or nails were found.

Selenite, selenate, seleno-DL-methionine, and seleno-DL-cystine were embryolethal *in vitro* at 20, 300, 1000, and 1000 µmol/L Se, respectively. Deformed optic vesicle and swollen rhombencephalon were found. These abnormalities were considered to correspond to *in vivo* malformations seen in hamsters or in birds and suggest that rat embryos are susceptible to Se teratogenicity (Usami and Ohno, 1996).

No indication of teratogenicity of selenium has been shown in humans even in the areas of high selenium intake in China (Yang *et al*., 1989). Among a small group of women handling selenite during laboratory work, only one pregnancy of four certain and one possible pregnancy went on to term, and the infant born had bilateral clubfoot. The question was raised as to whether these effects could be related to selenium exposure, but urine analysis indicated no increased uptake of selenium in this group (Robertson, 1970).

7.7 Interactions with Metals

Interactions between metals and selenium have been extensively studied since the late sixties, and especially after Ganther and coworkers (1972) showed that selenium could protect against methylmercury toxicity. Research has focused on cadmium and mercuric compounds. Interactions with cadmium and mercuric compounds have been extensively reviewed (Magos and Webb, 1980). Besides the finding of metal selenide complexes (e.g., selenium and silver or mercury) in tissue depositions, few investigations on humans have been performed, and, therefore, the relevance of the huge number of laboratory experiments on selenium metal interactions is difficult to assess. For a definition of interaction, the reader is referred to the general discussion of this problem in Chapter 7. Interactions between selenium and other elements can take place both in the environment and within the animal or human being. This section does not cover interactions in the environment.

7.7.1 Arsenic

The protective effect of arsenic (5 mg/kg. sodium arsenite) against selenosis produced by feeding seleniferous grain (15 mg/kg) was first discovered by Moxon in 1938. Arsenite can increase the biliary and the fecal excretion of selenium up to 10-fold and because of effective inhibition of methylation, exhalation of methylated selenium compounds is reduced (Hsieh and Ganther, 1977; Levander and Argrett, 1969; Levander and Baumann, 1966). Arsenic and selenium are excreted as a glutathione complex in bile (Gailer *et al*., 2002). Selenite inhibited arsenic methylation in primary rat hepatocytes (Styblo and Thomas, 2001). Arsenic compounds increased the toxicity of methylated selenium species *in vivo* (Obermeyer *et al*., 1971).

7.7.2 Bismuth

Interaction has been studied in the rat (Szymanska *et al.*, 1977). Selenite increased the whole-body retention of bismuth, but reduced the level in the kidneys. A redistribution of bismuth from a low-molecular-weight protein (mol. weight, 7000) (metallothionein-like protein) to proteins of higher molecular weight was seen in the kidneys. Similar interaction has also been reported for inorganic mercury and cadmium (Magos and Webb, 1980).

7.7.3 Cadmium

Interaction between selenium and cadmium has been extensively studied since it was found that selenium could prevent the damaging effects of cadmium on the rat testicular tissue (Magos and Webb, 1980). Subsequent experiments have revealed that simultaneous or prior administration of selenite protects against many of the acute effects of cadmium (e.g., lethality, gonadal necrosis, placental haemorrhage, teratogenicity, pancreatic dysfunction, elevated systolic blood pressure, and effects on heart metabolism) (Magos and Webb, 1980; Skowerski *et al*., 2000).

Other selenium compounds have also been tested for their ability to counteract testicular damage caused by cadmium. Selenide, selenite, selenate, and selenocysteine were most efficient, whereas selenomethionine was much less active. The former compounds both increased the testicular uptake and caused a redistribution of cadmium to high-molecular-weight proteins (Ridlington and Whanger, 1981). Cadmium-induced acute kidney damage measured as proteinuria and enzymuria was reduced with concurrent selenite administration (Flora *et al*., 1982). There are, however, few reports that indicate that selenium protects against the kidney damage seen after prolonged low-level exposure to cadmium (Magos and Webb, 1980).

Coadministration of selenite changes the cadmium levels in the tissues; selenite changes the protein binding of cadmium from binding to low-molecular-weight proteins to higher molecular-weight ones (mol. weight, 115,000) in the liver and the kidneys and to a Cd-Se-protein complex (mol. weight, 130,000) in blood plasma *in vivo* (Chen *et al*., 1975; Gasiewicz and Smith, 1976; 1978).

Cadmium also reduces the toxic effects of selenite probably by complexing Se^{2-}; however, cadmium administration does not seem to induce signs of selenium deficiency (Magos and Webb, 1980). In smokers, who are exposed to cadmium, cadmium apparently had a depressive effect on selenium in blood, because smoking alone did not operate as a true predictor of this effect (Ellingsen *et al*., 1997).

7.7.4 Cobalt

Necrosis of cardiac and skeletal muscles, similar to that of selenium deficiency, has been found in pigs and ducklings fed high doses of cobalt (200 or 500 mg/ kg). Selenite supplementation (2 mg/kg of the diet) provided complete protection (Van Vleet *et al*., 1981).

7.7.5 Copper

High dietary levels of copper have resulted in signs of selenium deficiency in chicks (Jensen, 1974; Witting and Horwitt, 1964), whereas dietary copper alleviated selenium toxicity in chicks and ponies (Jensen, 1975; Stowe, 1980). Rats fed a low-selenium diet are more sensitive to copper toxicity than controls, and selenite caused a redistribution of endogenous copper to the kidneys and inhibited the biliary excretion (Alexander and Aaseth, 1980).

7.7.6 Lead

Both selenium and vitamin E decrease the toxicity of lead in rats. Selenium was only partially protective in lead-poisoned vitamin E–deficient rats or rats with lead-induced nephropathy (Levander *et al*., 1977), whereas dietary selenium did not protect against lead-induced toxicity in Japanese quail exposed to lead in diet. Selenium had some protective effects in rats exposed to lead naphthenate (Flora *et al*., 1983; Rastogi *et al*., 1976; Stone and Soares, 1976). Coadministration causes a change both in selenium and in lead distribution in the body (Flora *et al*., 1983; Naganuma *et al*., 1983; Rastogi *et al*., 1976).

7.7.7 Mercury

7.7.7.1 Inorganic Mercury Compounds

Inorganic mercury exposure occurs with two forms of the element: mercury salts (Hg^{2+}) and elemental mercury (Hg^0). The kidney is the critical organ for Hg^{2+}, whereas the CNS is the critical organ for Hg^0. Mercury vapor is probably dissolved in the blood after uptake and is transported into the brain where it is oxidized to Hg^{2+}.

When selenite is coadministered or given shortly after mercuric mercury, it effectively protects against acute tubular necrosis, damage of the kidney, and death. However, repeated simultaneous administration of mercuric chloride with selenite resulted only in partial protection of the kidney damage caused by mercury (Chmielnicka *et al*., 1978). Mercuric administration inhibited GSH metabolizing enzymes and GSHPx in the kidney. This effect was reversed by selenite administration (Chung *et al*., 1982). Coadministration of the two elements usually leads to increased whole-body retention of both elements and especially of mercury. Both renal and fecal excretion of mercury are reduced, and changes in the organ distribution are seen (Hansen *et al*., 1981; Kristensen and Hansen, 1979; Magos and Webb, 1980). When mercury and selenium are coadministered, selenium and mercury form a high-molecular-weight complex with a plasma protein, thereby reducing Hg in target organs such as the kidney. High-molecular-weight complexes of Hg and Se are also seen in other organs. The protein in plasma is selenoprotein P (Yoneda and Suzuki, 1997). Timing of Hg and Se exposure seems to be critical, because only exposure hours apart strongly reduces the protective effect of selenium (Magos and Webb, 1980; Watanabe, 2002). Mercuric chloride decreases the toxic effects of selenium and reduces the formation of methylated selenium metabolites, probably by forming complexes with selenide, and potentiates the toxicity of methylated selenium compounds (Levander and Argrett, 1969; Magos and Webb, 1980).

It is not known whether the toxic effects of mercury vapor on the CNS are influenced by selenite. Toxicokinetic studies with Hg^0 and selenium have been performed on rats and mice. Experiments on mice showed that selenite pretreatment did not change the immediate uptake and distribution of mercury but markedly increased the retention in the whole body and especially in the lungs (Hansen *et al*., 1981; Khayat and Dencker, 1983). In rats, mercury retention tended to be elevated in liver and serum (Nygaard and Hansen, 1978). In retired miners previously exposed to elemental mercury, Kosta *et al*. (1975) found a coaccumulation of mercury and selenium in molar ratio close to unity in the kidneys, the pituitary, the brain, and the thyroid. The brain levels of mercury were remarkably high, up to 13 μg/g. Analysis of biopsy specimens from skin pigmentations found in mercury-exposed workers revealed deposits containing mercury and selenium (Kennedy *et al*., 1977). Contrary to a previous finding, workers exposed to elemental mercury vapor seem to excrete significantly more selenium in urine than unexposed controls (Alexander *et al*., 1983).

7.7.7.2 Organic Mercury Compounds

Organic mercury compounds usually include alkyl mercurials, methoxyethylmercury, and phenylmercury. The latter two compounds have seldom been studied with respect to toxicity and interaction with selenium. Of the alkylmercury compounds, most work has been done with methylmercury, which has also been most extensively studied with respect to its interaction with selenium (Ganther, 1980; Magos and Webb, 1980; Skerfving, 1978). Methylmercury selectively damages the nervous system in man, leading to ataxia, dysarthria, constriction of the visual field, and paresthesias. The protective effect of selenium against methylmercury toxicity was first reported by Ganther *et al*. (1972). Later, such protection was found in chick, quail, rat, mice, and cat (Ganther, 1980; Magos and Webb, 1980; Skerfving, 1978). When methylmercury is given in a single dose or in a limited number of doses, selenite effectively prevents the onset of neurological disorders. During long-term dosing with methylmercury, selenium offers some protection and at least delays the onset of symptoms (Chang, 1983; Magos and Webb, 1980). Remarkably small amounts of selenite are required to provide a protective effect even in cell cultures (Alexander *et al*., 1979; Ganther, 1980). The protective effects occur even if coadministration of selenium leads to heightened levels of mercury in brain (Alexander and Norseth, 1979; Chen *et al*., 1975; Ganther, 1980; Magos and Webb, 1980). However, decreased brain levels have also been reported (Komsta-Szumska and Miller, 1984). The elimination of mercury after several doses of methylmercury can be described by a one-compartment model (half-time, 23.6 days). Coadministration of several doses of selenite and methylmercury revealed a two-compartment model for elimination of mercury (half-time, 8.7 and 40.8 days) (Komsta-Szumska and Miller, 1984). The formation of a lipophilic $(CH_3Hg)_2Se$ complex, probably easily penetrating the blood–brain barrier, has been reported to take place both *in vitro* and *in vivo* (Magos and Webb, 1980; Masukawa *et al*., 1982; Naganuma and Imura, 1980). Reduced biliary excretion of MeHg is a prominent effect of selenium compounds, and, concomitantly, an increase in exhalable methylated selenium metabolites is observed. It has

been speculated that MeHg might serve as a methyl donor for selenium (Alexander and Norseth, 1979; Gregus *et al*., 2001; Urano *et al*., 1997).

Decreased levels of GSH-px in several organs including the brain are seen after methylmercury exposure. These are restored even when small amounts of selenium are added (Ganther, 1980). Also other selenoproteins, such as the deiodinases, can be affected by MeHg exposure (Watanabe, 2002). In macaques exposed to MeHg, a coaccumulation of inorganic mercury and selenium in thalamus and also in the occipital pole of their brains (Bjorkman *et al*., 1995). Interactions between selenium and methylmercury have not been described in humans.

7.7.8 Platinum

Cu-Diamminedichloroplatinum (cisplatin) is a potent anticancer drug widely used in human cancer therapy. However, a serious form of dose-related cumulative renal toxicity limits its use in patients. Several selenium compounds could effectively reduce the renal toxicity of cisplatin without compromising antitumor activity of cisplatin in animal models (Berry *et al*., 1984; Naganuma *et al*., 1983; 1984). The efficacy of selenocysteine conjugates seems to depend on activation to selenols by ß-lyase (Rooseboom *et al*., 2002).

7.7.9 Silver

In a number of animal species (e.g., rat, turkey, poults, ducklings, and pigs), signs of selenium or vitamin E deficiency (e.g., liver necrosis, myopathy, and exudative diathesis) can be induced by feeding high levels of silver. Such effects of silver could be antagonized by administering even modest doses of selenium relative to silver (Diplock, 1976; Ganther, 1980). Conversely, silver ions can protect against selenium toxicity (Jensen, 1975). Coadministration of selenium and silver causes changes in organ levels of both elements. Retention of silver in the body and especially in the blood plasma and kidneys is seen. Selenite inhibited biliary excretion of silver (Alexander and Aaseth, 1981; Naganuma *et al*., 1983). Ag_2Se precipitates were found in the glomerular basement membrane of an argyria patient (Aaseth *et al*., 1981). This has later been confirmed in other studies showing silver-selenium complexes in argyria (Sato *et al*., 1999).

7.7.10 Tellurium

In ducklings and pigs, tellurium, which belongs to the same group as selenium in the periodic system, induced signs of selenium and vitamin E deficiency, which could be counteracted in the ducklings by dietary selenium (Van Vleet *et al*., 1981).

7.7.11 Thallium

Thallium toxicity is prevented by selenate, and coadministration of selenate and thallium increased the tissue levels of both compounds (Levander and Argrett, 1969; Rusiecki and Brzezinski, 1966).

8 PREVENTION, DIAGNOSIS, PROGNOSIS, AND TREATMENT

To prevent health problems related to occupational selenium exposure maximum levels for the working environment have been established.

Occupational exposure limits (OELs) (according to WHO, IPCS, 1994 International Chemical Safety Cards, http://www.cdc.gov/niosh/ipcsneng/neng0072.html): TLV: 0.2 mg/m^3 as TWA; (ACGIH 2004). MAK: (inhalable fraction) 0.05 mg/m^3; Peak limitation category: II(4); Carcinogen category: 3B; pregnancy risk group: C; (DFG 2004). Swedish OEL: 0.1 mg/m^3. OSHA PEL*: TWA 0.2 mg/m^3 *NOTE: The PEL also applies to other selenium compounds (as Se) except selenium hexafluoride. NIOSH REL*: TWA 0.2 mg/m^3 *NOTE: The REL also applies to other selenium compounds (as Se) except selenium hexafluoride. NIOSH IDLH: 1 mg/m^3 (as Se).

The few known cases of acute poisoning in humans have shown uncharacteristic neurological and gastrointestinal symptoms. The characteristic garlic odor of the breath is not always reported. In more long-term exposure, garlic odor of the breath and nail, hair, and skin changes seem to have been of diagnostic value. Except for two intoxications (Carter, 1966; Yang *et al*., 1983) that resulted in death, the outcome of described intoxications has been without sequel. This indicates that the prognosis, in general, is good. Although the antidote effects of chemicals such as arsenite and cyanide are well documented in studies on laboratory animals, there are no reports on cases where these antidotes have been used in the treatment of systemic intoxications in humans. Dimercaprol may enhance the toxicity of selenium in animals (Carter, 1966); but in one successfully treated case (Civil and McDonald, 1978), vitamin C (4 g/day) and dimercaprol (150 mg × 4/day) were used. Symptomatic treatment should be given. After oral exposure, administration of charcoal in slurry is recommended in Hazardous Substances Data Bank (HSDB). Clothes should be removed to decrease dermal exposure. Painful skin, nail, and eye disorders have been successfully treated with 10% solutions or ointments of thiosulfate (Glover, 1970).

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CHAPTER

39

Silver

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ABSTRACT

Silver compounds may be absorbed through inhalation, but there are no quantitative human data on the extent of this phenomenon. Silver salts may be absorbed by up to 10–20% after ingestion. The highest concentrations of silver are usually found in the liver and spleen, and to some extent in the muscles, skin, and brain after ingestion. The biological half-time for silver ranges from a few days for animals up to approximately 50 days for the human liver; it is possible that skin deposits have an even longer half-time, but there are no quantitative data on this for man. Silver binds to high-molecular-weight proteins and metallothionein in tissue cytosol fractions. Excretion of silver from the body is primarily biliary.

Water-soluble silver compounds such as the nitrate have a local corrosive effect and may cause fatal poisoning if swallowed accidentally. Chronic exposure of humans leads to argyria, a clinical entity characterized by grey-blue pigmentation of the skin and other body viscera. Repeated exposure of animals to silver may produce anemia, cardiac enlargement, growth retardation, and degenerative changes in the liver.

1 PHYSICAL AND CHEMICAL PROPERTIES

Silver (Ag): atomic number, 47; atomic weight, 107.868; density, 10.5; melting point, 961.93°C; boiling point, 2212°C; crystalline form white metal, cubic 0.54; oxidation states +1, +2. Pure silver has the highest thermal and electrical conductivity of all metals. The metal forms important alloys with copper and other metals. The compounds of silver discussed here include silver nitrate, silver lactate, silver picrate, silver acetate, and the silver halides.

2 METHODS AND PROBLEMS OF ANALYSIS

There are spectrographic, colorimetric, and polarographic techniques for measurement of silver. The detection limit for spectrographic and dithizone colorimetric methods has been put at 0.01 mg/L for a 20-mL sample (APHA, 1966; Rooney, 1975). A summary of measurements of silver in various biological media is provided in the Toxicological Profile (ATSDR, 1990). The most recent approaches involve modified atomic absorption, inductively coupled plasma/atomic emission spectrometry, and inductively coupled plasma/mass spectrometry. The best examples of these approaches have been developed as standardized methods for biological samples (NIOSH, 1994) and water and solid waste (USEPA, 2003).

3 PRODUCTION AND USES

3.1 Production

The main ore from which silver is produced is argentite. Silver is recovered from this ore by cyanide extraction, zinc reduction, or electrolytic processes. The world production by mining of silver in 2004 is reported to be 634.4 million fine ounces (The Silver Institute,

2005), corresponding to approximately 19,600 metric tons. The estimated United States mining production for 2004 was 1200 metric tons (U.S. Geologic Survey). Mexico, Peru, Australia, China, and Poland were the top five silver mining countries in 2004 (The Silver Institute, 2005).

3.2 Uses

The primary use of silver is in industrial applications. The industrial applications include electrical applications, particularly as conductors, switches, and contacts; plating applications; silver brazing or soldering; and miscellaneous uses such as mirrors, batteries, catalyses in chemical reactions, and as bactericide and algaecide. The second most important use is the production of coins, jewelry, and tableware. Silver is extensively used in photographic processing, with the radiographic market being the most significant market followed by the consumer market (The Silver Institute, 2005). Silver salts are also used as disinfectants. Recently, production of silver nanoparticles has been started for application in various nanotechnologies, incorporation into fibers (Reip, 2005), and suggested as antibacterial agent in bone cement for use in arthroplasty (Alt *et al.,* 2004).

4 ENVIRONMENTAL LEVELS AND EXPOSURES

4.1 General Environment

4.1.1 Food and Daily Intake

A detailed mapping of various dietary sources of silver is not available. Silver may be ingested through consumption of marine organisms containing low concentrations (see later) and in small amounts released from dental fillings (Leirskar, 1974) and eating utensils (Harvey, 1970). Tipton *et al*. (1966) found that two subjects had ingested between 10 and 20 µg/day from food during a 30-day period. Wester (1971), by NAA, found a daily intake from food of 1–16 µg. US EPA estimates silver intake from diet ranged from 10–44 µg/day (US EPA, 1980).

4.1.2 Water, Soil, and Ambient Air

Seawater has been reported to contain silver concentrations of 0.055–1.5 µg/L (Schutz and Turekian, 1965). Much higher concentrations (0.03 mg/L) have been reported in wastewater effluents entering southern California coastal basins (Bruland *et al*., 1974; Galloway, 1972; Young *et al*., 1973). Silver has also been reported at concentrations of 14–20 mg/kg in bottom sediments in these areas (Bruland *et al*., 1974; Galloway, 1972). Molluscs collected from coastal areas of the North Sea have been reported to contain silver concentrations of up to 2.0 mg/kg (Dutton *et al*., 1973; Segar *et al*., 1971). Drinking water, not treated with silver for disinfection purposes, usually contains extremely low concentrations of silver. A range of nondetectable to 5 µg/L was reported for 380 finished waters, waters that have been treated and are ready to be delivered to consumers, from the U.S. sources (Carson and Smith, 1975). Treated waters may contain 50 µg/L or even slightly higher concentrations (Just and Szniolis, 1936). The U.S. Environmental Protection Agency has set the drinking water standard, the maximum contaminant level, at 0.1 mg/L (US EPA). Typical background levels found in rivers of the United States are 1–2 ng/L (Shafer *et al*., 1998). Concentrations of 5–150 µg/L have been reported in sewage sludge (Berrow and Webber, 1972) and 28-8 µg/g dry wt. in Stockholm 1995–2005.

Soil concentrations of silver vary greatly by geological area (Wells and Elliott, 1971). Granite igneous rock in Nevada has been reported to contain up to 50 mg/kg Ag.

Coal fly-ash has been reported to contain up to 15 mg/kg Ag (Headlee and Hunter, 1953), and emission of silver from coal-fired power plants may lead to its accumulation in soil of adjacent areas.

Silver has been measured in air as a result of anthropogenic actixzxvity. Air concentrations of silver over southern California have been reported to be approximately 2 ng/m^3 (Bruland *et al*., 1974). The emission of silver iodide crystals during cloud seeding has been estimated to result in a silver concentration in air of approximately 0.1 ng/m^3 (Sargent, 1969; Standler and Vonnegut, 1972). Silver concentrations in rainwater as a result of this process were estimated to be between 0.04 pg/mL and 5 ng/mL.

4.1.3 Tobacco

Concentrations of silver in cigarettes are low. Because of its high boiling point, most of the silver in cigarettes is probably not inhaled.

5 METABOLISM

5.1 Absorption

5.1.1 Inhalation

The deposition fraction of 0.5 µm spherical silver particles in the lungs of dogs has been found to be approximately 17% (Phalen and Morrow, 1973). Newton and Holmes (1966) studied the long-term retention in a human subject who had accidentally inhaled radioactive silver (chemical form not known). Their data indicated that the liver uptake was maximal

less than 2 weeks after inhalation, suggesting absorption of silver compounds deposited in the alveoli, although clearance and partial absorption of the silver compounds by the gastrointestinal tract cannot be excluded. Takenaka *et al*. (2001) found rapid clearance of ultrafine silver particles from the lungs of rats, with 96% clearance in 7 days. The fate of inhaled silver nanoparticles has not been studied.

5.1.2 Ingestion

The intestinal absorption of silver by mice, rats, monkeys, and dogs has been recorded at approximately 10% or less after ingestion of radioactive silver (Furchner *et al*., 1968). A value of 18% was estimated from studies in one single human subject (East *et al*., 1980) given silver acetate.

5.2 Distribution

Dogs exposed to silver by inhalation accumulated most of the administered dose in the liver, with lower concentrations in the lung, brain, and muscle (Phalen and Morrow, 1973). Studies on rodents have also indicated a high initial concentration of silver in the liver that decreases greatly within 10 days, whereas silver concentrations in the spleen and brain are retained for longer periods (Furchner *et al*., 1968; Gammill *et al*., 1950). Other studies (Matuk, 1983; Oh *et al*., 1981; Tanaka *et al*., 1983) have shown that approximately 60% of the silver in liver and kidneys of silver-injected rats may be recovered in the cytosol fraction bound to both high-molecular-weight proteins and the metallothionein fractions. Only approximately 30% of total tissue silver could be recovered in cytosol fractions of rat spleen and forebrain (Matuk, 1983). In a human being exposed to radioactive silver, >50% of the body burden of silver was found in the liver 16 days and later after exposure (Newton and Holmes, 1966).

5.3 Excretion

Mice, rats, monkeys, and dogs given radioactive silver salts by oral, intravenous, or intraperitoneal routes were found to excrete >90% of the absorbed dose through the feces (Furchner *et al*., 1968). The fecal elimination is mainly explained by biliary excretion (Scott and Hamilton, 1948). Fecal elimination has also been found to be the primary excretory pathway after exposure by inhalation to silver in dogs (Phalen and Morrow, 1973) and in humans (Newton and Holmes, 1966). Alexander and Aaseth (1981) reported that rats injected intravenously with radioactive silver nitrate excreted silver in the bile mainly bound to a low-molecular-weight complex that seemed to be glutathione.

5.4 Biological Half-Time

After rabbits had inhaled 4-μm mass median aerodynamic diameter (MMAD) monodispersed silver-coated Teflon particles, Camner *et al*. (1974) found that an average of 30% of the particles deposited were cleared from the lung in 1 day and another 30% during the rest of the first week of the exposure. After exposure by inhalation, dogs cleared 59% of an administered dose of radioactive silver from the lungs in 1.7 days (Phalen and Morrow, 1973). The liver had a somewhat slower clearance. An apparent biological half-time of approximately 1 day was found by whole-body scintillation counting in mice, rats, monkeys, and dogs after oral ingestion. This short half-time is partly due to fecal elimination of unabsorbed silver (Furchner *et al*., 1968). Somewhat longer half-times were observed for these species after intravenous injection of silver, with monkeys and dogs having half-times of 1.8 and 2.4 days, respectively. Silver injected into rats as radioactive silver nitrate had a half-time of 2.2 days (Habighorst and Buchwald, 1971). Matuk (1983) reported, for rats given a single intraperitoneal injection of radioactive silver nitrate, a 50% reduction in radioactivity of whole blood, plasma, kidney, and liver after approximately 40 hours. A 50% reduction in the radioactivity content of spleen occurred after approximately 70 hours, whereas that of brain took approximately 84 hours. The biological half-time of silver in the lungs of a human exposed to silver has been estimated to be 1 day, whereas that in the liver was 52 days (Newton and Holmes, 1966). In a carcinoid patient, Polachek *et al*. (1960) estimated a half-time of 48 days in the liver by external counting after intravenous injection of radioactive silver. On post-mortem examination, 195 days after injection, the highest concentration of radioactive silver was found in the liver and the second highest in the skin, indicating a relatively long half-time for the skin as well.

6 LEVELS IN TISSUES AND BIOLOGICAL FLUIDS—REFERENCE VALUES

The concentrations of silver in the kidneys, liver, and spleen of "normal, healthy people" have been reported by emission spectroscopy to be approximately 0.4, 0.7, and 2.7 mg/kg, respectively, on a dry-weight basis (Indraprasit *et al*., 1974). Lower values (nd 0.05 [kidney], nd 0.032 [liver] and nd 0.06 [lung] mg/kg wet wt) have been reported by neutron activation analyses by Brune *et al*. (1980). Normal concentration in skin was reported as 0.035±0.015 mg/kg dry weight by Schropl *et al*. (1968). Hamilton *et al*. (1972) reported

0.006±; 0.002 mg/kg wet weight in liver, 0.001 ± 0.002 in kidney, and 0.002 ± 0.000l in lung. Plasma samples from an unexposed population were all <1 µg/L. (Wan *et al*., 1991).

Tipton *et al*. (1966) found between 0.006 and 0.015 mg/day in urine and between 0.02 and 0.11 mg/day in feces. Wester (1971) by use of NAA found 0.9–1 µg/day in urine and 0.9–97 µg/day in feces of two patients with pancreatic insufficiency. In urine, Bostrom and Wester (1968) found 1 µg/day in normal persons and 0.7–7.5 µg/day in patients with untreated hyperthyroidism. Wan *et al*. (1991) observed urinary excretion levels in a control group generally to be <2 µg/day.

7 EFFECTS AND DOSE-RESPONSE RELATIONSHIPS

7.1 Local Effects and Dose-Response Relationships

Silver salts are pronouncedly caustic and have been used widely in the treatment of warts. Silver salts are used for the treatment of burns and have a strong antiseptic effect. Dressings soaked with 0.5% $AgNO_3$ have been extensively used without any evident local or systemic silver toxicity (Hartford and Ziffren, 1972).

The use of health guidance values provides information in the risk assessment when individuals may be exposed to hazardous substances. The reference dose (RfD) developed by the US EPA is an estimate of a daily oral exposure to the human population that is likely to be without an appreciable risk of deleterious effects during a lifetime. An RfD for silver has been derived (US EPA, 2005) as 0.005 mg/kg/day or for a standard 70-kg person, 0.35 mg/day.

Repeated occupational handling of silver objects, especially if repeated minor injury is involved, may give rise to so-called local argyria, which is bluish grey discoloration of the skin at the exposed site. Although not esthetic, this condition is considered harmless (Holzegel, 1970). Case reports have observed localized argyria as a result of silver earrings (Morton et al. 1996, Shall et al. 1990, van den Nieuwenhuijsen et al 1988), silver filigree (Ellison et al 1993) and acupuncture needles (Suzuki et al. 1993, Yamashita et al. 2001, Kakurai et al. 2003).

7.2 Systemic Effects and Dose-Response Relationships

7.2.1 Animals

The primary toxic effects of silver seem to be exerted on the cardiovascular, hepatic, and hematopoietic systems. Cardiac enlargement and ventricular hypertrophy have been reported in turkeys after dietary exposure to 900 mg/kg silver nitrate for 18 weeks (Jensen *et al*., 1974; Peterson *et al*., 1973). Centrilobular hepatic necrosis and ultrastructural alteration of mitochondria and lysosomes have been described in livers of vitamin E–deficient rats exposed to silver (Grasso *et al*., 1970). Repeated intraperitoneal injections of silver lactate to rats for prolonged periods have been found to produce silver deposition in lysosomes and basal lamina of the brain and spinal cord (Rungby and Danscher, 1983) and anterior pituitary (Thoriacius-Ussing and Rungby, 1984). Anemia of the microcytic, hyperchromic type is a common finding among animals chronically exposed to dietary levels of silver compounds at several hundred milligrams per kilogram (Jensen *et al*., 1974; Peterson *et al*., 1973; Shouse and Whipple, 1931). Deposits of silver granules in the glomeruli and tubules of the kidney, as well as pathological changes in renal tubules, were reported by Fuchs and Franz (1971) in rats exposed to 0.2% silver nitrate in drinking water in various groups from 10–50 weeks. On the basis of silver toxicity mechanisms for freshwater fish and crustaceans, researchers have suggested that the whole-body sodium ion uptake rate serves as a good predictor of sensitivity to acute silver toxicity (Bianchini *et al*., 2002). Silver nanoparticles tested *in vitro* in mammalian germline stem cells (Braydich–Stolle *et al*., 2005) were the most toxic type of metal particles compared to other metal nanoparticles and Mo-nanoparticles the least toxic ones. Effects of inhaled silver nanoparticles have not been studied in animals.

7.2.2 Humans

7.2.2.1 Acute Effects

Fifty milligrams or more of collargol (Ag salt) has been reported to be lethal after intravenous injection for therapeutic purposes. Autopsy findings in such cases have included pulmonary edema, hemorrhage, and necrosis of bone marrow, liver, and kidney (Hill and Pillsbury, 1939; Patein and Roblin, 1909). Intrauterine administration of approximately 7 g of silver nitrate has been reported to be rapidly fatal in man (Reinhart *et al*., 1971).

7.2.2.2 Chronic Effects

Repeated exposure to silver salts or colloidal silver by inhalation or ingestion brings about effects classically described as generalized argyria. This clinical entity is characterized by a grey-blue discoloration of skin, most pronounced in areas exposed to light (Buckley and Terhaar, 1973; Hanna *et al*., 1974; Holzegel, 1970; Honigsmann *et al*., 1973; Kugler *et al*., 1972; Lehnert, 1973; Rich *et al*., 1972). The discoloration is explained by deposits of microscopically detectable silver-containing granules in the corium and particularly around the hair follicles (Nasemann *et al*., 1974) and in the sebaceous

and sweat glands (Schell and Hornstein, 1974). Deposition of silver compounds causing discoloration and sometimes also functional impairment and/or clinical symptoms may also occur in other organs. Such particles found in the basement membrane of the kidney were demonstrated to consist of silver selenide (Aaseth *et al*., 1981).

Deposition of silver compounds in the cornea and the anterior capsule of the lens as may be detected by slit-lamp examination (Koelsch, 1956; Larsen, 1927; Rosenman *et al*., 1979) causes some impairment of vision in exceptional cases.

Argyria of the respiratory tract, liver, kidneys, and gastrointestinal tract has been reported and may be associated with certain clinical symptoms such as chronic bronchitis (Montandon, 1959) and abdominal discomfort (Franken and Langhof, 1964). However, because these case reports have not been properly evaluated in relation to reference groups, it is impossible to say whether there is a true causal relationship between exposure to silver and these symptoms. The exposure conditions giving rise to argyria have not been well defined. Evaluation of the older literature (Hill and Pillsbury, 1939) indicated that a total dose of 1–8 g Ag would be required to induce the condition in a long-term inhalation exposure situation.

The dosage required to induce argyria by ingestion seems to be somewhat higher (i.e., between 1 and 30 g of soluble silver salts) (Lehnert, 1973). Silver in metallic form is harmless with the possible exception of colloidal silver preparations. Blond people are considered more susceptible than others.

Concentrations of silver in body organs associated with discoloration and/or clinical symptoms are not known. A certain increase above normal seems to be without harmful effects, however. Nordberg *et al*. (1978) and Brune *et al*. (1980) reported tissue concentrations in the kidneys, liver, and lungs of autopsy samples from nonferrous smelter workers, of up to 0.2–0.3 mg/kg wet weight without obvious adverse effects.

The minimal tissue concentrations leading to argyria of the skin are not well known. The silver concentration in the skin of persons with argyria was 64 mg/kg dry weight (Schropl *et al*., 1968). East *et al*. (1980) reported 72 mg/kg wet weight in one case. These values are several thousand times higher than the normal value reported by Schropl (see Section 6). Ultrastructural examination of skin biopsies from patients with argyria has demonstrated silver deposition in the basal lamina of the various skin layers (Honigsmann *et al*., 1973), as well as in the sweat glands (Lehnert, 1973). Silver-containing granules have also been detected in lysosomes and in connective tissue fibers of the skin (Raymond *et al*., 1980).

7.3 Interactions with Selenium, Copper, and Vitamin E

Dietary administration of silver acetate has been found to antagonize selenium toxicity (Diplock *et al*., 1967). Conversely, addition of selenium, copper, and vitamin E to the diet in varying concentrations has been reported to decrease the toxicity of silver (900 mg/kg in diet as silver acetate) in turkey poults (Jensen *et al*., 1974). The addition of selenium to the diets of rats exposed to silver in drinking water prevented growth retardation but increased hepatic and renal concentrations of silver (Wagner *et al*., 1975). Alexander and Aaseth (1981) reported that concomitant administration of selenite increased retention of silver in the kidneys, brain, and blood of rats, apparently as a result of formation of insoluble silver-selenium complexes. Silver has been observed to alter copper metabolism, and no moderation of the effect is observed with zinc administration (Hirasawa *et al*., 1994).

8 TREATMENT

There is no recognized effective treatment for argyria. The condition seems to be relatively stationary when exposure to silver is discontinued. Chelation therapy is considered ineffective (Aaseth *et al*., 1981; Lehnert, 1973).

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CHAPTER

40

Tellurium

LARS GERHARDSSON

ABSTRACT

The main source of exposure in the general population is food (e.g., meat, dairy products, and cereals). In the working environment, inhalational exposure predominates. Small amounts of organic tellurium compounds can also be absorbed through the skin. No quantitative data have been published regarding the inhalational or gastrointestinal absorption of tellurium or tellurium compounds in humans. In animal experiments, the intestinal absorption has been estimated to range between 10 and 25%.

The highest tissue concentrations have been observed in the kidneys. Increased levels have also been noted in blood, heart, lungs, liver, spleen, muscle, and bone. The main accumulation is in bone, which harbors >90% of the total body burden. Tellurium can pass both the placenta and the blood–brain barriers. Parenterally administered tellurium is predominantly excreted in the urine, whereas orally ingested tellurium salts through biliary secretion are mainly excreted in the feces. Small amounts, probably approximately 0.1%, of absorbed tellurium are exhaled, presumably as dimethyltelluride. In rat experiments, biological half-times ranging from 9 days in blood to 23 days in kidney have been reported. The whole-body retention model for man estimates a biological half-time of approximately 3 weeks. The elimination from bone is slow, with an estimated half-time of approximately 600 days.

Acute systemic effects of tellurium toxicity in rats include listlessness, decreased locomotor activity, somnolence, anorexia, weight loss, gastrointestinal disturbances, changes in fur, and occasionally epilation and hind leg paralysis. Long-term studies of chronic effects are sparse. Dominant and critical effects have been reported from the nervous system, including peripheral neuropathy characterized by segmental demyelination and minor axonal degeneration. In the brain, black changes caused by dark tellurium particles that were localized in lipofuscin granules in neuron cytoplasm have been observed. Other effects have been reported from the liver (fatty degeneration and necrosis), kidney (proximal tubular lesions, oliguria, or anuria), and heart (cell necrosis, edema, and congestion).

Reproductive effects including, for example, hydrocephalus, edema, exophthalmia, and ocular hemorrhage have been described.

Acute exposure to tellurium in occupational settings may cause acute respiratory irritation followed by the development of garlicky odor of the breath and sweat, drowsiness, headache, malaise, lassitude, weakness, and dizziness. Gastrointestinal symptoms such as anorexia, nausea, vomiting, metallic taste, dry mouth, and constipation may appear. Dermatitis and blue-black discoloration of the skin may follow from exposure to tellurium hexafluoride. Severe intoxication may lead to depression of the respiratory system and circulatory collapse. No specific antidote for tellurium poisoning has been found. After inhalational exposure, treatment with fresh air, oxygen supply, assisted ventilation, beta$_2$ agonists, and oral or parenteral corticosteroids can be tried. Reviews of tellurium toxicology have been published by Browning (1969), Izrael'son (1973), Fishbein (1977), Alexander *et al*., (1988) and Kobayashi (2004).

1 PHYSICAL AND CHEMICAL PROPERTIES

Tellurium (Te): periodic system group VIb; atomic weight, 127.6; atomic number, 52; density, 6.2 (20°C); melting point, 449.5°C; boiling point, 989.8°C; specific gravity, 6.24 (20°C). Tellurium exists in two allotropic forms, as a silvery white crystalline metal and as a black amorphous powder. Twenty-one isotopes of tellurium are known, with atomic masses ranging from 115–135. Natural tellurium consists of eight isotopes (mainly ^{130}Te, ^{128}Te, and ^{126}Te), one of which (^{127}Te) is unstable. It is present to the extent of 0.87% and has a half-life of 1.2×10^{13} years. Chemically, tellurium resembles selenium and sulfur. Tellurium burns with a greenish blue flame in air and forms tellurium dioxide, which is only sparingly soluble in water. It forms compounds in oxidation states –2, +2, +4, and +6. Of toxicological interest are elemental tellurium, hydrogen telluride, and tellurium hexafluoride (the last two being colorless gases)—tellurium dioxide, tellurous and telluric acids, and sodium and potassium tellurites and tellurates, which are soluble in water. Organotellurium compounds are well documented in the literature. Several organometallic and complex compounds of tellurium have been described (e.g., aryl-, diaryl-, and triaryl tellurium(IV) chlorides). Recently, a new series of organotellurium compounds have been prepared (e.g., bis[2-hydroxymethylphenyl] telluride, bis[2-hydroxymethylphenyl] ditelluride[and bis]4-hydroxymethylphenyl] telluride) (Al-Rubaie and Al-Jadaan, 2002).

Elemental tellurium, which is insoluble in water (Cooper, 1971) does not easily form an oxide when heated in air. Tellurium dioxide is only sparingly soluble in water at room temperatures up to 40°C (e.g., at 18°C at pH 6.5); its solubility is 3.75×10^{-5} mol/L.

2 METHODS AND PROBLEMS OF ANALYSIS

Analyses of biological samples by photometry, conventional flame atomic absorption spectrometry, or inductively coupled argon plasma spectrometry are often complicated by the low sensitivity of the methods. Other methods with a higher sensitivity include neutron activation analysis (NAA; Nason and Schroeder, 1967a), inductively coupled argon plasma mass spectrometry, hydride generation atomic absorption spectrometry, and graphite furnace atomic absorption spectrometry (GF-AAS; Welz and Sperling, 1999). To avoid disturbances from interfering elements, it is often necessary to separate tellurium from these elements before the determinations by use of ion-exchange or solvent extraction methods for example. Lockwood and Limtiaco (1975) found a detection limit for tellurium of 0.50 µg/mL of the solubilized sample solution with electronically excited oxygen and atomic absorption spectrophotometry. Later Siddik and Newman (1988) used platinum as a modifier to increase the sensitivity when determining the tellurium concentrations in biological samples by flameless atomic absorption spectrophotometry. Detection limits in urine, plasma, and tissues in mice were approximately 50, 5, and 170 ng/mL or gram, respectively. Low detection limits, <50 ng/L, have also been found when using inductively coupled plasma mass spectrometry (ICP-MS) for the determination of tellurium in urine of nonoccupationally exposed subjects (Schramel *et al*., 1997). Recently, hydride generation atomic absorption spectrometry has been combined with preconcentration of the analyte by coprecipitation (Kaplan *et al*., 2005), giving a detection limit of 0.03 µg/L and a precision of 3.5% at the 10 µg/L level.

3 PRODUCTION AND USES

3.1 Production

Tellurium is rarely found in a free state, and there are no ore deposits that can be mined. It is sometimes found in its native copper tellurium lead form, but more often as the telluride of gold (calaverite) or bound to other metals such as silver and bismuth.

The main part of commercial tellurium is recovered from electrolytic copper refinery slimes. The tellurium concentration of the slimes can range up to 10%. Through autoclaving of slimes at elevated temperatures, the extraction of tellurium can range from 50–80%. Tellurium is recovered from the solution by cementation with copper. Copper telluride is leached with caustic soda and air to form a sodium tellurite solution. This solution with excess free caustic soda is thereafter electrolyzed in a cell using stainless steel anodes to produce tellurium metal (Kirk-Othmer, 1997).

To form commercial tellurium metal (99.5%), tellurium dioxide is dissolved in hydrochloric acid. The tellurium solution is saturated with sulfur dioxide gas to yield commercial tellurium powder, which is washed, dried, and melted (Kirk-Othmer, 1997). The estimated global production of tellurium was approximately 124 tons in 1999 (US Dept of the Interior, Bureau of Mines, 2001).

3.2 Uses

Tellurium compounds can be used in catalysts and catalytic processes (e.g., tellurium oxide, tellurium tetrachloride), as metal coatings (e.g., tellurium

chloride), as pigments (e.g., cadmium-telluride); in thermoelectric materials and as semiconductors (e.g., tellurides of silver, bismuth, copper, germanium, manganese, lead, and antimony); as semiconductors and solar cells in pocket calculators and cameras (e.g., tellurides of Bi and Cd); for corrosion resistance of electroplated nickel (e.g., sodium telluride); in therapeutic applications (e.g., asatine-211-tellurium colloid); and in the vulcanization and curing of rubber, in primary (nonrechargeable) and secondary (chargeable batteries) and fuel cells (Fishbein, 1991). It can be used to improve the crop strength of tin and the mechanical properties of lead. Tellurium can also be used as a coloring agent in chinaware, porcelains, enamels, glass, and for producing black finish on silverware (O'Neil, 2001). The largest use for tellurium (approximately 50% of the market) was as an additive to free-machining steel. Chemicals and catalyst use made up approximately 25% of the market. The remaining part is divided between additives to nonferrous alloys (10%), photoreceptor and thermoelectric applications (10%), and other uses (5%; USGS, 2002).

Organic tellurium compounds (e.g., tellurides) have been used as initiators for tellurium radical polymerization. Electron-rich monotellurides and ditellurides possess antioxidant activity.

4 ENVIRONMENTAL LEVELS AND EXPOSURES

4.1 General Environment

4.1.1 Food and Daily Intake

In 1967a, Schroeder *et al*. estimated the tellurium content in a number of food items. The concentrations reported were 4.2 mg/kg in meats, 4.8 mg/kg in dairy products, 2.8 mg/kg in cereals, 1.8 mg/kg in fats and oils, 1.1 mg/kg in fruits, and 0.44 mg/kg in hospital diets. These figures are, however, probably a considerable overestimation of the trues values. After revising these analyses, Nason and Schroeder (1967b) estimated the daily intake in humans to be approximately 100 µg.

4.1.2 Water, Soil, and Ambient Air

Reported concentrations in natural water include 0.31 ng/L in China (Yu *et al*., 1983), 0.5 ng/L in the Florida Bay (Andreae, 1984), 0.9 ng/L in the Red Sea (Sugimura and Suzuki, 1981), 0.22 ng/L in the North Atlantic (Lee and Edmond, 1985), and 0.17 ng/L in the Panama Bay (Lee and Edmond, 1985). Somewhat higher levels have been reported from seawater in South China (80 ng/L) and East China (40–70 ng/L; An and Zhang, 1983). Values approximately 10 ng/L have been reported from the Japanese inland sea of Seto (Chung *et al*., 1984).

Values of the same magnitude have also been observed in rainwater. Andreae (1984) found concentrations of 3.3 ng/L in Florida in February 1984 and slightly lower levels, 0.51 ng/L, at repeated measures in March 1984.

Soil concentrations of 0.035 mg/kg have been reported from Yokohama (Japan). Similar levels of 0.031 mg/kg have been found in Seoul (Korea; Hashimoto *et al*., 1989).

Reported levels in ambient air ranges from 0.12 ng/m^3 in Yokohama, Japan (Hashimoto *et al*., 1989), 0.46 ng/m^3 in Missouri (Chou and Manuel, 1986), and up to 0.35–50 ng/m^3 in NASN (Scott *et al*., 1976). In 1971, Seljankina and Alekseeva found a concentration approximately 2 µg Te/m^3 at a distance of approximately 2 km from an electrolytic copper-refining plant.

4.1.3 Plants

Data concerning the tellurium concentration in plants is very scanty. In one study of plants growing on natural tellurium-containing soil, the tellurium concentrations in saltbush and cactus were 2 and 25 mg Te/kg, respectively (Beath *et al*., 1935).

4.2 Working Environment

In 1942, Steinberg *et al*., determined air concentrations in an iron foundry. The values ranged from 0.01–0.1 mg/m^3. Approximately 70% of the values were observed in the range 0.01–0.05 mg/m^3. Values of the same magnitude, ranging from 0.01–0.7 mg/m^3, were found in a more recent study from an iron foundry (Blackadder and Manderson, 1975).

5 METABOLISM

5.1 Absorption

5.1.1 Inhalation

No quantitative information is available as to the human respiratory absorption of inhaled tellurium or tellurium compounds.

5.1.2 Ingestion

No quantitative data have been published regarding the intestinal absorption of tellurium or tellurium compounds in humans. Studies in animals indicate that up to 25% of orally administered tellurium (elemental

tellurium and tellurium dioxide) is absorbed in the gut (Hollins, 1969; Taylor, 1996). The gastrointestinal absorption of tellurium is completed within 2 hours after the intake. In rats, the main part of the absorption takes place in the duodenum and jejunum, and the total absorption was estimated to be in the range 10–25% (Slouka and Hradil, 1970). In sheep and pigs, these compounds are mainly absorbed further down in the colon (Wright and Bell, 1966).

5.1.3 Skin Absorption

The handling of tellurium compounds may cause skin burns or rashes, followed by a garlic odor in the breath indicating skin absorption. Blackadder and Manderson (1975) reported two cases of chemists who were accidentally exposed to tellurium hexafluoride gas when approximately 50 g leaked from a cylinder in the laboratory. They both showed the characteristic garlic odor of the breath, and one of them also developed an intradermal bluish black pigmentation in the skin of the fingers, face, and neck that took several weeks to disappear. The authors suspected that the skin absorption was caused by volatile tellurium esters.

5.2 Distribution

In rat experiments, approximately 90% of tellurium in blood enters the erythrocytes, probably bound to hemoglobin (Agnew and Cheng, 1971; Slouka, 1970). The main part of the remainder is bound to plasma proteins. Tellurium can pass both the placenta and the blood–brain barriers (Agnew, 1972; Agnew *et al*., 1968). The highest tissue concentrations have been found in the kidney. The levels observed in heart, lung, and spleen have been approximately 10–30% of the kidney concentrations. Levels in liver have been approximately 50% of those in the lungs, heart, and spleen. Measured concentrations in cardiac muscle have been approximately 20 times higher than in skeletal muscle. In the nervous system, when injected intracerebrally, tellurium accumulates in the gray matter. Tellurium also accumulates with time in bone (Browning, 1969), which harbors more than 90% of the total body burden (Schroeder *et al*., 1967).

5.3 Excretion

The excretion pattern depends on the chemical forms and mode of administration of tellurium and tellurium compounds. Parenterally administered tellurium is predominantly excreted in the urine, whereas orally ingested tellurium salts are mainly excreted in the feces (Durbin, 1960). After oral intake in rats, approximately 60–80% of ingested tellurium is rapidly excreted in the feces. Tellurium is transferred to the intestine by biliary excretion (Hollins, 1969; Slouka, 1970). Small amounts may also be excreted in milk and sweat.

Small amounts, probably approximately 0.1%, of absorbed elemental tellurium and tellurite are exhaled, presumably as dimethyltelluride, causing a characteristic garlic-like odor of breath and sweat (Cooper, 1971; DeMeio, 1946).

5.4 Biological Half-Time

The whole-body excretion of tellurium and tellurium compounds followed a two-phase pattern in experiments on rats (Hollins, 1969; Slouka, 1970). The fast phase had a biological half-time of approximately 19 hours (42–49% of the dose), and the figures for the slow phase were 13–15 days (51–58%). The whole-body retention model for man (ICRP, 1968) estimates a biological half-time of approximately 3 weeks. In rat experiments, biological half-times of 9.2 days were found in blood, 10.2 days in liver, 17.7 days in muscle, and 23 days in kidney (Hollins, 1969). The elimination from bone seems to be very slow, with an estimated half-time of approximately 600 days (Hollins, 1969).

6 BIOLOGICAL MONITORING

6.1 Levels in Tissues and Biological Fluids

Tellurium concentrations in whole blood in normal subjects may range from 0.15–0.3 µg/L (van Montfort *et al*., 1979). Tellurium levels in urine in normal subjects ranged from <0.1–10 µg/L (Fodor and Barnes, 1983; Kobayashi and Imaizumi, 1991). Observed concentrations in liver, lung, and kidney were 4–21 ppm, 0.04–1 ppm, and 0.6–16 ppm (µg/g wet weight), respectively (Iyengar *et al*., 1978). In a study of tellurium concentrations in hair and nails of normal Japanese adults, the levels as measured by hydride generation atomic absorption spectrometry were lower than the detection limit of the instrument (<1 ng/g; Fukabori and Nakaaki, 1990; Nakaaki and Fukabori, 1990).

The estimated whole-body content in normal subjects, as determined by neutron activation analysis, is approximately 500 mg (Nason and Schroeder, 1967a).

6.2 Biomarkers of Exposure

Blood and urinary analyses may be used for biological monitoring, but data are very scanty. Tellurium values for normal subjects have been reported

for blood (0.15–0.3 µg/L; van Montfort *et al*., 1979) and urine (<0.1–10 µg/L; Fodor and Barnes, 1983; Kobayashi and Imaizumi, 1991). Only few and incomplete data are, however, available from the working environment and regarding the relationship between tellurium in air, blood, and urine. Accordingly, it is not clarified whether these relationships are linear or nonlinear.

6.3 Biomarkers of Effects

Quantitative data on the relationship between internal dose and adverse health effects are still lacking and, thus, risk assessment cannot be carried out. More studies are needed to produce data that can be used for dose-effect and dose-response studies in humans. An early and classical sign is the garlicky odor of the breath, which may appear after ingestion of 40 µg of soluble tellurium.

7 EFFECTS AND DOSE-RESPONSE RELATIONSHIPS

Tellurium is not regarded as an essential trace element. Thus, tellurium deficiency is not a problem in humans or animals.

7.1 Local Effects and Dose-Response Relationships

7.1.1 Animals

Inhalation of elemental tellurium and tellurium dioxide aerosols (10, 50, and 100 mg/m^3; 2 hours daily; 13–15 weeks) caused desquamatory bronchitis and lobar pneumonia in rats (Izrael'son, 1973; Sandrackaja, 1962a). Inhalation of high concentrations of tellurium hexafluoride (50 mg/m^3; 1 hour) produced pulmonary edema in rabbits, guinea pigs, rats, and mice (Kimmerle, 1960). Decreased body weights and black deposits in lung tissue have been observed in male Harlan Wistar albino rats 6 months after a single intratracheal instillation of tellurium and tellurium-dioxide (Geary *et al*., 1978).

7.1.2 Humans

Exposures to tellurium vapor and hydrogen telluride may lead to irritation of the respiratory tract (Izrael'son, 1973; Popova *et al*., 1965), leading to bronchitis and pneumonia (Lewis, 1996). Dermatitis and blue-black skin discoloration may occur from exposure to tellurium hexafluoride. Ingestion of no more than 40 µg of soluble tellurium may lead to the unusual breath odor. It has been suggested that the formation of organic tellurium compounds (e.g., dimethyl telluride) may be responsible for the garlic-like odor to breath, possible by inhibiting the enzyme squalene epoxidase (Taylor, 1996).

7.2 Systemic Effects and Dose-Response Relationships

7.2.1 Animals

Acute and subacute studies of systemic toxicity of tellurium and tellurium compounds in rats show a number of symptoms (e.g., listlessness, decreased locomotor activity, somnolence, anorexia, weight loss, gastrointestinal disturbances, changes in fur, and occasionally epilation and hind leg paralysis) (Amdur, 1958; DeMeio, 1946; DeMeio and Jetter, 1948; Sandrackaja, 1962a).

7.2.1.1 Liver

Studies on rats, rabbits, and Peking ducks exposed to different tellurium compounds (tellurium dioxide, tellurium tetrachloride, sodium tellurite) showed liver changes ranging from simple cellular swelling to hydropic and fatty degeneration and cell necrosis (Carlton and Kelly, 1967; DeMeio and Jetter, 1948; Elnicnyh and Lencenko, 1969; Lencenko, 1967; Sandrackaja, 1962b). Other changes detected included impairment of glycogen function, detoxifying functions, and of protein metabolism as indicated by dose-related reduction of galactose tolerance, hippuric acid excretion, decrease of albumin/globulin ratio in serum, urinary bilirubin excretion, and inhibition of cholinesterase (Lencenko, 1967; Lencenko, and Plotko 1969; Sandrackaja, 1962b).

7.2.1.2 Kidney

Rats exposed to tellurium dioxide through inhalational exposure (condensation aerosol, 10 and 50 mg/m^3, 2 hours daily, 13–15 weeks) showed signs of focal vacuolization of cells and hemorrhage in the glomeruli, followed by albuminuria and hematuria (Sandrackaja, 1962a,b). In another study, rats were fed tellurium dioxide (375–1500 mg Te/kg diet, 24–128 days; De Meio and Jetter, 1946). The treatment resulted in changes ranging from cellular swelling to necrosis, accompanied in some rats by oliguria and anuria. The findings indicated severe lesions in the proximal tubular epithelium (De Meio and Jetter, 1948).

7.2.1.3 Blood

Rats exposed to tellurium dioxide and elemental tellurium aerosol (50–100 mg/m^3, 2 hours daily, 13–15 weeks) developed a normochromic, possibly

hemolytic, anemia with signs of dose-related reductions in hemoglobin concentration and erythrocytes, as well as hematuria (Sandrackaja *et al*., 1962a,b).

7.2.1.4 Heart

Peking ducks fed tellurium tetrachloride in the diet (50–1000 mg Te/kg, 2–4 weeks) developed myocardial hemorrhage, hydropericardium, and cardiac muscle necrosis (Carlton and Kelly, 1967). Similar changes, including cardiac damage with hydropericardium and myocardial hemorrhage, were observed in a study of newly hatched ducklings fed a commercial diet with 500 mg of tellurium tetrachloride per kilogram of feed for 2–4 weeks. A histopathological investigation showed areas of acute hemorrhagic necrosis in the ventricular myocardium, as well as signs of edema and congestion (Van Vleet and Ferrans, 1982).

7.2.1.5 Nervous System

Rats fed 0.2 g/day of tellurium showed black brain changes caused by dark tellurium particles that were localized in lipofuscin granules in neuron cytoplasm throughout the brain. The changes are probably due to effects of tellurium on brain mitochondria (Duckett and White, 1974).

Peroxidation-related effects on the brain have been studied in 15 male Wistar rats fed drinking water containing telluriumtetrachloride at a level of 100 mg/L. Control rats were given tapwater. The rats were killed in groups of five 7, 21, and 35 days, respectively, after the exposure. The succinic dehydrogenase activity was above the control range after 21 days, whereas the creatinine-kinase activity decreased or remained stable. The brain glutathione content was above the control range after 35 days (Valkonen and Savolainen, 1985).

Rats fed a diet containing 1.25% elemental tellurium starting on postnatal day 20 developed garlic odor within 48 hours and usually hind limb paresis within 72 hours. Treated rats showed progressive increases in blood nerve barrier permeability 24–72 hours after exposure. The blood–brain barrier, however, was not affected. Other effects included increased numbers of intracytoplasmic lipid droplets, intracytoplasmic membrane delimited clear vacuoles, and cytoplasmic excrescences within myelinating Schwann cells after 24 hours, axon demyelination after 48 hours, and endoneurial edema after 72 hours. The synthesis of cholesterol was sharply inhibited after 12 hours (Bouldin *et al*., 1989).

Weanling rats fed a diet containing elemental tellurium may develop peripheral neuropathy characterized by segmental demyelination and minimal axonal degeneration. An early neuropathy sign is an increasing number of cytoplasmic lipid droplets in myelinating Schwann cells. They are probably derived from newly synthesized lipid rather than from the early breakdown and internalization of myelin lipids. The earliest observed biochemical abnormality in tellurium neuropathy is an inhibition of the cholesterol synthesis at the squalene epoxidase step, causing an accumulation of squalene within the nerve (Goodrum *et al*., 1990). The metabolic changes observed in the sciatic nerve, for example, probably initially involve the inhibition of the conversion of squalene to 2,3-epoxysqualene. This block in the cholesterol biosynthesis pathway may, directly of indirectly, lead to the inhibition of the synthesis of myelin components and to the breakdown of myelin (Harry *et al*., 1989; Morell *et al*., 1994).

Starting at postnatal day 21, weaned rats were fed a diet containing 1.1% of elemental tellurium. After 7 days of tellurium treatment and at several time points of post-tellurium treatment, the animals were processed for ultrastructural analysis, Schwann cell nuclei quantification, and teased fiber preparations. Quantitative determination of Schwann cell nuclei per transverse section of sciatic nerve showed a dramatic increase of Schwann cells 2 days after tellurium treatment compared with control nerves. The number of Schwann cell nuclei then decreased progressively during the long-term period of recovery studied (330 days after tellurium treatment). Dying cells exhibited morphological features of apoptosis. Both healthy immature Schwann cells and endoneurial macrophages were involved in the phagocytosis of apoptotic Schwann cells. Also other distinct biological mechanisms such as the persistence of supernumerary Schwann cells in the endoneurium and the shortening of internodal lengths are involved in the regulation of Schwann cell numbers during the remyelination stage (Berciano *et al*., 1998).

Developing rats fed a diet containing 1.1% tellurium get a primary demyelination of peripheral nerves, which is followed by a period of rapid remyelination. The demyelination that is caused by the limiting of the supply of cholesterol leads to repression of the expression of mRNA for myelin-specific proteins. Tellurium exposure was followed by an increase in total RNA (largely rRNA) in sciatic nerve, which could not be accounted for by cellular proliferation. The increased concentrations of rRNA may be a reactive response of Schwann cells to toxic insult and may relate to the higher levels of protein synthesis required during remyelination. On the other hand, steady-state levels of mRNA, determined by Northern blot analysis, for P0 and myelin basic protein were markedly decreased. Message levels increased during the subsequent period of remyelination and reached almost normal

levels 30 days after the beginning of the tellurium exposure. The coordinate alterations in message levels for myelin proteins indicate that Schwann cells can down-regulate and then up-regulate the synthesis of myelin in response to alterations in the supply of membrane components (Toews *et al*., 1990).

7.2.1.6 Reproductive and Developmental Effects

Pregnant rats fed 500–3000 ppm tellurium in the diet developed a high incidence of hydrocephalic offspring (Mackison *et al*., 1981).

The teratogenicity and lethality of tellurium diethyldithiocarbamate (TeDE) have been investigated in 3-day chicken embryos. TeDE had a greater toxicity as regards the effect parameters: early deaths, late deaths of malformed embryos, late deaths of nonmalformed embryos, and malformed survivors, compared with, for example, copper and zinc acetate (Korhonen *et al*., 1983).

The developmental toxicity of tellurium was studied in Crl Sprague–Dawley rats and New Zealand white rabbits. Groups of pregnant rats were fed a diet containing 0, 30, 300, 3000, or 15,000 ppm of tellurium on days 6 through 15 of gestation. Artificially inseminated rabbits were fed a diet containing 0, 17.5, 175, 1750, and 5250 ppm of tellurium during days 6–18 of gestation. Signs of maternal toxicity were detected in a statistically significant and dose-related manner at dietary concentrations of 300 ppm and greater in rats and 1750 ppm and greater in rabbits. Skeletal (mainly skeletal maturational delays) and soft tissue malformations (primarily hydrocephalus) were observed in the offspring of pregnant rats exposed to the highest levels (3000 and 15,000 ppm) of tellurium. Rabbit fetuses in the highest exposed group (5250 ppm) showed slightly elevated signs of skeletal delays and nonspecific abnormalities (Johnson *et al*., 1988).

The effects of multiple maternal subcutaneous injections of tellurium dioxide (TeO_2) suspended in olive oil (0–1,000 µmol/kg) from day 15–19 of gestation have been studied in Wistar rats. Multiple maternal injections at doses exceeding 10 µmol/kg caused a dose-related appearance of hydrocephalus, edema, exophthalmia, ocular hemorrhage, umbilical hernia, undescended testis, and small kidneys in fetuses on day 20 of gestation. At 500 µmol/kg, reduction in maternal weight was also noted. At this level, the incidence of the anomalies previously mentioned was 100%. The 100-µmol/kg dose of Te resulted in a 100% incidence of hydrocephalus and edema without causing any apparent maternal toxic responses. Accordingly, tellurium can be teratogenic to the rat fetus without causing concomitant maternal toxicity (Perez-D'Gregorio and Miller, 1988). Tellurium, however, may induce both maternal toxicity (e.g., reduction in maternal weight gain) and teratogenic effects in the rat when the teratogenicity is not mediated by alterations in the diet (Perez-D'Gregorio *et al*., 1988).

Teratogenic effects have also been observed in Wistar rats after single maternal subcutaneous injections of 0.12 mg/kg diphenyl ditelluride, diluted in canola oil at days 6, 10, or 17 of gestation. A single maternal injection at day 10 caused malformation in fore limbs and hind limbs, absent or short tail, subcutaneous blood clots, exophthalmia, hydrocephalus, and absence of the cranial bone and cutaneous tissue in fetuses on day 20 of gestation (Stangherlin *et al*., 2005). Effects of diphenyl ditelluride on the *in vitro* incorporation of ^{32}P into intermediate filament proteins from slices of cerebral cortex of 17-day-old rats have been studied by Moretto *et al*. (2005). Concentrations of 1, 15, and 50 µmol/L ditelluride induced hyperphosphorylation of the high-salt Triton insoluble subunits, glial fibrillary acidotic protein (GFAP) and vimentin, without altering the immunocontent of these proteins.

Neonatal rats were fed tellurium through the mother's milk, from the day of birth until sacrifice at 7, 14, 21, and 28 days of age. Investigations with light and electron microscopy revealed degeneration of Schwann cells and myelin in the sciatic nerves at each age studied. In the CNS, hypomyelination of the optic nerves was convincingly demonstrated at 14, 21, and 28 days of age, accompanied by some evidence of myelin degeneration. These changes were also observed in the ventral columns of the cervical spinal cords, although less markedly. There was little evidence of oligodendrocyte pathology in the CNS, and it seems that degeneration of these cells is not the primary cause of the CNS hypomyelination in contrast to the PNS where Schwann cell degeneration has been shown to precede the myelin pathology (Jackson *et al*., 1989). Tellurium damages the endothelium, crosses the vascular wall of endoneurial and perineurial vessels in weanling rats, causes a perivascular edema, cytoplasmic anomalies in the Schwann cells, destruction of myelin, and apparently invades axons (Duckett, 1982). Tellurium has been found to penetrate more quickly and in larger amounts the walls of blood vessels in the sciatic nerve of weanling rats intoxicated with tellurium than with the same nerve in other weanling and adult rats.

7.2.1.7 Miscellaneous Biochemical Effects

Rats fed sodium tellurite orally (daily doses ≥0.005 mg Te/kg) for 7 months showed reduced activities of catalase and free thiol groups in the blood (Lencenko, 1967). Similar findings have been reported by Sandrackaja (1962a,b). Other studies in rats exposed to maximum tolerable doses of 0.5 mg and 0.1 mg Te/kg body weight

showed a significant reduction of acetylcholine esterase and monoamine oxidase in serum and brain, as well as a significant decrease in hepatic glutathione, glutathione-S-transferase, and alkaline phosphatase in liver and kidney (Srivastava *et al*., 1983). Weanling rats fed sodium tellurite (150 µg Te/kg body weight per day) in diet and drinking water for 11–30 months showed increased serum cholesterol in male but not in female animals (Schroeder, 1968).

Khayat and Dencker (1984) compared the effects of tellurium and selenium in lung and other organs. The results showed that tellurium (IV) was as effective as selenium (IV and VI) (all given in a dose of 10 µmol/kg body weight) in retaining inhaled $^{203}Hg^{0}$ (1.5 µmol/kg body weight) in the lung (probably transformed to $^{203}Hg^{2+}$ after oxidation). Tellurium (IV) had to be given in a dose of 100 µmol/kg body weight to give the same effect. At a dose level of 10 µmol Te(IV) per kg body weight, the mercury retention ratios (treated/control) were 140 for the lung and 8.6 for the whole body. The corresponding figures for Te (VI) for the doses 10, 30, and 100 µmol/kg body weight were 10, 73, and 120 and 3.7, 3.9, and 4.3, respectively. The retention of $^{203}HgCl_2$ injected intravenously was increased in a dose-dependent manner by preadministration of tellurium. Te (IV) was 3–10 times more effective than Te(VI). The kidney and spleen were the dominant organs, as was the case after Se pretreatment (Khayat and Dencker, 1984).

7.2.2 Humans

7.2.2.1 Occupational Exposures

Thirteen workers had a suspected exposure to tellurium, possibly in the form of hydrogen telluride and tellurium dioxide dust, when working at blast furnaces where the slime of an electrolytic lead refinery was treated (Shie and Deeds, 1920). Seven of the exposed workers showed classical signs of tellurium intoxication with symptoms such as garlic odor of the breath, sweat, and urine; metallic taste; and dryness of the mouth. Five of the workers showed a decreased sweat production, and three of them had a dry and itchy skin, anorexia, nausea, vomiting, depression, and somnolence. Similar symptoms were reported by Steinberg *et al*. (1942) in iron foundry workers exposed to 0.01–0.1 mg Te/m^3 for almost 2 years. Of the 98 exposed workers, garlic odor of breath was found in 84 workers and garlic odor of sweat in 30 workers. All exposed workers showed an excretion of tellurium in urine, but none of the referents. The same disease pattern developed in three laboratory workers after approximately 30 minutes of accidental exposure to tellurium-containing fumes (Amdur, 1947). The tellurium concentrations in urine ranged between 8 and 16 µg/L.

Two cases of nonfatal intoxication after occupational exposure to tellurium vapor were reported by Popova *et al*. (1965). Signs and symptoms included garlic odor of breath, general weakness, cough, shivering, amnesia, pallor of the skin, and black-green discoloration of the mucosa of the tongue and the nasopharynx. Temperature and pulse rate were increased, and moderate leukopenia, neutrophilia, and leukocytosis were present.

Two postgraduate chemists were hospitalized for 2 and 3 days, respectively, after accidentally inhaling tellurium hexafluoride gas, when 50 g leaked from a cylinder in the laboratory (Blackadder and Manderson, 1975). Both showed typical signs and symptoms of intoxication; in particular, the stench of sour garlic was noted on breath and from excreta. One of the subjects showed an unusual bluish black discoloration of the webs of the fingers and streaks on the face and neck. Neither of the patients developed any signs of permanent damage, and both of them made a full spontaneous recovery without treatment.

7.2.2.2 Accidental Nonoccupational Exposure

Tellurium intoxications are rare and almost exclusively confined to occupationally exposed workers. Only a few cases of nonoccupational poisoning have been reported so far. Three cases of accidental tellurium poisoning have been presented by Keall *et al*. (1946). By mistake they were given a solution of sodium tellurite instead of sodium iodide during retrograde pyelography. For two of the patients the estimated dose was approximately 2 g (30 mg/kg). Two of the patients developed symptoms such as vomiting, renal pain, stupor, loss of consciousness, irregular breathing, and cyanosis, and died after approximately 6 hours. At autopsy all tissues emitted a strong garlic odor. A deposition of black tellurium was found in the mucosa of the bladder and of the ureter. Congestion was found in the lungs, liver, spleen, and kidney.

Predominant symptoms during intoxication include loss of appetite, dryness of the mouth, suppression of sweating, a metallic taste in the mouth, and, most notably, a sharp garlic odor of the breath, sweat, and urine. These symptoms were observed in a 37-year-old, nonoccupationally exposed woman with tellurium intoxication (Muller *et al*., 1989).

7.2.3 Summary of Systemic Effects

Data are not sufficient for presenting a dose-response pattern for the different systemic effects reported. In humans, data are particularly sparse, mainly confined to case reports of accidental occupational or nonoccupational exposure. In animals, long-term studies are lacking. Critical and dominant effects seem

to appear in the nervous system, as described previously. An early and critical sign in peripheral neuropathy is a block in the cholesterol biosynthesis pathway, causing an accumulation of squalene within the nerve, followed by the breakdown of myelin.

8 CARCINOGENICITY AND MUTAGENICITY

Tellurium has not been reported as a human or animal carcinogen. Mutagenicity studies are sparse, and more information is needed before firm conclusions can be made. In 1980, Kanematsu *et al*. carried out rec assays on 127 metal compounds with *Bacillus subtilis* to check their DNA-damaging capacity and mutagenicity. Reverse mutation assays with *E. coli* and *Salmonella* strains showed that tellurium compounds ($Na_2H_4TeO_6$, Na_2TeO_3) were potent mutagens. More recently Tiano *et al*. (2000) studied the ability of three diaryl tellurides to protect trout (*Salmo irideus*) erythrocytes against oxidative stress, induced thermally and by variation of pH. At low concentrations (<10 μmol/L), all three tellurides had a protective effect on DNA damage without altering the hemolysis rate. In higher concentrations, they accelerated the hemolysis rate and two of the diaryl tellurides were strongly genotoxic.

There are also several experiments indicating an anticarcinogenic effect of tellurium.

Bloomer *et al*. (1981) found in animal experiments on mice that astatine-211-tellurium colloid (an alpha-emitting radiocolloid) had a curative effect versus experimental malignant ascites without causing undue toxicity to normal tissue. Similarly, *in vivo* experiments showed that Te significantly prolonged the survival of mice implanted with tumors (Sun *et al*., 1996).

Experiments on cancer cell lines have indicated a protective effect by some organotellurium compounds (e.g., diaryl telluride, alkyl aryl telluride, and dialkyl telluride) (Engman *et al*., 1997; 2000; Powis *et al*., 1997; Sun *et al*., 1996).

9 DIAGNOSIS, PREVENTION, AND TREATMENT OF TELLURIUM POISONING

Tellurium intoxications are rare and almost exclusively confined to occupationally exposed workers. There have been no reports of workers dying from exposure to tellurium or tellurium compounds. Accidental deaths have occurred, however. Two of three patients given sodium tellurite instead of sodium iodide during retrograde pyelography died (Keall *et al*., 1946). Acute exposure to tellurium may cause acute respiratory irritation followed by the development of garlicky odor of the breath and sweat. Systemic effects may include fatigue, headache, malaise, lassitude, weakness, dizziness, somnolence, alopecia, and gastritis. Chronic exposure may lead to garlic breath, metallic taste in the mouth, decreased sweating, dryness in the mouth, fatigue, anorexia, and nausea. The skin may develop a blue-black discoloration after exposure to tellurium hexafluoride (Rumack, 2003; Yarema and Curry, 2005).

The garlicky odor of the breath and sweat is an early classical sign and may also be found in urine and feces. Ingestion of as little as 40 μg of soluble tellurium may be sufficient to cause the garlic odor. Tellurium hexafluoride is especially toxic by inhalation and may produce respiratory depression, pulmonary edema, cardiovascular collapse, and death in experimental animals (Rumack, 2003).

After a significant exposure to tellurium hexafluoride, chest X-ray, arterial blood gases, or pulse oximetry may be checked. Also hematological parameters should be monitored.

For treatment of tellurium poisoning after oral exposure, administration of activated charcoal as a slurry (240 mL water/30 g charcoal) may be tried. The usual dose is 25–100 g in adults, 25–50 g in children (1–12 years), and 1 g/kg in infants (<1 year old).

Most cases require no treatment after inhalational exposure. As a first step, the patient should be moved to fresh air. If signs of cough or difficulty in breathing appear, check whether the patient has developed respiratory tract irritation, bronchitis, or pneumonitis. Oxygen supply and assisted ventilation may be required. Bronchospasm can be treated with $beta_2$ agonists and oral or parenteral corticosteroids (Rumack, 2003).

If the eyes have been exposed, they should be irrigated with copious amounts of room temperature water for at least 15 minutes. If irritation, pain, swelling, lacrimation, or photophobia persist, the patient should be sent to a health care facility.

After dermal exposure, contaminated clothing should be removed, and the exposed area should be thoroughly washed with soap and water. If irritation or pain persist, the patient should be sent to a health care facility (Rumack, 2003).

10 STANDARDS—THRESHOLD LIMIT VALUES

Elemental tellurium, Te dioxide and Te chloride, has a TWA of 0.1 mg/m^3 (ACGIH TLV, 2006; OSHA PEL,

2006) and a German MAK value (2006) of 0.1 mg/m^3. The corresponding TWA of Te hexafluoride is 0.2 mg/m^3 (ACGIH TLV, 2006; OSHA PEL, 2006). Te in dust or fume has a TWA of 0.1 mg/m^3 (OSHA PEL, 2006) and a German MAK value (2006) of 0.1 mg/m^3.

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CHAPTER

41

Thallium

GEORGE KAZANTZIS

ABSTRACT

After rapid, almost total, absorption from the gastrointestinal tract, soluble thallium compounds are widely distributed in the body, the highest concentration being accumulated initially in the kidneys. Excretion occurs through both urine and feces, the disappearance of thallium from the tissues following first-order kinetics. The biological half-time of ^{204}Tl in the rat has been calculated at 3–4 days. In man, increased concentrations of thallium may be found in urine and feces for several weeks after absorption. Excretion also occurs through hair, which in unexposed subjects has been shown to contain the highest concentration of thallium in any tissue. In the rat, 21 days after dosing by parenteral or oral routes, up to 60% of the remaining body burden was found in the hair. There are similarities between the ionic transport of thallium and of potassium through cell membranes, but once intracellular, thallium is less rapidly released than potassium.

Thallium has caused acute and often fatal poisoning as a result of unintentional, criminal, or suicidal ingestion. Fatalities have also occurred after the now obsolete therapeutic administration of thallium. Gastroenteritis, collapse, and later peripheral neuropathy are the principal features in acute poisoning, but with longer survival, alopecia becomes a characteristic feature after 2–3 weeks. In chronic poisoning, the main features are vague ill health, paresthesias, and in some cases loss of hair; this pattern is seen after low-level occupational exposure. The widespread use of thallium as a rodenticide has been responsible in some countries for the death of domestic and wild animals and their natural predators.

Exposure of the general population to thallium may occur as a result of atmospheric pollution from coal-burning power plants and from copper, lead, and zinc smelters. Contamination of drinking water may occur from smelting and refining operations with these metals. Thallium intake in such polluted areas may exceed the normal dietary intake in the general population that has been estimated to be $<5\,\mu g/day$, mostly from foodstuffs, which does not constitute a threat to health. Where environmental monitoring shows thallium levels significantly above background, biomonitoring of the population should be carried out, and where this reveals evidence of excessive exposure, emissions should be reduced.

1 PHYSICAL AND CHEMICAL PROPERTIES

Thallium (Tl): atomic weight, 204.4; atomic number, 81; density, 11.9; melting point, 303.5°C; boiling point, 1457°C; crystalline form blue-white metal, tetragonal; oxidation states +1, +3. Compounds to be taken up in this chapter are thallous sulfate, thallous nitrate, and thallous acetate. Other common compounds are thallium(I) oxide, thallium(III) oxide, thallous carbonate, and thallous sulfide.

Thallium shares group IIIa of the Periodic Table with indium, gallium, aluminum, and boron. Thallium metal forms a brownish black oxide on exposure to air. Thallium is highly reactive, readily soluble in acids, and forms monovalent thallous and trivalent thallic salts, the latter being less stable (Lee, 1971).

2 METHODS AND PROBLEMS OF ANALYSIS

Thallium analyses should be accompanied by a quality assurance program and require careful sample pretreatment. Thallium losses during sampling, sample pretreatment, and determination are a major source of analytical error, and contamination hazards have to be taken into account, because thallium may be present in laboratory ware. In environmental samples, thallium concentrations are in the microgram per kilogram range, and in biological samples a few micrograms per kilogram, when no preconcentration of thallium is applied (WHO, 1996). Sample collection, analysis, and data presentation should be carried out in accordance with a protocol to ensure adequate validation of biological monitoring procedures (Vesterberg *et al.*, 1993). It is currently possible to determine thallium concentrations of approximately 0.1 µg/L or 0.1 µg/kg.

Graphite furnace atomic absorption spectrometry (GFAAS), the most widely used analytical method, is still suited for applications where high sensitivity is required for small sample amounts with thallium concentrations of a few micrograms per kilogram. Analytical methods for determining thallium concentration with GFAAS in biological and environmental samples with detection limits have been summarized (WHO, 1996).

Inductively coupled plasma mass spectrometry (ICP-MS) has good precision and accuracy for determining concentrations of thallium in the microgram per kilogram range. A detection limit of 0.09 µmol/kg (18 µg/kg) was reported for thallium concentrations in rat liver and blood plasma samples (Templeton *et al.*, 1989), and a detection limit of 0.1 µg/L was reported for thallium analyzed by ICP-MS in more than 250 water samples from lakes acidified with nitric acid (Henshaw *et al.*, 1989). For both GFAAS and ICP-MS, direct analysis of the diluted sample is feasible.

Differential pulse anodic stripping voltammetry (DPASV) is a sensitive method for quantitative determination of thallium in urine and water samples. Simultaneous determination of several metals from one solution is also possible with DPASV. The lower detection limit for thallium by DPSAV is 10–100 ng/L (Griepink *et al.*, 1988).

3 PRODUCTION AND USES

Thallium is found in the United States, China, and Brazil in sulfide minerals (e.g., lorandite and crookesite). However, it is usually recovered from flue-dust residues from zinc and lead smelters and as a by-product of cadmium production. Thallium, which is present as a sulfate, is separated by electrolysis. It is also obtained from the residues in the production of sulfuric acid by the lead-chamber process.

Thallous sulfate was used on a large scale as a rodenticide, but this has been replaced in some countries (e.g., the United States, Great Britain, France, and several other European countries) by less toxic compounds. However, in some other countries, after the development of warfarin resistance in rats, the use of thallium is on the increase again. In 1975, the total industrial consumption of thallium in the United States was estimated at not more than 500 kg (1000 lb), of which 70% was used in the electrical and electronics industries (De Filippo, 1976). Thallium is used in photoelectric cells, lamps, and in electronics, in semiconductors, and scintillation counters. As a catalyst in organic synthesis, thallium is used for oxidation of hydrocarbons and olefins, for polymerization, and for epoxidation (Wade and Banister, 1973). Thallium compounds are used in infrared spectrometers, in crystals, in other optical systems, and for coloring glass. Thallium has limited uses in alloys and has been used in mineralogical analysis. The industrial uses of thallium are limited, but these may increase, in particular as catalysts in organic synthesis. ^{201}Tl has been used increasingly in myocardial imaging. Currently thallium is used mainly in specialized electronic research equipment. Thallium ions show excellent nuclear magnetic resonance (NMR) properties and have been used as a probe to emulate the biological functions of alkali metal ions, especially potassium and sodium (Nriagu, 1998).

4 ENVIRONMENTAL LEVELS AND EXPOSURE

4.1 General Environment

Thallium is widely, but sparingly, distributed over the earth, mainly in rock formations and soils containing potassium feldspars and micas, with a crustal abundance in the order of 1 mg/kg. Thallium concentrations in soil are of the order of 0.l–≈l .0 mg/kg, with higher values in the vicinity of metallic ore deposits. Thallium uptake by vegetation increases with increasing soil acidity and is also determined by plant species, with high uptake, up to 45 mg/kg in green kale (Kazantzis, 2000). Concentrations of thallium ranged from 40–124 mg/kg in soils from a mining area in Guizhou, China, compared with 1.5 to 6.9 mg/kg in undisturbed natural soils (Xiao *et al.*, 2004). They found thallium in crops to be species dependent, with a thallium level in green cabbage

up to 500 mg/kg dry weight, surpassing the levels in soils in which the green cabbage was grown. Thallium is also found in potash, lead, and zinc ores and in fossil fuels. It is present in the sea in a concentration of approximately 0.01 µg/L; in freshwater, levels have ranged from 0.01–14 µg/L. Air levels reported from Nebraska were on the order of 0.04–0.48 ng/m^3. The dietary intake of thallium has been estimated at approximately 2 µg/day, on the basis of the sparse data available (Carson and Smith, 1977; Hamilton and Minski, 1972/73).

The most important sources of thallium exposure in the general population are air emissions from coal-burning power plants, and copper, lead, and zinc smelters. Polluted atmospheres may contribute as much thallium or more to intake than a normal human diet (Carson and Smith, 1977). Thallium contamination of drinking water may occur in the vicinity of copper, zinc, and cadmium mining, smelting, and refining operations. Concentrations of toxic metals other than thallium are also likely to be high. Smaller amounts of thallium may enter food chains from potash-derived fertilizers, but concentration factors are not high. Although not more than approximately 15 tons thallium are now produced worldwide annually, it is estimated that approximately 2000–5000 tons are mobilized currently by industrial processes (Kazantzis, 2000).

Concentrations of 0.7–88 µg/L were reported in river water draining a metal mining area in New Brunswick (Zitko *et al*., 1975). In algae and moss from these rivers, concentrations ranging between 9.5 and 162 µg/kg dry weight were found. High concentrations of thallium have been found in lake trout in areas of Great Lakes, Canada, impacted by human activities and urban development, suggesting that a risk of thallium poisoning from fish consumption may be higher than generally recognized (Lin and Nriagu, 1999). Greater bioaccumulation factors of inorganic thallium are possible in water containing low amounts of potassium. Water quality guidelines seeking to protect biota from deleterious effects of thallium should consider the role of potassium (Twiss *et al*., 2004). The use of thallium to kill rodents implicates its spread to their natural predators such as foxes, weasels, and martens. Furthermore, wild and domestic animals may feed on the poison directly.

Although pesticides containing thallium are no longer being sold in some countries, they can still be found stored in homes. As a result of such storage, some unintentional contamination of food has occurred. Colorless and tasteless thallium compounds have also been used for homicidal purposes. The use of thallium as a rodenticide should be prohibited worldwide (WHO, 1996).

4.2 Working Environment

Inhalation of thallium has resulted from the handling of flue dusts and of dusts from the roasting of pyrites. Exposure through inhalation may also occur in the extraction of the metal, in the manufacture of thallium-containing rodenticides and thallium-containing lenses, and in the separation of industrial diamonds (Richeson, 1958). Glöme and Sjöström (1955) considered skin contact as the route of absorption of thallium in a group of workers manufacturing rodenticides. Intoxication resulting solely from skin contact was reported in six men working with solutions of organic thallium compounds in the separation of industrial diamonds (Richeson, 1958). Skin absorption followed by gastrointestinal absorption was considered to be the main source of intoxication in thallium workers (Ewers, 1988).

5 METABOLISM

5.1 Absorption

Absorption of thallium compounds is rapid after ingestion, inhalation, or skin contact and may be complete after ingestion. In experiments on the rat, Lie *et al*. (1960) administered ^{204}Tl as a thallous nitrate solution by six different routes. Whatever the route of administration, the body burden (as a proportion of the administered dose) was similar and followed a single exponential function extrapolating to 100% at zero time. From these data, although indirect, the authors deduced that complete absorption had occurred from the gastrointestinal tract after ingestion and from the respiratory tract after intratracheal injection. The rapidity of absorption from the gut of the rat was shown by Lund (1956), who observed thallium in the urine within 1 hour after oral administration of thallous sulfate.

5.2 Distribution

Thallium is widely distributed in the body after its rapid absorption in animals. Acute and chronic studies agree that the highest concentration is found in the kidneys. In the rat study reported previously (Lie *et al*., 1960), the kidney concentration was 5½ times the next highest concentration in the salivary gland. This was followed by testis, muscle, bone, lymph nodes, gastrointestinal tract, heart, spleen, and liver, all of which had relatively small differences in concentration between them. There was little variation in the relative thallium concentrations in these tissues on successive days during the first week, with the exception of hair. This latter increased with time, so that after 21 days it contained up to 60% of the body burden. The relative

organ concentrations remained constant whatever the route of administration. Blood contained very little thallium, indicating rapid equilibration with tissues.

A steep fall in thallium concentration in the blood was also observed by Rauws (1974) in the first minutes after intravenous injection of ^{204}Tl with thallium nitrate in the rat. An exponential decrease was approached only after 2 hours. At a steady state, which was reached after 24 hours, the thallium in the blood was located predominantly in red blood cells. In chronic feeding experiments in the rat, Downs *et al*. (1960) found the highest concentration in the kidneys, followed by bone, liver, lung, spleen, and brain.

In man, too, thallium is widely distributed in the tissues, with the highest concentration in the kidneys. In the fatal case reported by Smith and Doherty (1964), tissue thallium concentrations were 7.8 mg/kg in the kidneys, 5.2 mg/kg in the brain, 7 mg/L in the bile, and 2.29 mg/L in the urine. In the second fatal case reported by Cavanagh *et al*. (1974), the concentration of thallium in the kidneys was 20 mg/kg; heart, 13 mg/kg; brain (grey matter), 10 mg/kg; skin, 6 mg/kg; and liver, bone, muscle, 5 mg/kg; with lower concentrations in other tissues.

5.3 Excretion

Thallium is excreted in both animal and man by the kidneys and intestine and also in small part through hair and into milk. It can also cross the placental barrier (Heyroth, 1947).

In the rat study described previously (Lie *et al*., 1960), excretion of ^{204}Tl maintained a similar pattern regardless of the route of administration. Approximately 6% of the administered dose had been excreted in the urine after 1 day, excretion falling to approximately 0.5% by the tenth day. Again, by whichever route thallium was administered, fecal excretion exceeded urinary excretion during the 21 days of the study, the fecal/urinary ratio rising from 2:5 because of a gradual decrease in thallium excreted in the urine. Thyresson (1951) and Barclay *et al*. (1953) in observations on rats also found fecal excretion to exceed urinary excretion. From observations on the rat after the intraperitoneal injection of 10 mg thallous sulfate per kg body weight, Lund (1956) found the principal routes for the elimination of thallium to be the gut and the kidneys. Concentrations of the metal in gastrointestinal secretions corresponded with those in the plasma. In rabbits given an intravenous infusion of thallous nitrate (0.5 g/L), thallium was shown to be excreted by glomerular filtration, approximately half the filtered amount being reabsorbed in the tubules. With the addition of 1% potassium sulfate to the infusion, the renal clearance of thallium was doubled; this was believed to result from the simultaneous tubular secretion of thallium and potassium (Lund, 1956). Rauws (1974) gave intravenous $^{204}TlS0_4$ to a small group of rats and simulated thallium kinetics on the basis of a three-compartment model. He concluded that a significant exchange of thallium occurred between the tissues and the intestinal contents, which he described as an enteroenteral cycle.

Few observations have been made in man. Thallium was still excreted in urine and feces by a 19-year-old male 5 months after ingestion of a thallium-containing rat poison (Arnold *et al*., 1964). Barclay *et al*. (1953) gave radioactive thallous nitrate and thallous sulfate orally to one patient with metastatic osteogenic sarcoma and found the rate of excretion in the urine to be 3.2%/day of the amount remaining in the body. Thallium excretion in both urine and feces may persist for many weeks despite low plasma levels in poisoned patients. In unexposed persons, the highest tissue concentration of thallium has been found in hair, throughout its length (Weinig and Zink, 1967) (see Section 6). In the long term, therefore, hair, and to a lesser extent nails, provides an important additional route for the slow excretion of thallium from the body.

Intravenous infusion in pregnant rats of thallium sulfate containing ^{204}Tl for periods up to 32 minutes showed fetal concentrations of thallium in the order of one fifteenth of the maternal plasma levels (Gibson and Becker, 1970). The uptake of thallium by maternal red blood cells, which is known to occur, could not adequately explain the low level of thallium that crossed the placenta.

5.4 Biological Half-Time

The body burden as a percentage of administered dose was determined in rats after the administration of ^{204}Tl by a variety of routes over a period of 21 days (Lie *et al*., 1960). Body clearance was found to occur exponentially with a half-time of 3.3 days whatever the route of administration. At the end of 21 days (the total period of observation), 1% of the administered dose remained. Because, except for hair, there was no day-to-day variation in relative organ concentration, individual organs were considered to have similar biological half-times. With a biological half-time of 3.3 days, it was calculated that with daily dosing an equilibrium would be attained at approximately 20 days. Studies by Hologgitas *et al*. (1980), based on radioactive thallium scanning and human reports, indicated a half-time on the order of 1.7 days. A biological half-time of approximately 4 days was also found by Rauws (1974) in his observations with ^{204}Tl given intravenously in rats (quoted in Section 5.2). In his three-compartment model, Rauws considered the

brain as a compartment on its own, because of its role as a target organ and because thallium ions penetrate into it more slowly. He found the brain concentration to be always lower than the mean concentration in the organism calculated from body load and body weight. There are not enough data to estimate the biological half-time in humans, but observations on a single case of thallium poisoning—on the basis of urinary excretion of thallium over a 3-month interval—were consistent with a half-time of 30 days (Clinical Conferences at the Johns Hopkins Hospital, 1978).

6 BIOLOGICAL MONITORING

Few data are available on levels of thallium in normal subjects. In a study on six people of various ages dying from unrelated causes, Weinig and Zink (1967) estimated tissue concentrations of thallium by mass spectroscopy. The highest values were found in hair, with concentrations ranging from 4.8–15.8 µg/kg. Concentrations in nails ranged from 0.72–4.93 µg/kg, and in the wall of the colon from 0.56–5.40 µg/kg wet tissue. The mean tissue concentration was calculated as 1.2 µg/kg, from which it was derived that the thallium content in a 75-kg person would be on the order of 0.1 mg. The same investigators found the thallium concentration in early-morning urine samples in nine subjects to range from 0.13–1.69 µg/L. Johnson (1976), in a study in New Zealand on 11 subjects with no known exposure to toxic metals, found a mean thallium concentration in liver of 0.47 mg/kg dried tissue, with a range of 0.4–0.9 mg/kg.

The concentration of thallium in whole blood in a population of 320 children in New Jersey was measured by AAS. Values ranged between nondetectable (<5 µg/L) and 80 µg/L, with a mean concentration of 3.0 µg/L. Approximately 80% of the children showed no detectable thallium in the blood, whereas 17.5% had between 5 and 20 µg/L. Five children (1.6% of the total group) had blood thallium levels between 40 and 80 µg/L, but all were without evidence of thallium toxicity (Singh *et al*., 1975).

In three carefully controlled population-based studies involving a total of 686 unexposed subjects, the range of urinary thallium concentrations was 0.06–1.2 µg/L, with a mean thallium urinary concentration of 0.3–0.4 µg/L. With a short biological half-life and an assumed steady state in such population-based samples, the urinary excretion value can be considered as an indicator of total dose after inhalation and total dietary intake (WHO, 1996).

In a population sample living in the vicinity of thallium emission into the atmosphere, the mean urinary thallium concentration was 5.2 µg/L ± 8.3 µg/L, with a range of 0.1–76.5 µg/L. From a questionnaire, a clear dose-response relationship was identified between thallium concentrations in urine and the prevalence of tiredness, weakness, sleep disorder, headache, nervousness, paraesthesia, and muscle and joint pain, with a similar dose-response relationship when thallium in hair was taken as an indicator of exposure (WHO, 1996).

From these limited studies, it has been suggested that an approximately 15-fold increase in urinary thallium excretion above the mean unexposed level of 0.3–0.4 µg/L may be related to subjective symptoms that could possibly be considered as early adverse effects. The Task Group (WHO, 1996) considered that exposures with urinary thallium concentrations <5 µg/L are unlikely to cause adverse health effects. In the range of 5–500 µg/L, the magnitude of risk and severity of adverse effects are uncertain, whereas exposures with urinary thallium concentrations >500 µg/L have been associated with clinical poisoning.

Extensive data on thallium levels in persons from the general population is available from the NHANES studies in the United States. Thallium levels in urine, blood, and hair are useful biomarkers of exposure to thallium.

7 EFFECTS AND DOSE-RESPONSE RELATIONSHIPS

7.1 Laboratory Animals

In acute poisoning, the principal effects are seen in the digestive and nervous systems with, in addition, a necrotizing renal papillitis. In chronic poisoning, the most striking feature is loss of hair. This phenomenon, so characteristic of thallium poisoning, is probably the result of cessation of cell proliferation in the hair follicles (Cavanagh and Gregson, 1978) and is thus closely similar to the hair loss caused by X-irradiation and radiomimetic chemicals.

In mammalian species, the acute LD_{50} for thallium compounds for all routes of administration ranges between approximately 5 and 70 mg/kg (Adamson *et al*., 1975; Hart *et al*., 1971; Truhaut, 1959).

In chronic poisoning, the daily ingestion of thallium acetate added to the diet of male and female rats was tolerated at the 10 mg/kg level but was lethal in male rats at the 30 mg/kg level by 15 weeks (Downs *et al*., 1960). Rats given 10–20 mg thallium acetate followed by weekly subcutaneous injections of 5 mg/kg developed irritability, diarrhea, alopecia, and poor weight gain. Abnormalities, in particular degenerative changes in the mitochondria (Herman and Bensch, 1967), were seen in kidney, liver, and intestinal epithelial cells by electron microscopy. Kennedy and Cavanagh (1977)

produced thallium poisoning in cats by giving weekly subcutaneous injections of thallous acetate (4 mg/kg body weight) and studied the neurological features induced. The principal functional disturbances with this degree of intoxication were hypotonia and ataxia. The pathological changes were confined to the primary sensory neurons and consisted of "dying back" of both central and peripheral axons but with a remarkable sparing of many sensory nerves of muscle sense organ; motor nerves seemed to be spared in this species. In the spinal cord, there was axonal degeneration in the dorsal columns but not in other long tracts.

7.2 Domestic and Wild Animals

Clausen and Karlog (1974) examined 60 wild martens and badgers in Denmark over a 2-year period and found a thallium load in 48% of these, with evidence of poisoning in 22% of the total. Acute hemorrhagic intestinal inflammation was seen in most of the cases of poisoning. In the 13 poisoned animals, liver concentrations ranged from 4.7–57 mg/kg; kidney concentrations ranged from 1.8–92 mg/kg; and intestinal concentrations varied between 0.2 and 42 mg/kg. The thallium concentration was estimated in 34 red foxes in Denmark that had been found sick or dead during 1971 (Munch *et al*., 1974). In four of seven red foxes where the cause of death could not be immediately established, a thallium content was found in excess of 1 mg/kg in at least one organ. Of the 27 foxes where an alternative diagnosis had been made, thallium concentrations were >1 mg/kg in five animals, and between 0.5 and 1 mg/kg in eight.

A pathological study was performed on dogs (Zook and Gilmore, 1967) and on cats (Zook *et al*., 1968) accidentally poisoned by rodenticides or by eating vermin poisoned by thallium. The more severely poisoned animals developed hemorrhagic gastroenteritis with hepatic or renal damage. The longer survivors developed gross skin lesions with inflammation, bleeding, crusting, and shedding of fur. Incoordination, trembling, and paralysis developed at this stage. The most important findings were the following: In the kidneys, tubular necrosis with glomerular hyalinization; in the brain, focal edema and chromatolysis of neurons were reported; degenerative changes were seen in the peripheral nerve; necrosis of skeletal and cardiac muscle fibers; edema and bronchopneumonia in the lungs; and hemorrhage and ulceration in the gut. In contrast to the severe damage seen in the other organs, liver damage in the dog was mild, and only moderate centrilobular necrosis was seen in the cat.

7.3 Humans

Thallium compounds are extremely toxic. Acute poisoning is characterized by gastroenteritis, with nausea, vomiting, diarrhea, and abdominal pain within hours of absorption. Involvement of the nervous system then becomes apparent after a few days, with paresthesia, exquisitely painful and tender extremities, weakness, mental confusion or delirium, convulsions, with respiratory and circulatory involvement followed by death. When the period of survival extends beyond 1 week or so, a varied neurological picture may develop with headache, ataxia, tremor, paresthesias, and muscular atrophy predominating. There may also be cranial nerve involvement, with ptosis, ophthalmoplegia, retrobulbar neuritis, or facial paralysis later on in the intoxication. Tachycardia and hypertension seen in the more severely affected cases are thought to be due to denervation of the glossopharyngeal and vagus nerves. The autonomic neuropathy is a key component of thallium toxicity.

The neurological picture in the subacute case is characteristically a distal neuropathy that begins with sensory loss and numbness in the fingers and toes that later spreads proximally. A little while later, there develops a motor weakness, distal at first, that again spreads proximally. This neurological pattern of changes may spread further to involve cranial nerves, respiratory muscles, and even eye muscle innervation. This steady proximal spread is reminiscent of Landry's ascending paralysis, a condition now not often recognized.

After an interval of 1–3 weeks, alopecia develops, and the head hair can be readily pulled away; pubic and axillary hair, and also the medial third hairs of the eyebrows that do not actively grow, are relatively spared. Ridges (Mees's stripes) appear on the fingernails. Recovery may be complete, or neurological defects may remain for long periods with mental abnormality, ataxia, and tremor (Bank *et al*., 1972; Cavanagh *et al*., 1974; 1984, personal communication; Clinical Conferences at the Johns Hopkins Hospital, 1978; Grunfeld and Hinostroza, 1964; Mathews and Anzarut, 1968; Reed *et al*., 1963; Smith and Doherty, 1964).

Three cases, two of which were fatal, were described in detail by Cavanagh *et al*. (1974). The more severely poisoned of these was diagnosed as a case of Guillain-Barré polyneuritis, and at postmortem, there was little evidence of neuronal degeneration; but the second fatal case, who was less acutely poisoned, showed extensive distal degeneration of all nerves examined, with chromatolysis of many nerve cells. Neuronal degeneration was of the distal, dying-back type and was still actively continuing 3 weeks after the onset of paresthesia. The more acutely affected of these two fatal cases was estimated to have ingested 0.93 g Tl as the acetate in two divided doses, whereas the fatal case in which symptoms developed less rapidly had taken the same total amount but in three divided doses. A survivor had ingested 0.31 g Tl in a single dose. In the latter, who experienced severe loss of head and beard

hair, thallium could not be detected in the serum 7 weeks after exposure but was present in the urine in a concentration of 3.0 mg/L.

The neuropathy produced by thallium was studied by Kennedy and Cavanagh (1976) in a further case: a 32-year-old man who died 1 month after ingesting thallium sulfate at a calculated dose rate of 33 mg/kg. The presenting features consisted of distal numbness, paresthesias, and pains in the limbs followed by convulsions. The principal clinical findings consisted of tachycardia, hypertension, peripheral neuropathy, and cranial nerve involvement. The diagnosis was not made until the 21st day, when alopecia developed. Cerebral edema and petechial hemorrhages were the only abnormalities seen in the cerebral hemispheres, perhaps related to assisted respiration measures. There were striking degenerative changes in the dorsal columns of the spinal cord affecting both major tracts. Chromatolysis of spinal anterior horn cells was associated with severe Wallerian degeneration of both motor and sensory peripheral nerve fibers.

A symmetrical, mixed peripheral neuropathy is characteristic, with distal nerves more strongly affected than proximal nerves (Cavanagh, 1988); another characteristic feature is an extreme sensitivity of the legs, followed by the "burning feet" syndrome and paresthesia. The so-called therapeutic dose for ringworm of the scalp, recommended to produce hair loss in 2–3 weeks, was 8 mg Tl/kg. However, Munch (1934) reviewed six deaths among children given this dose, and found, in 8006 children treated with thallium, 447 cases of poisoning (5.5%). Altogether he collected reports on 778 cases of thallium poisoning, with a mortality of 6%. The minimal lethal dose for soluble thallium salts for an adult is in the order of 1 g Tl (Gettler and Weiss, 1943) or 10–15 mg/kg. Doses in the order of 1 g Tl are likely to be fatal within a few days. Doses ranging between 0.5 g and 1 g are likely to give rise to a progressive neurological deficit ending in death in 2–3 weeks, whereas smaller doses will show progression to alopecia and are more likely to be followed by recovery. There is no therapeutic dose for thallium salts.

Thallium poisoning has occurred after the unintentional contamination of food. In one outbreak, barley containing 1% thallium sulfate for pest control gave rise to 27 cases of poisoning with 7 deaths (Munch *et al.*, 1933). Thallium salts have been used for suicide; their criminal use has been reviewed by Prick *et al*. (1955).

Industrial thallium poisoning has occurred after absorption by inhalation of dust or fume, through ingestion from contaminated hands or food, or as a result of skin contact. Munch (1934) recorded 12 cases of industrial poisoning up to 1934. The principal clinical features in these cases were fatigue, anorexia, pains in the legs, and loss of hair. There were no deaths, but one worker lost his sight as a result of optic atrophy. A worker who, wearing protective clothing, had handled approximately 6 kg thallium per day for many years, developed ascending polyneuropathy, visual disturbance, and finally hair loss (Egen, 1955).

No information on exposure levels is available. Glömme and Sjöström (1955) described four cases in which absorption was thought to follow skin contact in the manufacture of rodenticides. Minor degrees of polyneuropathy and varying degrees of alopecia were noted, with a maximal urinary thallium excretion of 380 µg/L. Other reports with less specific effects after industrial exposure to thallium are difficult to evaluate. Occupational thallium poisoning has usually followed long-term, lower level exposure producing a cumulative effect with a milder clinical course than that described for acute poisoning, characterized mainly by subjective symptoms, in some cases with polyneuropathy and partial alopecia. More data on environmental and biological levels of thallium are required in such cases.

As described in Section 6 of this chapter, human exposures with urinary Tl concentrations <5 µg/L are unlikely to cause adverse effects. In the range of 5–500 µg/L, risks are uncertain, whereas exposures with urinary Tl >500 µg/L have been connected with clinical poisoning.

7.4 Interaction with Potassium and Other Effects

There are similarities between the ions of thallium and of potassium: they have similar ionic radii, and there is experimental evidence to suggest that the biological handling of thallium and potassium ions is interrelated. Mullins and Moore (1960) found in frog muscle that potassium and thallium ions traversed cell membranes in a similar way; Gehring and Hammond (1964) showed that the active transport mechanism for potassium into rabbit erythrocytes also transported thallium. The same authors (1967) studied the relationship between the level of potassium and the excretion of thallium in rats, dogs, and sheep. Infusion of potassium increased the renal clearance of thallium and also its mobilization from tissues. It was concluded from these experiments that the disappearance of thallium followed a first-order kinetic process. However, although the ionic movements of thallium and potassium ions are related, once inside the cell, thallium is less readily released than potassium. Thallium can substitute for potassium in causing activation of adenosine triphosphatase, indicating that the mechanism involved in the active transport of potassium cannot differentiate between the two ions. In poisoned patients, T-wave inversion on the ECG suggests that

Tl^+ ions may be disturbing the function of K^+ions in cardiac contraction mechanisms. In rats, an increased potassium intake increased the LD_{50} of thallium, suggesting a translocation away from the toxic receptor site (Gehring and Hammond, 1967). However, the excessive mobilization of thallium ions with subsequent redistribution in the tissues may be detrimental (Cavanagh *et al*., 1974). Thallium has also been found to replace potassium in the stabilization of ribosomes, as well as in physiological functions such as muscle contraction (Arzate and Santamaria, 2000).

Thallium has other effects on tissues in addition to those related to potassium transport. It uncouples oxidative phosphorylation (Melnick *et al*., 1976), adversely affects protein synthesis (Hultin and Näslund, 1974), and inhibits a number of enzymes, including alkaline phosphatase and succinic dehydrogenase (Truhaut, 1959).

Schoental and Cavanagh (1977) have drawn attention to the common features in the neuropathy produced by thallium, arsenic, and thiamine deficiency, in all of which the "dying back" type of neuronal degeneration is observed. Thallium reacts specifically with riboflavin to form an insoluble compound (Kuhn *et al*., 1933); a hypothesis has been propounded by Schoental and Cavanagh that thallium acts by specifically interfering with the availability of riboflavin- and flavoprotein-cofactors in the axons of peripheral nerves. Its effects will, thus, as with arsenic and thiamine deficiency, be to reduce the level of available energy within the cell, because the important pyruvate decarboxylation process will be particularly impaired in all these conditions.

8 DIAGNOSIS, TREATMENT, AND PREVENTIVE MEASURES

The diagnosis of thallium poisoning may be difficult, because it is often unsuspected. The cardinal features are gastroenteritis, peripheral neuropathy, and then later, alopecia, the last occurring at a stage when treatment is likely to be ineffective. The possibility of thallium poisoning should be borne in mind in obscure neurological illnesses, especially where there is peripheral neuropathy with alopecia; in addition, thallium should be looked for in the urine. A simple screening test for thallium in urine (see Section 2) may lead to early diagnosis. However, thallium, being slowly excreted by the kidneys, may be present in the urine in significant amounts for some weeks after absorption; a level >7–10 µg/L would be confirmatory evidence of poisoning. A brownish black pigmentation close to the hair root is characteristic of thallium exposure and may appear as early as the third or fourth day (Gerdts, 1974; Mathews and Anzarut, 1968). The diagnosis of thallium poisoning was established 1 week after ingestion by the microscopic examination of hair after the application of 10% sodium hydroxide, which revealed dark bands of pigmented material characteristic of the presence of thallium traces (Uges and Huizinga, 1976).

The criteria for the diagnosis of mild thallium poisoning resulting from low-level occupational exposure have been given by Glömme (1983). An occupational history involving the handling of rodenticides; the production or use of thallium or its various salts; and work on lead, zinc, or cadmium production—together with neurological symptoms dominated initially by subjective effects in the form of paresthesia—should alert to the possibility of occupational thallium poisoning. A urinary excretion of thallium in the order of 300 µg/L would provide confirmatory evidence.

The treatment of thallium poisoning was initially unsatisfactory. Large doses of potassium chloride may hasten the excretion of thallium but may cause a transient increase in blood levels and a redistribution in tissues that may lead to a temporary exacerbation of symptoms (Papp *et al*., 1969). Chelating agents, including BAL, EDTA, diethyldithiocarbamate (dithiocarb), and dithizone, are contraindicated in the treatment of thallium poisoning. Although dithiocarb has been shown to increase the urinary excretion of thallium in the rat (Schwetz *et al*., 1967), Kamerbeek *et al*. (1971a) reported clinical deterioration with EEG changes, coincident with administration in man caused by a redistribution of thallium with an increased concentration of a lipophilic chelate in the brain.

Kamerbeek *et al*. (1971b) reported increased fecal excretion of thallium with clinical improvement in three cases by giving Prussian blue by mouth. Van der Merwe (1972) reported a successful outcome in two patients treated with Prussian blue. Each had swallowed 700 mg thallous sulfate. Stevens *et al*. (1974) treated 11 patients with thallium poisoning with Prussian blue given by mouth. Their results were good when the agent was given within a few hours of ingestion, even when a lethal dose had been taken. In other cases, improvement was obtained, although treatment had been started late. Fecal elimination of thallium exceeded urinary excretion in all cases except those in which constipation altered the pattern of elimination. Rauws (1974) from his pharmacokinetic studies in the rat estimated a 70% inhibition of thallium reabsorption from the gut in rats pretreated with Prussian blue. Prussian blue is a crystal lattice of potassium ferric ferrocyanide(II) (potassium ferric hexacyanoferrate [II]). When given orally in the treatment of thallium poisoning, it adsorbs thallium ions during their

enteroenteral cycling through the gut, exchanging these for potassium ions on the surface of the crystal lattice. Neither Prussian blue nor its complex with thallium are absorbed systemically, and side effects have not been reported.

Stevens *et al*. (1974) recommended for the treatment of acute thallium poisoning gastric lavage followed by administration of Prussian blue, 250 mg/kg daily, dissolved in 50 mL of 15% mannitol divided into four doses and given orally. Prussian blue is best given in its soluble or colloidal form. They also recommended intravenous potassium chloride during the first days of treatment when renal function is normal, and the energetic treatment of constipation. If Prussian blue is not available, Stevens *et al*. (1974) advise the administration of activated charcoal, 0.5 g/kg twice daily, with potassium chloride, 3–5 g daily, given by mouth. Treatment with activated charcoal, short-term peritoneal dialysis, and potassium chloride diuresis ensured survival after the ingestion of a lethal dose of thallium iodide (Koshy and Lovejoy, 1981), although dialysis and diuresis were considered ineffective in eliminating thallium in this case. Long-term hemodialysis with forced diuresis and administration of Prussian blue by mouth resulted in complete recovery in a woman who had ingested 2 g thallium sulfate (Pedersen *et al*., 1978); 21% of the ingested thallium was recovered through the artificial kidney and urinary tract. Thompson (1981) in his review considered giving Prussian blue the most effective treatment, with activated charcoal as an alternative. Forced diuresis and hemodialysis were considered to be of minimal value, and the cautious use of potassium was advised in view of its effect on the redistribution of thallium.

Activated carbon has been shown to adsorb thallium *in vitro* (Hoffman *et al*., 1999). Prussian blue's safety profile has been considered to be superior to that of other proposed therapies and should be the drug of choice in acute thallium poisoning (Hoffman, 2003). The optimal therapy for thallium intoxication is currently considered to be the administration of Prussian blue, combined with forced diuresis with furosemide and mannitol, which may be supplemented by hemodialysis. Activated charcoal may be used in place of Prussian blue (IPCS, 1992; Wainwright *et al*., 1988).

In one case of acute thallium poisoning, with peripheral neuritis and progressive alopecia, in which Prussian blue contraindicated, double filtration plasmapheresis was effective in inducing remission (Tian *et al*., 2005).

In the prevention of thallium poisoning in the community, the use of thallium-containing pesticides should be discontinued wherever possible, and restrictions must be placed on their availability (Lancet, 1974).

In the prevention of thallium poisoning at the workplace, substitution by less toxic compounds should be advocated wherever possible. When thallium compounds must be used, strict safety precautions should be observed to prevent ingestion, inhalation, or skin contact. Food, drink, and cigarettes should be prohibited at the worksite. Personal hygiene should be ensured; protective clothing and respiratory protective equipment should be worn when inhalation of thallium-containing dust may occur.

When environmental monitoring shows thallium levels significantly above background, biomonitoring of the population in the vicinity should be carried out and emissions reduced when there is evidence of excessive exposure. At industrial facilities, such as metal mining and smelting and cement plants using pyrite, the concentration of thallium in raw materials, stack gases, and wastewater should be monitored and controlled, and waste materials containing thallium compounds should be sealed and marked. Appropriate protective measures should be taken to reduce occupational exposure to thallium and to ensure worker safety. In the general population, the total intake of thallium, mostly from foodstuffs, has been estimated to be <5 µg/day, and this level does not constitute a threat to health. Follow-up epidemiological studies should be performed on populations exposed to raised levels of thallium in their environment to determine whether there may be an increased health risk (WHO, 1996).

Petera and Viraraghevan (2005) in their review of public health and environmental concerns regarding thallium, because little is known about the behavior of thallium in an aquatic system point to the need for a greater understanding of thallium transport pathways. Technologies for the effective removal of thallium from waters and wastewaters need to be developed, and recycling of waste thallium in industries should be investigated. They conclude that further study on the health impact of thallium on an exposed population is needed, even in areas with moderate thallium concentration.

9 PROGNOSIS

The estimation of outcome in thallium poisoning is difficult. In general, cases with a fulminating onset are rapidly fatal, and the longer the course of the illness, the greater the chance of survival, although recovery may be incomplete where there is peripheral neuropathy. Prognosis has been improved with current treatment as described previously, providing this can be started at an early stage.

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CHAPTER

42

Tin

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ABSTRACT

Tin is not an essential metal and is widely used in industry. In nature, it occurs both in inorganic and organic forms. The gastrointestinal absorption of soluble tin salts is only a few percent of the ingested dose. In chronic exposures, bone is the major storage organ for tin, and its biological half-life in bone is approximately 100 days. The excretory routes of tin compounds may vary depending on the type of tin compounds and the mode of exposure. Most of the inorganic tin compounds are nontoxic because of their low solubility and absorption. In workers who inhale tin oxide, it accumulates in the lung and causes "stannosis," a benign pneumoconiosis without any tissue reaction or pulmonary dysfunction.

Although insoluble tin compounds are nontoxic, soluble tin compounds can be toxic. Oral administration of 45 mg/kg of stannous chloride can induce vomiting and diarrhea in cats. In human, high intakes of inorganic tin compounds can cause abdominal pain and anemia. Inhaled tin hydride gas can cause damage to nerves. Epidemiological study shows a positive relationship of increased tin intake to a decrease in copper status, changes in cholesterol metabolism, and ischemic heart disease.

Tin can be converted by chemical and biological reactions in the environment to form more toxic organotin compounds. Short-chain alkyltin compounds such as trimethyl and triethyl tins are well absorbed from gastrointestinal tract, whereas long-chain alkyltin compounds are not much absorbed. The toxicity of organotin compounds differs, depending on their physicochemical properties. Alkyl and aromatic tin compounds are neurotoxins. Triethyltin can cause encephalopathy and cerebral edema. Severe dermatitis has been described in workers exposed to tributyltin and painters applying formulations. Tributyltin is widely used as an antifoulant in marine paints to prevent any growth on ocean vessels. However, the use of tin-containing paints for smaller marine vessels is restricted in many countries.

Organotin compounds can penetrate cell membranes because of their lipophilicity and cause damage to cell membrane, interrupt oxidative phosphorylation, and damage mitochondria. They can inhibit synthesis of heme oxygenase and can be immunotoxic and genotoxic. There is no definite evidence that inorganic or organic tin compounds can cause carcinogenic or teratogenic effects, although in a few studies effects have been reported. Tributyltin and triphenyltin are implicated in endocrine disruptions, and hence, cause reproductive failure in marine organisms.

Further detailed information on tin compounds can be found in reviews by Klevay (2000), Hoch (2001), and WHO documents (WHO, 1999; 2001; FAO WHO, 2005).

1 PHYSICAL AND CHEMICAL PROPERTIES

Tin, Sn: atomic weight, 118.69; atomic number, 50; density, 5.8–7.3; melting point, 231.9°C; boiling point, 2602°C (*Handbook of Chemistry and Physics*). Tin exists in three allotropic forms: white metallic, tetragonal (tin white), and white rhombic (tin brittle), and when tin is cooled below 13.2°C, it is converted to allotropic form called gray tin or α-tin, cubic. Tin is a metal that

is insoluble in water, and it is not very electropositive and, therefore, may be obtained by reduction with a wide variety of reducing agents, including certain metals (Al, Zn, etc.). Tin may exist either as the metal or in the +2 or +4 oxidation states.

Tin forms a large number of inorganic and organic compounds. Inorganict in compounds are divided into two series: stannous or tin (II) compounds and stannic or tin (IV) compounds. At pH >6, stannous compounds are easily oxidized. Tin does not exist in ionized form, but rather in colloidal complexes. The most common inorganic tin compounds are stannous chloride ($SnCl_2$), stannous oxide (SnO), stannous fluoride (SnF2), stannic chloride ($SnCl_4$) and stannic oxide (SnO_2). The organic compounds of tin are classified as R_4Sn, R_3SnX, R_2SnX_2, and $RSnX_3$, whereas R is an alkyl or aryl group (e.g., methyl, butyl, ethyl, octyl, or phenyl group) and X is an anionic group (e.g., chloride, fluoride, oxide, hydroxide, carboxylate, or thiolate). In the organic compounds of tin, there are 1–4 covalent carbon–tin bonds. The best-known anthropogenic compounds are tributyl species (used in antifouling paint formulation). There are naturally occurring organotin compounds, which are mostly produced by methylation of inorganic tin (Quevauviller *et al*., 1989). However, the most of organotins entering the environment are man-made products.

2 METHODS OF ANALYSIS

A number of analytical techniques and methods have been used to determine inorganic tin and organotin compounds. In the past, quantitative determination of small amounts of tin always presented problems. Determination problems for tin were related to the weakness of instrumentation and to the difficulties associated with quality control in the absence of interlaboratory comparisons. The rapid and constant development of instrumentation and analytical methods has been observed in recent years. Progress in improvement of sample preparation and development of methods resulted in good progress in the detection limit of measurement techniques used for tin.

The atomic absorption spectrophotometric (AAS) method was widely used in the past for the determination of inorganic tin in food and biological materials. However, because of the unsatisfactory combustion of tin oxide in flame and the detection limit of AAS (Engberg, 1973), new techniques coupled with AAS were developed. Among them is graphite furnace atomic absorption spectrometry (GF-AAS), which has been used for the detection of tin in soft drinks and milk (Dabeka and Mckenzie, 1992). Waite *et al*. (1989) reported the use of electrothermal atomic absorption spectroscopy (ET-AAS) to obtain the total tin content in sediments. This technique, developed in the 1990s, is still used in approximately 40% of the total publications on analysis of trace elements in biological materials. Combined use of high-performance liquid chromatography (HPLC) separation and ET-AAS detection provides higher selectivity and sensitivity. Hydride generation (HG)/graphite furnace atomic absorption spectrometry (GF-AAS) has been described for the determination of tin followed by gas chromatography (GC) (Quevauviller *et al*., 1993). GF-AAS is able to analyze solid samples directly (Hirano *et al*., 2001). Inductively coupled plasma-atomic emission spectroscopy (ICP-AES) analysis can also be used for the determination of tin in water, vegetation, animal tissues, air filters, soils, sediments, leachates, and effluents (Yokoi *et al*., 1990a). As with emission spectroscopy, the sample is atomized in this method. However, instead of looking at the light emission at specific wavelengths, analyte ions, produced by the argon plasma directly in the entrance orifice of a quadruple mass spectrometer, are accelerated, magnetically separated, and counted with an electron multiplier. This technique provides high selectivity and sensitivity approximately 10–10,000 times higher than with conventional ICP and flame atomic absorption (AAS) techniques. A quantitative detection limit for this technique is approximately 0.1 µg/L of tin (Yokoi *et al*., 1990a). ICP-AES technique has been applied to food samples (Perring and Basic-Dvorzak, 2002). Inductively coupled plasma-mass spectrometry (ICP-MS) has been also reported for detection of tin in food samples (Biego *et al*., 1996). At present, the coupling HPLC-atomic detector is probably the most common approach for liquid samples, and ICP-MS the more powerful detector system (Sanz-Medel, 1998). Electron microscopic analysis by energy-dispersive X-ray (EDX) in SEM and electron energy loss spectroscopy (EELS) in TEM is used for detection of tin (Jonas *et al*., 2002). The specificity of tin detection by EELS can be confirmed by the measurement of electron loss spectrum in comparison with the standard curve for this element.

In the past, many spectrophotometric methods were developed for the analysis of tin, using dithiol, catechol violet, and quercetin methods (Adcock *et al*., 1970; Engberg, 1973; Kirk and Pocklington, 1969). However, most of them are not very sensitive.

Many analytical methods have been used for the determination of organotin compounds. Atomic absorption and other spectroscopic methods combined with chromatography have been used for the estimation of diorganotin compounds. Pesticide residues have been determined by spectroscopic methods and gas-liquid or thin-layer chromatography. Several reviews (Leroy

et al., 1998; Quevauviller *et al*., 1995) have described the major analytical methods for the determination of organotin species. Most techniques are based on separation by gas chromatography (GC). Organotin compounds are mostly analyzed after extraction or digestion with either the Grignard method (e.g., derivatization with pentylmagnesium bromide) or the ethylborate method (Rudel, 2003). The resulting derivatives from both methods are volatile and can be analyzed by the GC method with atomic emission (GC-AES) or mass spectrometry detection (GC-MS). Methods for detection of phenyltin using GH and electron capture detector (ECD) were reported (Soderquist *et al*., 1974). Use of an electrothermal quartz furnace for atomic absorption can increase the efficiency for determination of methylated tin in water (Chau *et al*., 1982). An analytical method published by Muller (1987) allows simultaneous measurement of 19 organotin compounds by capillary GC and flame photometric detection (FPD). HPLC is also widely used for analysis of organotin species (Astruc *et al*., 1990). However, there is a risk of degradation for butytin on reversed phase material, and that resulted in modification of new procedures involving HPLC and ID-ICP-MS (isotope dilution ICP-MS) (Brown *et al*., 1994). It has been used successfully for the determination of di- and tributyltin in sediment and mussel (Brown *et al*., 1994) and represents a very promising analytical method for tin speciation. Besides that, after HPLC separation, tin can be analyzed by flame, quartz furnace, or electrothermal AAS, increasing the efficiency of tin determination (Astruc *et al*., 1989). Classical flame AAS is now of less interest because of its very low sensitivity (Leroy *et al*., 1998). An application of inductively coupled plasma atomic emission after HPLC increases the residence time of the analyte in the plasma, thereby lowering the detection limits (Lafreniere *et al*., 1987). Coupling HPLC with AAS or inductively coupled plasma atomic emission increased the efficiency and enabled the continuous measurement (Leroy *et al*., 1998). The electron capture detector (ECD) is another classical technique for detection of di- and trialkyltin chlorides, triphenyltin, and tricyclohexyltin. Other recent techniques, which have gained importance, are microwave-induced plasma atomic emission spectrometry and mass spectrometry coupled to capillary GC (Leroy *et al*., 1998). Differential pulse anodic stripping voltammetry (DPASV) has been applied for detection of dibutyltin chloride in water (Kitamura *et al*., 1984). The detection limit is in the range of 10^{-4}–10^{-6} mol/L (Leroy *et al*., 1998). Instrumental neutron activation analysis has also been used for determination of tin; however, the disadvantages of this method are limited availability, analysis, and very high cost (Hirano *et al*., 2001). Moreover, some basic analytical problems remain to be solved for reliable speciation with HPLC. These include absorption in the column packing, column or buffer solutions competing and displacing bioinorganic ligands, and incomplete separation.

Despite the considerable number of techniques with high efficiency for determination of organotin and total tin, there is not a recognized standard procedure as yet. This has resulted in enormous scattering of values and inability to compare data from different sources. Moreover, reliable methods have still to be developed for the quantitative extraction, separation, and determination of many individual tin species in mixtures containing both inorganic tin and organotin compounds that may occur in various media. Therefore, two main approaches may be used to provide validation or useful complementary information: the use of certified reference materials and the use of alternative different principles–based methods.

3 PRODUCTION AND USES

3.1 Production

The earth's crust contains approximately 2–3 mg/kg tin, which comprises 0.0006% of earth's crust (Budavari, 2001). Annual world production in 1990 of tin was approximately 225,000 tons, 70% of which is obtained from the ores and 30% being recovered from the scrap metal. The world's largest producer of tin in 2001 was China (36% of the world total). Metallic tin is derived from the mineral cassiterite (SnO_2) and to a lesser extent from the sulfide ore stannite (Cu_2S-FeS-SnS_2) (Graf, 1996). Most of the world supply of tin, mainly as cassiterite, comes from China, Indonesia, Peru, Brazil, Bolivia, and Australia. The largest world producers of recycled tin are France and the United States. Approximately 25,000 million food cans are produced in Europe each year, and approximately 20% of them have unlacquered tin-coated steel bodies (Blunden and Wallace, 2003).

Organometallic tin compounds have been used in increasing amounts since 1950 for a variety of applications, and the annual world production has risen from <50 tons in 1950 to approximately 50,000 tons in 1994 (Fent, 1996a). The annual world production of biocide triorganotins is in the range of 8000–10,000 metric tons: 70% corresponding to tributyltin and triphenyltin derivatives. Worldwide synthesis of tributyltin compounds is approximately 9000 metric tons annually for all applications (Laughlin *et al*., 1986).

3.2 Uses

Tin is used in tin-plated containers, in solders, alloys such as bronzes, babbit, pewter, and in more specialized

alloys such as dental amalgams and the titanium alloys used in aircraft engineering. Inorganic tin compounds, in which the element may be present in the oxidation states of +2 or +4, are used in a variety of processes for the strengthening of glass, as catalysts, stabilizers, and as dental anticariogenic agents. One of the common compounds of tin(II) is stannous chloride ($SnCl_2$), which is used in tin galvanizing and as a reducing agent in the manufacture of polymers and as a mordant in dyeing. Stannous oxide (SnO) is used in making tin salts for chemical reagents and for plating, and stannous fluoride (SnF_2) is the additive in fluoride toothpastes. Tin (IV) compounds, including stannic chloride ($SnCl_4$), are widely used as a stabilizer for perfumes and as a starting material for other tin salts. Stannic oxide (SnO_2) is used as a catalyst in certain industrial processes and a polishing powder for steel. Tin sulfide is used as a bronzing agent for wood coloring. Stannous chloride and other inorganic tin compounds are used in the manufacture of toothpaste, ceramics, porcelain, enamel, drill glass, and ink. Tin salts sprayed onto glass are also used to produce electrically conductive coatings, and these have been used for panel lighting and for frost-free windshields. Moreover, stannous dichloride is used as a reducing agent in the preparation of technetium-99m radiopharmaceuticals.

Mono- and dialkyltin compounds ($RSnX_3$ and R_2SnX_2) have been well established as high-performance stabilizers in the poly(vinyl chloride)-processing industry for more than 40 years. These additives are firmly bound in the plastic; certain methyl-, octyl-, and dodecyltin stabilizers are approved for use in food and beverage containers, food wrap, and blister packaging for pharmaceuticals and other consumer products. Diorganotins are used in the manufacture of antioxidants. Trisubstituted organotin compounds (R_3SnX) have biocidal properties. One major application for these compounds has been as antifoulants in marine paints to prevent the growth of barnacles, seaweed, and other organisms on the hulls of ocean vessels. The antifouling paints contain approximately 20% (w/w) tributyltin, which is slowly leached into the surrounding water and reaches concentrations at the part per trillion level (ppt) (Matthias *et al*., 1988). The use of tin-containing paints for smaller marine crafts is highly restricted in many countries. The regulation restricts the tributyltin content of paint up to 4–5 μg/cm^2. Other applications include the use of tributyltin compounds as industrial disinfectants, as well as the use of triphenyltin and tricylohexyltin compounds as agricultural fungicides and acaricides (Isensee *et al*., 1994). The tetrasubstituted organotin compounds (R_4Sn) are mainly used as intermediates in the synthesis of other organotin compounds.

4 ENVIRONMENTAL LEVELS AND EXPOSURES

4.1 General Environment

Tin is a component of many soils and may be released in dust from windstorms, roads, and farming activities. Tin bound to the soils and sediments is relatively immobile. Other sources of tin are processes such as burning of waste or burning of fossil fuels (coal or oil). Only a few toxicological studies with inorganic tin have been conducted. It is generally accepted that toxicity of inorganic tin is very low. With an EC_{50} of 22 mg/L, only low toxicity was observed in daphnids (Rudel, 2003).

The contamination of organotin compounds in the aquatic system causes reproductive failure and population decline in gastropods, mussels, oysters, and others (Hung *et al*., 2001; Oehlmann *et al*., 1991). The no-observed-effect level (NOEL) for spat of the oyster species is approximately 20 ng/L; however, the NOEL for the development imposex is <1.5 ng/L (WHO, 1990). The permitted amount and rate of tributyltin compounds that can leach out from the marine paints in contact with water is <4 μg of organotin cm^{-2}/d^{-1} according to the Organotin Antifouling Paint Control Act of 1988. There is an international agreement that organotins are harmful to the marine environment, and, therefore, environmental concern led to introduction of legislative controls in a number of countries including the United Kingdom, the United States, Canada, and the European community. Nevertheless, despite the legislation restricting the use of tributyltin in antifouling paints, monitoring programs revealed the presence of organotin compounds in costal water sediments far from the source of organotins. Tributyltin is widespread in the North Sea and in the marine environment of southwestern British Colombia and Italy, which have intensive shipping activity (Cocchieri *et al*., 1993; Stewart and Thompson, 1994; Strand *et al*., 2003). In most of the Asian countries except Japan, South Korea, and Hong Kong, there are no such restrictions on the use of trisubstituted organotin compounds. The world's largest shipbuilding industries are in Korea, Japan, and China, where the highest concentrations of tributyltin and triphenyltin have been found (Kannan *et al*., 1995a; Sudaryanto *et al*., 2002).

4.1.1 Food and Daily Intake

Estimated daily intake of tin from food and water (excluding canned food) is 1–8 mg/day, ranging from 2.9–1 mg/day in 3-year-old children to 7.5 mg/day for female vegetarians (Vannoort *et al*., 2000; van Dokkum *et al*., 1989; Ysart *et al*., 1999). The increase in the estimated total dietary tin intake (estimated as 0.19 mg/

day (Hamilton and Minski, 1972) has been observed in 1991 in the United Kingdom because of an elevated tin concentration in the canned vegetables (Ysart *et al*., 1999). The main sources of tin are canned foods, cereal grains, dairy, meat, vegetables, seaweed, licorice, and certain toothpastes. Average concentration of tin in fresh vegetables, meat, milk, and fish is <1 mg/kg and often below the limit of determination (Biego *et al*., 1999). Food packed in cans coated with lacquer contains <10 mg tin/kg food, whereas the tin content in preserved food in unlacquered cans is on average 24 times higher and ranges from 20–>200 mg/kg (Perring and Basic-Dvorzak, 2002). The FAO/WHO Expert Committee of food additives suggests weekly intake for tin of 14 mg/kg of body weight or 120 mg/day for adult with 60-kg weight (WHO, 2001). Foods with a low pH such as orange, pineapple, and tomatoes are more aggressive to tin coating. Such acidic foods can cause the dissolution of tin and increase concentration of tin, exceeding 200 mg/kg (Vannoort *et al*., 2000). The concentration of tin in drinking water is generally estimated as <0.01 mg/L (Sherlock and Smart, 1984). The intake of tin from water can range from 0.012–0.02 mg/day, with daily consumption of water at approximately 2 L/day. Teapots with a glazed surface can be additional sources of tin leaching into tea and other infusions (Ajmal *et al*., 1997). However, stainless steel teapots are harmless, and there is no metal release during the infusion process.

Dietary exposure to organotin compound may result from the consumption of organotin-contaminated meat and fish products. The butyltin and phenyltin compounds accumulate within the marine food chain, eventually accumulating in aquatic food products such as fish, oysters, and crab. The finding of triphenyltin in costal fish, as well as in open oceanorpelagic fish, suggests biomagnifications through the food chain (Takeuchi *et al*., 1991). The highest concentrations of 1 μg/g muscle of triphenyltin have been measured in fish species obtained from bay or inshore areas. The average intake of organotin by humans may be estimated from a standard market-basket survey. The average daily intakes of triphenyltin per 50-kg person in Japan have been determined to be approximately 0.6–10.4 μg from 1990–1997 (NIHS, 1998). The average consumption of tributyltin ranges from 0.18 μg/day/person in the United Kingdom to 2.6 μg/day/person in Korea (Keithly *et al*., 1999). Moreover, cookies, which were prepared with silicone-backed parchment, foodstuffs stored in containers made of PVC polymers, and several brands of wine have been reported to contain butyltin compounds (Kannan *et al*., 1999; Takahashi *et al*., 1999). Approximately two thirds of the global consumption of organic tin is used as heat stabilizers for polyvinyl chloride (PVC) polymer production (Hoch, 2001). The release of these compounds from PVC tubing used for drinking water systems has been reported (Sadiki and Williams, 1999). Approximately 40% of distribution waters supplied through PVC pipes contained organotin compounds. Monomethyl-, dimethyl-, monobutyl-, and dibutyltin levels ranged up to 291 ng/L, 49.1 ng/L, 28.5 ng/L, 52.3 ng/L, respectively (Sadiki and Williams, 1999). By use of the predicted tolerable daily intake value for dibutyltin of 5 μg/kg (as tin), an adult who weighs 60 kg and consumes 2 liters of drinking water per day can safely ingest water with a butyltin concentration of 150 μg/L (as tin) (Summer *et al*., 1996). However, there are no data on levels of triphenyltin in drinking water. The possible contamination of dibutyltin dilaurate has been reported in poultry and turkey in the United States (Epstein *et al*., 1991). The estimated daily intake of butyltin compounds in Japan is 6.7 μg/person, whereas in certain countries in Asia it ranges from 0.027–2.1 μg/person (Kannan *et al*., 1999). Trials of triphenyltin formulation in Germany and the United Kingdom on potatoes and sugar beets revealed that residues ranged from 0.016–0.3mg/kg in potatoes 7 days after application and less than detection limit in beets 35 days after application (ACP, 1990; FAO WHO, 2005).

4.1.2 Water, Soil, and Air

Inorganic and organic tin compounds can be found in the air, water, and soil near places where they are naturally present in the rocks, or where they are mined, manufactured, or used. In the atmosphere, tin exists as gases and fumes and attached to the particles of dust. These particles containing tin can be spread by wind or washed out by rain. Some inorganic tin compounds dissolve in water, whereas the most inorganic tin compounds bind to soil and to sediments in water.

Organotin compounds can degrade into inorganic tin compounds or remain unchanged for years in sediments. Marine biota tends to accumulate the highest concentration of organotin compounds. Analysis of seawater taken before restriction on butyltins used in antifouling paints had shown the concentrations of butyltin compounds ranging between 50 ng/L and 1 μg/L in European and North American marinas. Maximum concentrations in marine water rarely exceeded 100 ng/L along the English Channel and Atlantic coasts (Alzieu, 2000). Despite the legislation restricting the use of tributyltin in Europe, biomonitoring shows that the concentration of butyltins in the gulf of Naples ranges up to 3.4 μg/L in seawater, whereas the target value (EQT) was set at 0.097 ng/L in 1990 (Cocchieri *et al*., 1993). In marinas of southwest of The Netherlands, tributyltin

concentration ranged up to 2.5 µg/L in seawater in 1994 and 0.3 µg/L in seawater and 17.5 mg/g in sediments in 1998 (Ritsema *et al*., 1998). Considerable variability in butyltin concentrations has been detected on the French coast. Maximum concentrations exceed 100 ng/L (Michel and Averty, 1999). The levels of tributyltin in the harbor of Osaka City, Japan, is in the range of 2–33 ng/L in water and 0.002–0.966 mg/kg dry wt in sediments, whereas in costal areas of Otsuchi Bay, the level is in the range 8–74 ng/L and 0.01–0.64 mg/kg in seawater and sediments, respectively (Harino *et al*., 1998). It was suggested that high concentrations of organotins in water could be a result of their release from sediments. The sediments in the marinas act as a long-term source of dissolved-phase contamination, because the water phase is in equilibrium with the sediments (Ritsema *et al*., 1998). The retention of butyltins in seawater is highly variable, depending on temperature, pH, and light. The calculated half-lives range from 6 days in summer time for turbid water to 127 days in winter for nonturbid water (Alzieu, 2000). The retention of the total mineralization of tributyltins is in the range of 50–75 days (Rudel, 2003). Degradation in anaerobic sediments can range from 2 years up to decades. Half-lives of 10.2 and 5.1 days are calculated for tributyltin and dibutyltin, respectively, in an activated sludge system (Stasinakis *et al*., 2005), whereas the half-life of tributyltin added to estuarine samples ranged from 3 to 13 days (Lee *et al*., 1989). Organotins are hydrophobic substances and, hence, only slightly soluble in water. Therefore, organotins may remain in marine sediments for many years because of their relative stability under the anoxic conditions in sediments (Fent, 1996a). It has been shown that the retention time for tributyltin is approximately 2.1 years and those for dibutyl- and monobutyltin are 1.9 and 1.1 years, respectively, in sediments (Sarradin *et al*., 1995). In salt water, the toxicity of organotins is less than in freshwater because of reduced interactions between organotins and microorganisms (White *et al*., 1999).

The existing sediment certified reference material (CRM) is estimated as 1.27 mg/kg for controlling the quality of highly polluted sediments; however, it cannot be used for the quality control of analysis of coastal sediment with low butyltin content (USEPA, 1997). The average contamination of butyltins in harbor sediments of the U.S. costal area is approximately 54 µg/kg dry wt., whereas levels 10 times lower are recorded in sediments collected 25 km from Vancouver harbor (Canada) (Stewart and Thompson, 1994). The concentrations of organotin compounds are high in costal areas of the Asian countries, where no regulations on the use of trisubstituted organotin compounds have been adopted. Butyltin contamination is widely distributed along the costal water of Malaysia, with concentration in the range of 2.8–1100 ng/g dry weight for tributyltin in sediments (Sudaryanto *et al*., 2004). Contamination of 14.7 µg/L has been recorded in harbor water in Bahrain (Hasan and Juma, 1992). Triphenyltin levels in ambient water and sediments in the Tokyo Bay area were estimated at 1.8 ng/L and 0.19 ng/g in 1993, respectively. The concentrations of butyl- or phenyltins in water depend on both fresh input and degradation and absorption kinetics. However, their fresh input is predominant to overwhelm elimination by degradation and absorption. Concentrations of up to 0.2 µg/L of triphenyltin were detected in boat harbors, and a concentration of 1.5 µg/L was determined in areas where triphenyltin fungicides had been sprayed (Fent and Hunn, 1991). Triphenyltin concentrations in foliage and soils were determined as 8.5–37 µg/g dry weight and 1.2–12 µg/g dry weight, respectively (Kannan and Lee *et al*., 1996b). The largest contamination of organotin compounds in fresh water river systems has been documented in South Carolina in 2000 and was >10 mg/kg in surface-water bed sediments.In bed sediments of a beaver pond located approximately 1.6 km downstream of the release, the organotin concentrations reached 40 mg/kg (Landmeyer *et al*., 2004).

The concentration of organotins detected in raw municipal wastewater ranged between a few nanograms and a few micrograms per liter (Chau *et al*., 1992). Organotins accumulate on the suspended solids reaching concentrations up to 1.5 mg/kg in digested sludge (Fent, 1996b). Various organotin compounds are detected in surface and pore waters. For example, in Ganga Plain in Kanpur-Unnao industrial region, their concentrations in surface water range from 2.1–70.1 ng Sn/L for monobutyltin, 1.7–101.1 for dibutyltin, and 2.9–19.8 for tributyltin (Ansari *et al*., 1998).

4.2 Working Environment

The limit value (time-weighed average) for tin in air was set 0.1 mg/m^3 in 1979 specified as a maximum worksite concentration. The Occupational Safety and Health Administration (OSHA) and the National Institute for Occupational Safety and Health (NIOSH) have established a workplace exposure limit for organic tin compounds, which is 0.1 mg/m^3 as Sn, regarding tin and inorganic tin compounds, it is 2 mg/m^3 as Sn except tin oxide.

5 METABOLISM

5.1 Inorganic Tin

5.1.1 Absorption

5.1.1.1 Inhalation

Various case reports on effects of occupational exposure to tin dust have been published. Long-term

exposure to tin dust or fumes results in accumulation of the particles of tin compounds in the lung tissues, because it is poorly absorbed. Inorganic tin deposits in the lung because of its insolubility and lack of absorption (Browning, 1969; Krigman and Silverman, 1984). Thus, lungs are the main target organs for tin dust. Inhalation of SnO_2 dust is a hazard in the deep mining of tin, and a chronic industrial exposure to tin dust also causes benign pneumoconiosis called stannosis (Pendergrass and Pryde, 1948; Sluis-Cremer *et al*., 1989). Inhalation of tin oxides in molten metal refining can lead to the same condition. Silicosis and pulmonary heart diseases were reported as common causes of death among Chinese workers in tin mines in the late 1980s (Chen *et al*., 1992). There was nearly a sixfold increase in deaths from pulmonary heart disease and approximately 50% increase in mortality from nonmalignant respiratory diseases. However, the risk from pulmonary heart diseases among tin miners may be due to respirable silica dust in hard rock tin mines (Chen *et al*., 2005). It has also been found that the risk of lung cancer was increased among the tin miners and that silicosis seems not to be related to the increased risk of cancer (Chen and Chen, 2002; Fu *et al*., 1994). Later study analyzed mortality among the UK tin miners and revealed an increased risk of death from gastric cancer, especially among underground workers (Raj *et al*., 2003). The incidence of gastric cancer was increased by 40% from 1941 to 1986. However, such an increase can be associated with high concentration of radon in the UK tin mines and exposure to arsenic, a by-product of certain tin mines. Exposure of workers to a stannous chloride solution in a bottle factory has caused wheezing, exertional shortness of breath, cough, and thoracic pain. However, it has been shown that the causal agent was the hydrochloric acid released by the action of heat on the aqueous stannous chloride solution.

5.1.1.2 Ingestion

In fact, inorganic tin compounds are comparatively harmless because of their poor absorption, relative insolubility, and low retention in tissue (Johnson and Greger, 1982; Krigman and Silverman, 1984; Winship, 1988). Studies on animals and on human subjects show that the absorption of ingested inorganic tin is approximately 5% in mice, rats, dogs, and monkeys, whereas apparent absorption in humans is approximately 3% from a diet supplemented with 50 mg tin/day (Johnson and Greger, 1982). Stannous compounds are more readily absorbed from the gastrointestinal tract than stannic compounds (WHO, 1980). Results of a study on distribution of tin in green beans from cans and in tin-free green bean puree indicated that stannous ions are strongly bound to insoluble bean constituents, and such complex prevents liberation of free tin ions (Horio *et al*., 1970). The toxic effects of tin depend on the concentration of tin in the foodstuff ingested (WHO, 2001). There are several case reports of gastric irritation and vomiting in humans consuming canned foods, especially acidic fruit products packed in unlacquered or partially lacquered tinplate cans. Fifteen students were poisoned after the consumption of canned orange beverages in 1963, and a further 81 cases were reported elsewhere. The symptoms observed were vomiting, diarrhea, fatigue, and headache. Tin content of random samples of cans from the same manufacturers ranged from approximately 75–500 mg/kg; 113 cases were reported in Washington and Oregon in 1969 after ingestion of canned tomato juice (Barker and Runte, 1972). Analysis of tin from the juice consumed has shown a tin content of 477 mg inorganic tin per liter juice. In general, the levels of tin in foods, which are responsible for these symptoms, are between 250 and 2000 mg/L. In the fruit juice, tin mostly exists in the ionic form as complexes with acids (WHO, 2001).

Gastrointestinal irritation has been seen in cats, but not dogs, given 5 or 10 mL/kg of orange juice containing >400 mg/kg of tin (Benoy *et al*., 1971; Omori *et al*., 1973).

5.1.1.3 Skin Contact

Tin tetrachloride, stannous chloride, and stannous sulfate are strong acids, whereas sodium stannate and potassium stannate are strong alkalies. Therefore, such inorganic tin compounds can cause irritation of the skin or the eyes because of acid or alkaline reactions produced with water. Tin salts are irritants to the skin, especially dusts or mists of acidic and basic tin salts. However, despite widespread use of tin salts, only occasional cases of allergic dermatitis in human have been reported.

5.1.2 Distribution

Tin is present in small amounts in all human and animal organs, with approximately 352 mg total in an adult man. Animals fed on tin-containing food may accumulate tin in the body, and it is retained in the liver, kidneys, heart, bones, and least in the brain (Browning, 1969). Tin is widely distributed after parenteral injection, especially in the liver and spleen, where it deposits in the reticuloendothelial system, and most of it is excreted eventually in the urine and a little in the bile (Barnes and Stoner, 1959). In mice, it has been shown that intraperitoneally injected stannous chloride is accumulated in all organs except the testis (Chiba *et al*., 1990). A 1% solution of $SnCl_2$, when applied for 18 hours to dermal scratches in rabbits, produced a reaction with intraepidermal pustules but none on intact skin (Stone and Willis, 1968). It is generally accepted

that only a trace amount of inorganic tin crosses the placental barrier and is not a teratogen (Hiles, 1974). However, a report by Chmielnicka *et al*. (1981) demonstrated that considerable concentrations of tin have been found in embryos of rats exposed to stannous chloride.

5.1.3 Excretion

Inorganic tin is excreted mainly in the feces, with additional slow elimination in the urine (Browning, 1969; Winship, 1988). The fraction excreted with the bile is <15% (WHO, 1980). The average concentration of tin in the urine has been estimated at approximately 0.023 mg/L, with a range 0–0.04 mg/L (Baselt *et al*., 1989). In humans and animals, concentration of tin is always higher in urine than in hepatic bile (Ishihara and Matsushiro, 1986). Gastrointestinal absorption of inorganic tin was reported to be extremely low in rats. Approximately 90–99% of administered (^{113}Sn) tin chloride at a dose of 50 mg/kg weight by gavage in water was excreted in the feces within 48 hours, whereas only traces of ^{113}Sn were detected in the urine and in the organs (Fritsch *et al*., 1977). Only 3% of a single oral dose of 20 mg/kg has been shown to be absorbed, half of which has been excreted within 48 hours (Hiles, 1974). However, in 19.8% (mice) to 62.8% cases tin has been excreted through urine within 3 days after intravenous injection of ^{113}Sn(II) as chloride (Furchner and Drake, 1976). Mice injected intravenously with 0.1 or 0.2 mL of 1% tin citrate excreted no tin in their feces or urine (Browning, 1969). Thus, there are differences in the excretory routes of tin in various studies, and these may be due to the differences in the forms, doses, and routes of administration.

5.1.4 Biological Half-Life

The biological turnover of tin in tissue and organs has been studied by a number of research groups. In rats, the biological half-life has been estimated to be 84 days after an injection of ^{113}Sn, and half-life of 100 days was reported for tin in the skeleton (Durbin *et al*., 1957). Hiles estimated the half-life for stannous chloride in rat liver to be 10–20 days, whereas in bone, the half-life for both Sn(II)and Sn(IV) has been estimated as 34 and 40 days, respectively (Hiles, 1974). However, later, it was estimated that the half-life was approximately 85 days in rat liver and 50 days in spleen (Marciniak, 1981). The complete elimination of tin (^{113}Sn(II)) after intravenous or intraperitoneal injections has been observed after 90–100 days (Furchner and Drake, 1976).

5.1.5 Biotransformation

Inorganic tin can be methylated by chemical and biological reactions in the environment (Ashby and Craig, 1988). A mixed inoculum of microorganisms from Chesapeake Bay sediments was able to transform inorganic tin to organic compounds (Hallas *et al*., 1982). The methylation of tin compounds can contribute to the biocycling of tin in the environment by increasing the volatility, toxicity, adsorptivity, and mobility (White *et al*., 1999). In aqueous solutions at acidic pH, divalent tin forms $Sn(OH)_2$, had very low solubility.

5.2 Organotin Compounds

5.2.1 Absorption

5.2.1.1 Inhalation

In man, organotins can be also exposed by the inhalation route. The first documented case of organotin poisoning in humans was in 1880, when workers were exposed to triethyltin acetate vapors (Reiter and Ruppert, 1984). The exposed workers had general weakness, nausea, and diarrhea. There are few case reports of trimethyltin chloride exposure (Besser *et al*., 1987; Kreyberg *et al*., 1992; Yanofsky *et al*., 1991). One student was exposed to trimethyltin while he measured 300 mg of powdered organotin (Saary and House, 2002). Even minimal exposure to the extremely toxic trimethyltin causes cough, difficulty in breathing, abdominal pain, headache, and impaired memory. Collective poisoning with triphenyltin during crop treatment has been reported. One patient repeatedly exposed to triphenyltin had an enlarged liver and elevated serum enzymes. There was evidence of liver damage at biopsy that included increased collagen and signs of hepatocyte regeneration (Horacek and Demcik, 1970). Two cases of poisoning by inhalation of fungicide powder containing 60% triphenyltin have been reported (Manzo *et al*., 1981). Patients experienced a brief loss of consciousness, severe headache, weakness, and photophobia. There were no abnormal neurological findings.

There were no deaths, and only minor clinical signs were noted when a group of rats was exposed to atmospheres containing almost saturated vapors of tributyltin compounds for 7 hours (Schweinfurth, 1985). However, changes in exploratory behavior have been reported in mice when they were exposed to an aerosol of tributyltin in olive oil at concentrations ranging from 0.05–0.4 mg/L (50–400 mg/m^3) (Truhaut *et al*., 1979). Exposure to an aerosol of tributyltin resulted in significant lesions of tissues in the respiratory system, diffuse congestion of the pulmonary blood vessels, as well as distention and rupture of the alveoli (Truhaut *et al*., 1981).

5.2.1.2 Ingestion

The organotin compounds can be absorbed from the gastrointestinal tract (Kimbrough, 1976). The most

toxic organotin compounds are trimethyl- and triethyltin, which are well absorbed from the gastrointestinal tract, unlike inorganic tin. The poorly absorbed alkyl and aryltin compounds are less toxic when given orally (Winship, 1988). Monoethyltin is absorbed to approximately 8% in the rats, whereas absorption of octyltin compounds is <0.1% (Bridges *et al*., 1967). The absorption of dibutyltin and triphenyltin compounds is approximately 10% in rats. The digestive gland of mollusks is the primary site of organotins uptake (Sole *et al*., 1998).

5.2.1.3 Skin Contact

Short-chain alkyltin compounds can be absorbed from the skin easily, as indicated by their high toxicity after dermal application (Hall and Ludwig, 1972). Triethyltin contaminants caused 217 poisonings and 111 deaths when diethyltin diiodide combined with linoic acid was used to treat furuncles (Barnes and Stoner, 1959). Exposure to dimethyltin at 80 mg/kg caused dermal necrosis with black scar formation in rats. Organotins such as tributyl and dibutyltin can cause skin burns that heal quickly if contact is ceased. Extended exposure to organotins can cause erythematous dermatitis. Studies on rat dorsal skin have shown that tributyltin is much more irritating than dibutyltin (Middleton *et al*., 1978). Treatment with tributyltin at 33 nmol/cm^2 produced epidermal necrosis and dermal inflammation, whereas dibutyltin produced similar effects only at 10 times the higher dosage. The contact with antifouling paints containing tributyltin resulted in irritation, erythema, and even mild pustular lesions (Lewis and Emmett, 1987). A single dermal application of the commercial pesticide Lastanox containing approximately 20% of tributyltin oxide caused edema, erythema, papules with inflamed borders, hemorrhages, and hypermia (Pelikan and Cerny, 1969). Complete recovery has been observed within 20 days after exposure.

5.2.2 Distribution

It has been shown that in fish and marine mammals, tributyltin is predominantly accumulated in blubber and muscle, whereas dibutyltin concentration is higher in the liver and kidneys (Guruge *et al*., 1996; Kannan *et al*., 1996a). Preferential accumulation of butyltins in the liver and kidneys may be associated with the presence of increased amounts of metal binding proteins such as metallothionein-like proteins or glutathione, which may play an important role in the detoxification of alkylating agents (Kannan *et al*., 1996a; Takahashi *et al*., 2000). The trialkyltins are transported in the body through enterohepatic circulation (Iwai *et al*., 1982). Tributyltin metabolism is rapid, and tributyltin itself and its metabolites are detectable in blood within 3 hours after administration. The percent of tributyltin composition to total butyltins in the serum was higher than in tissues (Shim *et al*., 2002). In the kidneys, the concentrations of triethyltin were approximately 30% of that in the liver. The metabolism and excretion of butyltins are less efficient in the mammalian species than in water birds (Iwata *et al*., 1997). In wild birds, muscle and feathers accumulate high concentrations of butyltins (Guruge *et al*., 1996). Among butyltins, monobutyltin is dominantly retained in birds.

The highest concentration of triethyltin was found in the liver and blood of rats exposed to 20 mg/kg triethyltin hydroxide for 5 days, followed by 10 mg/kg for another 12 weeks (Cremer, 1957). A similar pattern of distribution was observed in rats orally exposed to tetrapropyltin, tetrabutyltin, and tetraethyltin at 10 mg/kg. The kidney contained relatively high concentrations of all three organotins, whereas the liver retained mainly tetrabutyltin. The distribution to the brain was poor, but the amount of triethyltin in the brain has been shown to increase with time (Arakawa *et al*., 1981). The highest levels of trimethyltin have been observed in the kidney, blood, and lungs 1 hour after administration, with penetration into the brain and adipose tissue 12 hours after exposure (Doctor *et al*., 1983). Dodecyltin compounds were mainly found in thymus and liver, followed by kidney and bone marrow (Schering, 1992). Residues of these compounds in the brain reached only 1.4% of those in the liver. Triphenyltin is mainly distributed in the kidneys followed by the liver, brain, and heart after oral administration to rats or after chronic exposure for 104 weeks (Kellner *et al*., 1989). Relatively high levels of triphenyltin were found in the liver, pancreas, kidneys, and brain of hamsters within 24 hours after treatment with 50 mg/kg triphenyltin (Ohhira and Matsui, 1996). High levels of triphenyltin were detected in brain tissues in rats given a single oral dose of 50 mg/kg triphenyltin chloride or 6 mg/kg fungicide containing 60% of triphenyltin (Lehotzky *et al*., 1982b; Ohhira and Matsui, 1996). The authors suggested that thriphenyltin is able to cross the blood–brain barrier because of its high lipophilicity. Tetraphenyltin accumulates more rapidly and mainly in liver of rats fed with 55.4 mg/kg tetraphenyltin, but metabolized faster than in kidney (Ohhira and Matsui, 2003a).

5.2.3 Excretion

The excretion route of tributyltin is mainly the bile rather than the urine. An initial rapid elimination of tributyltin oxide injected intraperitoneally to mice has been shown with a slow phase in the feces (Brown *et al*., 1977). Thus, approximately 90–96% of ingested tributyltin is excreted in the feces (Evans *et al*., 1979).

Similar to butyltins, orally administrated dodecyltin, octyltin, and phenyltin compounds are eliminated almost exclusively through feces, with only a small amount in urine (Eckert. *et al*., 1992; IPCS, 1999; Penninks *et al*., 1987). Approximately 10% of dioctyltin is excreted in the urine, whereas <1% of triphenyltin is excreted in urine, mainly as unchanged parent compound. Ethyltin trichloride administered intraperitoneally was excreted almost exclusively in the urine in rats, whereas diethyltin is excreted through the bile (Bridges *et al*., 1967; Cremer, 1957). Carboxyethyltin, after daily intravenous administration, was detected mainly in urine, suggesting a rapid excretion of the parent compound without any metabolism (Penninks and Seinen, 1985b). However, daily gavage of 15 mg/kg carboxyethyltin resulted in excretion in urine and feces as a result of fast hydrolysis of the parent compound in the gastrointestinal tract. Therefore, excretion of organotins depends on their chemical structure, solubility (hydrophilic or hydrophobic nature), and mode of administration.

5.2.4 Biological Half-Life

For triphenyltin, the biological half-life has been estimated to be approximately 3 days in rat brain and is considerably longer in guinea pig. The half-life of tin in the liver and kidneys has been estimated as 3–4 days after oral absorption of diethyltin chloride in rats, but is longer in muscles and bone. The half-life of injected bistributyltin oxide in mice is approximately a few days with an initial rapid elimination followed by a slow elimination rate of 3–4 weeks, similar to inorganic tin (Brown *et al*., 1977). For tricyclohexyltin, half-life ranges from 5–40 days with slow removal from the brain.

5.2.5 Biotransformation

The metabolism of organotin compounds determines their environmental fate and tissue retention. Although in both aerobic and anaerobic conditions, organotins (such as tributyltin) biodegrade through a sequential dealkylation process to inorganic tin, the addition of organic nutrients in soil can slow down the process of degradation (Shizhong *et al*., 1989). The exposure of *Chlorella vulgaris* to tributyltin results in rapid biosorption with sequential degradation to dibutyl- and monobutyltin (Tsang *et al*., 1999). The reaction rates for the cleavage of carbon bonds are greater for tributyltin to dibutyltin than that for dibutyltin to monobutyltin or monobutyltin to inorganic tin. Recent evidence demonstrates that toxicity of butyltin compounds decreases with degradation (Suzuki *et al*., 1992; Whalen *et al*., 1999). Butyltins are metabolized by the microsomal cytochrome P450 enzyme hydroxylation system (Fish, 1984; Iwai *et al*., 1981; Ueno *et al*., 1995). However, debutylation of dibutyltin to monobutyltin by microsomal monooxygenase system is considerably slower than that of tributyltin to dibutyltin (Kannan *et al*., 1996a). Isolated rat liver microsomes metabolize tributyltin to hydroxybutyldibutyltin derivatives with further oxidation to 1-butanol, 1-butane, and ketones. Tributyltin is metabolized more rapidly than triphenyltin. Phenyltins biodegrade through sequential dephenylation with cleavage of the tin-carbon bond by biological, ultraviolet, chemical, or thermal mechanisms (Stasinakis *et al*., 2005). Triphenyltin is metabolized by the cytochrome P450 enzyme system (Suzuki *et al*., 1992), but it can convert cytochrome P450 into cytochrome P420 and thereby affect the function of the monooxygenase system (Prough *et al*., 1981). The metabolic products are less toxic than parental compounds. In humans, the cytochrome P 450 system enzymes are not involved in dealkylation and dearylation of organotins (Ohhira *et al*., 2003b).

6 LEVELS IN TISSUE AND BIOLOGICAL FLUIDS

The average concentration of tin in urine is approximately 4.2–42.2 nmol/L and in hair is approximately 0.42–3.37 nmol/g. The average concentration of tin in blood of normal subjects is 0.14 mg/L and is found mainly in the erythrocytes (Baselt *et al*., 1989). Tissue analysis has shown that tin is present in lungs, adrenal glands, and liver at concentrations of 37, 23, and 23 mg/kg, respectively. The highest amount of tin (in dry ash) has been found in the cecum (130 mg/kg), ileum (79 mg/kg), rectum (57 mg/kg), and sigmoid colon (45 mg/kg) (Schroeder *et al*., 1964). Inorganic tin does not accumulate in soft tissues with increasing age (Baselt *et al*., 1989). A dose-dependent increase in the tin content of the tibia and kidneys of weanling rats has been reported after administration of tin in the diet ranging from 1–2000 µg/g diet (Johnson and Greger, 1985).

Concentrations of organotin compounds in organisms are very high near sources such as ports, pleasure-boat marinas, shipyards, and much-traveled shipping routes. The bioconcentration factors for triphenyltin have been found to be 2090 in carp kidney and in the range of 80,000–440,000 in the crab hepatopancreas (Kannan *et al*., 1995b; Tsuda *et al*., 1987). The levels of triphenytyl in mussels and samples of sea birds from coastal areas of Japan are approximately 0.45 µg/g and 0.05 µg/g, respectively. In fish from the same coastal areas, the maximum concentration of triphenyltin has been reported to be 2.6 µg/g in 1989 and 0.25 µg/g in 1995. Butyltin compounds in the tissue of mussels

from Egremont are in the range 0.217–0.508 mg/kg dry wt, slightly higher than the range of 0.11–0.201 mg/kg dry wt at New Brighton (Harino *et al*., 2005). The concentration of tributyltin reached 1000 ng/kg in bream muscles from the Elbe estuary (Shawky and Emons, 1998). The concentration of trisubstituted organotins is higher in digestive glands than in other tissues. Tributyltin concentration in digestive tissue and the gill has been reported to be twice as high as in mantle and muscle (Laughlin *et al*., 1986; Shim *et al*., 1998). The average estimated level of triphenyltins in fish and other marine products ranges from 0.03–1.3 mg/kg (Ishizaka *et al*., 1989). In fish from sampling sites of the North Sea, concentrations of tributyltin range between 27–202 ng/g in muscles and 54–223 ng/g in liver (Shawky and Emons, 1998). Concentrations of tributyltin in the muscle of fish from the Gulf of Mexico were from 0.1–0.196 mg/kg, whereas in fish from Switzerland, the concentration reported is 0.17 mg/kg (Fent and Hunn, 1991). A recent study from Denmark determined the levels of total butyltin in shellfish and fish, and they were approximately 10–440 ng/g (Nielsen and Strand, 2002). It was shown that concentrations of butyltin compounds in the liver of dolphins from the Italian coast of the Mediterranean Sea were in the range of 1.2–2.2 µg/g (Kannan *et al*., 1996a).

The total butyl content in the liver of mice exposed to tributyltin is approximately 1 mg/kg and remains unchanged 24 hours after administration. The contents of tin in the liver of rats and guinea pigs are lower at 3 hours and higher at 24 hours than those of mice (Ueno *et al*., 2003). The main form of butyltin in the liver of rats treated with tributyltin was dibutyltin. After a single oral dose of tributyltin, high levels of tributyltin were seen in the frontal and temporal lobes and in the cerebellum in the brain of rabbits.

Despite the possibility of human exposure to butyltins, only a few studies have examined butyltins in human tissues. The concentrations of butyltins in the liver of Japanese ranged from 59–96 ng/g wet wt, whereas monobutyltin and dibutyltin, degradation products of tributyltin, were predominant (Takahashi *et al*., 1999). The hepatic deposition of butyltin in a group of Danish men was from 1–33 ng/g, whereas total butyltin concentrations in human liver samples collected from Poland were in the range of 2.4–11 ng/g (Nielsen and Strand, 2002). The variation in concentrations of butyltin among individuals from different countries may probably reflect the differences in dietary intake of fish (Nielsen and Strand, 2002). The concentration of butyltin in blood collected from 32 volunteers during a blood drive organized by American Red Cross in central Michigan in 1998 ranged from 0–101 ng/mL (Kannan *et al*., 1999). After removing the highest concentration from the data, the average concentration was approximately 4.5 ng/mL. The occurrence of butyl compounds in human blood suggests the widespread exposure to these compounds from a variety of sources. When tributyltin in a mixture of 3 mL cherry brandy and 7 mL ethanol was given orally to a volunteer, only 5.1–5.4% of the dose was excreted in the urine, mainly as dibutyltin metabolites (Uhl, 1986). In 1999, more than 1000 people were exposed to methyltin contained in cooking oil in Southeast China. The concentration of trimethyltin in blood samples from these people after the incident was approximately 70 ng/g, whereas the levels of di- and trimethyltin in urine were approximately 80 ng/mL (Jiang *et al*., 2000). Analysis of tin in organs showed high concentrations of methyltins in liver (1.93 µg/g dimethyltin and 1.42 µg/g trimethyltin), kidney (1.05 µg/g dimethyltin and 0.47 µg/g trimethyltin), and heart (0.1 µg/g dimethyltin and 1.48 µg/g trimethyltin).

7 EFFECTS AND DOSE-RESPONSE RELATIONSHIPS

Tin has not been shown to be an essential element for humans. However, one report has claimed that 1–2 mg tin/kg diet is needed for growth in rats (Schwarz, 1974).

7.1 Inorganic Tin

7.1.1 Local Effects and Dose-Response Relationships

7.1.1.1 Human

There are several reports on outbreaks of food poisoning from tin contamination in canned food. The gastrointestinal disturbances such as vomiting and diarrhea have been reported after consumption of formulated orange juice and apple juice containing 250–390 mg/kg of tin in a large number of people in Kuwait (Benoy *et al*., 1971). A total of 113 cases of acute gastroenteritis during a 3-month period was documented after ingestion of canned tomato juice containing tin at concentration of 131–405 mg/kg (Barker, Jr. *et al*., 1972). It has been reported that canned peaches containing 563 mg tin/kg had caused gastrointestinal symptoms (Nehring, 1972). Volunteers given orange juice containing tin at concentrations up to 1400 mg/kg on various occasions experienced nausea and diarrhea (Benoy *et al*., 1971). However, volunteers who had ingested tin at doses of 120–200 mg/day (equivalent to approximately 1.6–2.9 mg/kg body weight per day) for 23 days did not show any adverse effects (Calloway and McMullen, 1966). Inorganic stannous and stannic chloride at a concentration of 10–50 µmol/L induce prolonged suppression of DNA synthesis in human lymphocytes

(McLean *et al*., 1983). Stannic chloride had been found to be a potential clastogen showing an age-related elevation of mitotic index, chromosome aberration, sister-chromatid exchanges, and micronuclei formation in blood lymphocyte culture from tin miners (Ghosh *et al*., 1988).

It is known that bad breath odor is mainly caused by volatile compounds formed by the catabolism of sulfur-containing amino acids by bacteria located in the crypts of the tongue and in periodontal pockets (van Steenberghe, 1997). Tin compounds can inhibit the formation of the volatile compounds through production of insoluble tin sulfide.

7.1.2 Systemic Effects and Dose-Response Relationships

7.1.2.1 Animals

Tin is considered an essential nutrient for growth in the rat, and a tin-deficient diet leads to reduced growth (Krigman and Silverman, 1984; Yokoi *et al*., 1990b). In animals, a low-tin diet results in poor growth, alopecia or bilateral hair loss, hearing loss, and reduced feeding efficiency. Dietary tin deficiency can cause imbalances of essential metals, causing an increase in calcium and a decrease in the concentration of zinc, copper, and manganese (Yokoi *et al*., 1990b). However, chronic administration of stannous chloride at the dose of 10 mg/100g can cause significant, but reversible, growth retardation of Wistar–Kyoto rats (Escalante *et al*., 1991). The addition of stannous chloride to rat diet at a concentration of 10–200 mg/kg for 28 days did not change the body weight of rats; however, there was a linear inverse response of feed intake (Pekelharing *et al*., 1994).

Oral administration of tin at a dose of 45 mg/kg weight induced vomiting and diarrhea in cats (Omori, 1966). Cats fed orange juice containing 540 mg/kg of tin, providing a dose of 5.4 mg/kg weight, had vomiting, and incidences of vomiting were increased with higher doses (Benoy *et al*., 1971). Neurological damage and renal damage have been reported in rats exposed to stannous chloride (Conine *et al*., 1976). There were marked differences in the toxicity of stannic and stannous oxide and salts in Wistar rats when given orally. Animals were fed diets containing 0, 0.03, 0.1, 0.3, or 1% tin salts or oxides for either 4 or 13 weeks showed no adverse effects, such as changes in mortality and body and organ weight at low doses but had growth retardation at high doses (de Groot *et al*., 1973). The insoluble tin compounds were shown to be relatively harmless, whereas cationic tin compounds soluble in water or dilute acids can be very toxic because of high absorption from gastrointestinal tract. The high toxicity of soluble tin compounds and ameliorating effect of supplementary dietary iron and copper are reported. Schafer *et al*. (1985) reported an oral LD_{50} of 775 mg/kg body weight for tin (II) oxide in deer mice.

ATSDR (Agency for Toxic Substances and Disease Registry) has evaluated the noncarcinogenic oral dose data for inorganic tin but did not derive a chronic oral minimal risk level (MRL), because the lowest dose tested, 0.7 mg Sn/kg per day as stannous chloride, reduced survival in a 42-month drinking water study in rats (Schroeder *et al*., 1968). However, ATSDR did derive an intermediate-duration of MRL of 0.3 mg/kg/day on the basis of a NOAEL of 32 mg Sn/kg/day (as stannous chloride) for hematological effects in rats in a 13-week dietary study (de Groot *et al*., 1973). Mammary adenocarcinoma, uterine sarcoma, and adenocarcinoma near the jaw occurred in a group of rats, which were fed a diet containing 2% chlorostannate for more than 1 year (Roe *et al*., 1965); 16% of B6C3F1 mice fed with stannous chloride at a concentration of 2000 mg/kg (or 0.2%) for period of 105 weeks developed hepatocellular adenomas or carcinomas (National Toxicology Program, 1981). A similar increase has been seen with the incidence of histolytic lymphomas among female mice. Injection of metallic tin powder into Lewis rats caused the enlargement of draining lymph nodes and intense hyperplasia of plasma cells (Levine and Saltzman, 1996). Another group has shown that no adverse effects were noted nor was there any difference in tumor incidence in a group of mice receiving over several generations sodium chlorostannate either at 1000 or 5000 mg/kg Sn in the drinking water or stannous oleate at 5000 mg/kg in the diet (Walters and Roe, 1965). It has been shown that the exposure of rats to tin at concentration 50–600 mg/kg in the drinking water resulted in a significant decrease in the bone compressive strength (Ogoshi *et al*., 1981).

Tin toxicity may involve the inhibition of certain key enzymes. The chronic administration of stannous chloride at a dose of 10 mg/100g body weight twice weekly for 4–6 weeks has been shown to cause the cardiac hypertrophy in stroke-prone hypertensive rats. The tin-induced increase in left ventricular heme oxygenase level, as well as cGMP content, has been accompanied by a decrease in left ventricular weight/body weight ratio (Seki *et al*., 1999).

In vitro exposure of K562 cells to stannous chloride resulted in loss of viability and DNA damage in a dose-response manner (Dantas *et al*., 2002). The DNA damage was linked to tin-mediated formation of reactive oxygen species. It has been suggested that tin can interfere with heme formation by competitive antagonism with iron, zinc, and copper (Pekelharing *et al*., 1994). The high-tin diet depresses copper status in rats. The

interaction between copper and tin affects the levels of liver superoxide dismutase and glutathione peroxidase (Reicks and Rader, 1990). Moreover, a genotoxic effect of tin chloride has been shown with a tester strain of *Escherichia coli* AB1157 (Assis *et al*., 1998). In rats fed a diet containing 20 mg tin/kg, hemoglobin, hematocrit, and red blood cell count were decreased, whereas total iron-binding capacity was not affected (Beynen *et al*., 1992).

7.1.2.2 Human

High intakes of inorganic tin compounds can cause abdominal pain, anemia, and liver and kidney problems (Williams *et al*., 1999). The inorganic gas of tin hydride is neurotoxic (causes nerve damage) when inhaled. It is similar to arsine gas, but the effects are less severe because it does not cause hemolysis (Baldwin and Marshall, 1999). It has been shown that tetra hydrogenated tin (SnH_4) is a hemolytic poison. High levels of tin can cause skin rash, stomach complaints, nausea, vomiting, diarrhea, abdominal pain, headache, and palpitations, whereas low levels of tin exposure result in fatigue, depression, low cardiac output (left), low adrenals, shortness of breath, asthma, headaches, and insomnia. Tin exposures show an epidemiological relationship with ischemic heart disease and the metabolism of cholesterol (Klevay, 2000). Tin at concentrations similar to those found in human diets can decrease copper status (Pekelharing *et al*., 1994), and that in turn can increase cholesterol in plasma (Klevay, 1984). There are also reports that suggest that exposure to tin can increase the risk of chronic renal failure (Nuyts *et al*., 1995).

7.2 Organotin

7.2.1 Local Effects and Dose-Response Relationships

7.2.1.1 Animals

The acute oral LD_{50} values for tributyltin for mice and rats range from 44–234 mg/kg body weight, whereas LD_{50} values for intravenous and intraperitoneal injections are much lower (Funahashi *et al*., 1980; Pelikan and Cerny, 1968; Schweinfurth H., 1985). For rats, the intraperitoneal LD_{50} value is 10 mg/kg and for mice the intravenous LD_{50} is estimated as 6 mg/kg (WHO, 1990). A single dose of tributyltin at 500 mg/kg caused numerous hemorrhagic lesions in the digestive tract in mice (Pelikan and Cerny, 1968). The toxic signs such as anorexia, emesis, tremor, and diarrhea have been observed after a single oral administration of triphenyltin. The triphenyltin oral LD_{50} for rats ranges from 140–300 mg/kg body and 81–93 mg/kg for mice, whereas dermal LD_{50} are 350 mg/kg in mice and >2000 mg/kg in rats (WHO, 1992). Acutely dosed triphenyltin (50 mg/kg) produces marked hyperglycemia in hamsters, whereas this was not found when hamsters were fed diets containing phenyltin concentrations <60 mg/kg (Ohhira and Matsui, 1996). The hyperglycemic action of triphenyltin may be dose dependent on the accumulation of this compound in the pancreas. In rats, neither tributyltin nor dibutyltin caused any abnormality in the intestinal tract despite the fact that tributyltin is a potential inducer of intestinal heme oxygenase (Krajnc *et al*., 1984; Rosenberg *et al*., 1984). However, atrophy of hepatocytes in the centrilobular region has been noted in livers at 80 mg/kg and 320 mg/kg (Krajnc *et al*., 1984). Feeding rats with dibutyltin caused inflammation and damage of the bile duct. Repeated exposure to dibutyltin at 50 mg/kg for 3 days produced severe damage of the bile duct as characterized by complete deterioration of the luminary epithelium largely in the pancreatic regions of the bile (Barnes and Magee, 1958). Dietary dibutyltin as low as 20 mg/kg caused thymus atrophy in rats within 2 weeks (Seinen *et al*., 1977b). The water-soluble monooctyltin and diethyltin, as well as highly lipophilic octyltin compounds, were less potent or may not produce thymus atrophy. Most organotin compounds have been shown to be highly toxic to rat lymphocytes, causing a decrease in immune response (Seinen *et al*., 1977a). The decrease in the weight and cellularity of the thymus has been seen in rats fed with 50–150 mg/kg dibutyltin dichloride or 150 mg/kg dioctyltin dichloride. However, mice and other species seem to be more resistant to the effects of ingested organotin compounds on the thymus.

The interspecies differences in susceptibility of these compounds might be due to differences in gastrointestinal absorption and metabolism (Boyer, 1989). It has been reported that the effect of dialkyltin compounds on the thymus is due to their interference with proliferation of thymocytes through cytostatic rather than cytotoxic mechanism (Boyer, 1989; Penninks *et al*., 1985a; Seinen *et al*., 1977b). Tributyltin at a dietary level of 20–320 mg/kg caused thymus weight loss and atrophy accompanied with reduction of peripheral lymphocytes and T cells in spleen (Krajnc *et al*., 1984). Therefore, tributyltin, in contrast to dialkyltins, induced immunodeficiency through direct action on lymphocytes in the thymus (Vos *et al*., 1984a). Hematological changes such as anemia, lymphopenia, and trombocytosis have been noted in rats fed tributyltin at a dose of 50 mg/kg (Wester *et al*., 1990). Behavior disturbance has been observed after exposure to 10 mg/kg triethyltin (Reiter *et al*., 1980). A diminished startle response to a puff of air or sudden noise proved to be a sensitive indication of triethyltin exposure (Squibb

et al., 1980). Tributyltin at 10–60 mg/kg given as a single dose to rat pups on postnatal day 5 caused persistent alterations in motor activity development and acoustic startle response (Crofton *et al*., 1989). Alterations in brain weight and brain histology have been found after intraperitoneal injection of tributyltin to 5-day-old rats (O'Callaghan *et al*., 1988).

7.2.1.2 Human

Organic tin compounds are toxic, because they inhibit synthesis of heme oxygenase and they can be also genotoxic. They cause severe irritation and burning to the skin, because they are absorbed through this route. This can lead to systemic toxicity, which is also produced when these compounds are inhaled or ingested. Major effects may be anemia and renal and hepatocellular damage. The production of catecholamines may also be stimulated, which may lead to hyperglycemia, changes in blood pressure, and damage to the immune system. Alkyl and aromatic tin compounds are highly potent neurotoxins. Trialkyltin compounds, such as triethyltin, can cause encephalopathy and cerebral edema. Excessive industrial exposure to triethyltin produces symptoms of headaches, visual defects, and nausea (Prull and Rompel, 1970). Triethyltin uncouples oxidative phosphorylation that leads to mitochondrial damage, cerebral edema, and nerve damage. Workers who were exposed to organic tin compounds (trimethyl and triethyl derivatives) developed psychomotor disturbances, tremor, convulsions, hallucinations, and psychotic behavior (Hu, 1998). Patients who inhaled fungicide powder containing 60% triphenyltin or were exposed to triphenyltin while spraying it complained about dizziness, nausea, and photophobia (Manzo *et al*., 1981). There are no neurobehavioral studies among children whose mothers were exposed to organotins during pregnancy (Sokas, 1998). Skin burns and severe lesions on the hands have been reported among workers occupationally exposed to liquid tri- and dibutyltin chlorides because of leaking gloves or failure to wear hand protection (Lyle, 1958). The development of severe dermatitis has been described among workers accidentally exposed to tributyltin and painters applying tributyltin formulations (Goh, 1985; Lewis and Emmett, 1987). Dermatitis and irritant and allergic reactions have been reported among Italian agricultural and ex-agricultural workers who used triphenyltin-containing pesticide formulations (Lisi *et al*., 1987).

7.2.2 Systemic Effects and Dose-Response Relationships

7.2.2.1 Animals

Tributyltin may be a contributory factor and perhaps a major cause for the reproductive failure of the European flat oyster (*Ostrea edulis*) in past decades in certain locations (Thain and Waldock, 1986). The no effect levels are <1 ng/L for mollusks and gastropods. Bioaccumulation of tributyltin in mussels (*Mytilos edulis*) has been shown to be higher through water than uptake through food (Laughlin *et al*., 1986). The study with marine mollusk *Hinia incrassate* has shown that tributyltin can bioaccumulate more than dibutyltin (Oehlmann *et al*., 1998). Another study with freshwater fish yielded bioconcentration factors of 9000 for tributyltin, 1800 for dibutyltin, and 700 for monobutyltin (Caussy *et al*., 2003). These calculations show that bioaccumulation of organotins depends on their chemical species. Toxicity increases as the number of carbon atoms in the chain increases. The absence of any cytochrome P450–dependent system might be an explanation for the capacity of the mollusks to bioaccumulate butyltin species (Alzieu, 2000). Bioaccumulation of tributyltin and triphenyltin also depends on pH. For example, bioaccumulation of tributyltin and triphenyltin in carp has been shown to be higher at alkaline pH values than with pH <7 (Tsuda *et al*., 1990). The toxicity of dioctyltin compounds has been shown only in the range of 1–8 mg/L for *Daphnia magna*, whereas monooctyltin is not toxic. Dioctyltin at doses of 20, 30, and 45 mg/kg is embryo-fetotoxic and induces developmental defects (Faqi *et al*., 2001).

Di-alkyltin dichlorides, such as di-*n*-butyltin dichloride, induce an acute interstitial pancreatitis in rats at approximately half the lethal dosage (EC_{50}) of 6 mg/kg weight (Merkord *et al*., 1997). The di-*n*-butyltin induces an acute and later a chronic pancreatitis in rats; however, it does not cause parenchymatous necrosis (Merkord *et al*., 1998).

Subcutaneous injection of tributyltin, tricyclohexyltin, or diethyltin at doses up to 30 mg/kg can induce hepatic heme oxygenase with a decrease in hepatocellular cytochrome P-450 and a transient decline of ALA syntheses activity, whereas it did not affect renal heme oxygenase (Rosenberg *et al*., 1982). *In vitro* studies have demonstrated that butyltin compounds may interact directly with cytochrome P-450, causing its degradation (Rosenberg and Drummond, 1983). In addition, it has been demonstrated that dibutyltin impairs the barbiturate metabolism in rats, suggesting that butyltin exposure may alter the response to xenobiotic substances.

Organotins, especially triphenyltin and tributyltin, are implicated in endocrine disruptions and consequently cause reproductive failure (Golub and Doherty, 2004; Lavado *et al*., 2004). Bioaccumulation of organotins can interfere with the synthesis and clearance of key hormones regulating sex differentiation and gamete growth. *In vitro* studies revealed that

triorganotins inhibit P-450 monooxygenase ethoxyresorufin *o*-deethylase, as well as aromatase and dehydrogenase, which convert estrone to estradiol and androstenedione to testosterone (Lavado *et al*., 2004; Nebbia *et al*., 1999; Thibaut and Porte, 2004). These effects of organotins suggest the possibility of alterations in steroid hormone metabolism. Indeed, it has been shown that tributyltin inhibits testosterone metabolism, which is associated with elevated testosterone levels in gastropods (Oberdorster *et al*., 1998). Gastropod imposex, an irreversible syndrome when male genital tracts are imposed on female organisms, is typically induced by very low concentrations of tributyl- or triphenyltin compounds (Horiguchi *et al*., 1997; Morcillo *et al*., 1999; Sole *et al*., 1998). However, aromatase activity is not decreased in gastropods exposed to tributyltin, even in those exhibiting clear evidence of imposex (Morcillo *et al*., 1999). Recently, it has been shown that trisubstituted organotin compounds act as agonists of retinoid X and peroxisome proliferator-activated receptors, which play an important role in the development of gastropod imposex (Kanayama *et al*., 2005). The effective concentration of receptor inhibition is approximately 10^{-8} mol/L. In fish yolk sac fry stage has been also shown to be the most sensitive to the toxic effects of tributyltin compounds (de Vries *et al*., 1991). Studies of reproduction in rats by administration either in food or oral dosing have demonstrated that triphenyltin at dose of 1 mg/kg and triphenyltin hydroxide at a dose >6 mg/kg cause death to pups at birth, but there was no reduced fertility or *in utero* lethality (Golub and Doherty, 2004). Winek *et al*. (1978) reported that rats fed triphenyltin hydroxide at 20 mg/kg became sterile. Diphenyltin at 16.5 mg/kg and greater administered on days 0–3 of pregnancy causes embryonic loss mediated through reduction of progesterone and suppression of uterine decidualization (Ema and Miyawaki, 2002). Triphenyltin acetate or chloride reduces the number of gametes at various stages of the sperm cycle, and rats can become completely sterile after administration of 20 mg/kg for 20 days (Pate and Hays, 1968; Snow and Hays, 1983). Moreover, triphenyltin can cause reduction in relative ovarian and uterine weight in guinea pigs fed 20 mg/kg and rats treated with 20 and 50 mg/kg (Newton and Hays, 1968; Verschuuren *et al*., 1966).

Trialkyltin compounds are toxic to the CNS of newborn and adult rats at doses of 3–7 mg/kg (Harry and Tilson, 1981; Swartzwelder *et al*., 1981). Prenatal administration of triphenyltin acetate causes abnormalities in spontaneous locomotor activity and conditioned avoidance in rat pups (Lehotzky *et al*., 1982a). Tributyltin and triphenyltin compounds are potent stimulators of placental hCG production and suggest that the placenta is a potential target organ for trialkyltin compounds (Nakanishi *et al*., 2002). Triphenyltin can induce hyperglycemia and hypertriglycemia in hamsters but not in rats (Matsui *et al*., 1984; Ohhira and Matsui, 1996). Trimethyltin and triethyltin are neurotoxic and can cause neuronal necrosis primarily affecting the hippocampus and endocrinal cortex (Bouldin *et al*., 1981; Winship, 1988). Triphenyltin compounds show relatively low neurotoxicological effects even at high doses compared with triethyltin, trimethyltin, tributyltin, and tripropyltin (Bouldin *et al*., 1981; Wada *et al*., 1982). Severe swelling of the brain accompanied by hind limb paralysis was observed in rats exposed to 20 mg/kg triethyltin for 3 weeks (Barnes and Magee, 1958). Unlike triethyltin treatment, oral exposure to trimethyltin in a single dose of 10 mg/kg caused severe and permanent damage in the CNS in rats as characterized by neuronal necrosis rather than intramyelinic edema (Bouldin *et al*., 1981). Trimethyltin at a single dose of 4.5 mg/kg produces neurodegeneration in the hippocampus, especially in CA1 stratum radiatum (Scallet *et al*., 2000). The toxic effects of trimethyltin include inhibition of transmembrane gradient of K+ in astrocytes with release of glutamate and aspartate into the extracellular fluid on release from astrocytes (Aschner *et al*., 1992). The lack of ability to maintain a transmembrane gradient triggers a destructive cascade of events that results in swelling of astrocytes (Matthews *et al*., 1991). Tributyltin and dibutyltin inhibit various parameters of cholinergic activity (Kobayashi *et al*., 1996). These compounds suppress K+-induced release and synthesis of acetylcholine in cortex, as well as inhibit the activity of choline acetyltransferase. Because organotins are difficult to eliminate from the central nervous system, exposure to these compounds can lead to permanent neurological deficits.

Neurotoxicity of organotin compounds is accompanied by impairment of liver function (Manzo *et al*., 1981). Significant depression of hepatic microsomal aminopyrine demethylase and aniline hydroxylase activities has been observed in rats fed with triphenyltin at dose 1 mg/kg for 3 days (Di Nucci *et al*., 1986). However, triphenyltin did not affect bile flow and liver weight. The administration of tributyl- and dibutyltin cause hepatotoxicity in mice at doses >180 or 60 µmol/kg, respectively (Ueno *et al*., 1995). Monobutyltin does not cause liver injury even at 7 mmol/kg. Both tri- and disubstituted butyltin compounds have been shown to induce inflammation of the bile duct associated with hepatic lesions (Krajnc *et al*., 1984). Tributyltin compounds reduce cyclic AMP production, change calcium metabolism, and also suppress hormone-induced calcium response in primary hepatocytes (Snoeij *et al*., 1986).

Leukopenia is the most prominent feature of triphenyltin poisoning accompanied by a decrease in the size of the spleen, thymus, and lymph nodes (Stridh *et al*., 1999; Vos *et al*., 1984b). Benzene metabolite of triphenyltin seems to be responsible for lymphocyte death (Stridh *et al*., 1999). Secondary Fanconi syndrome has been observed in guinea pigs after painting tributyltin on their skin (Mori *et al*., 1984). Microcytic anemia has been observed in rats exposed to 80–320 mg/kg tributyltin oxide (Krajnc *et al*., 1984). In erythrocytes, tributyltin >10 µmol/L induces hemolysis associated with intercalated within the lipid membrane multimolecular tin-containing aggregates, which transform red cells into crenellated spheres (Byington *et al*., 1974; Gray *et al*., 1986; Porvaznik *et al*., 1986).

Triphenyltin, tripropyltin, and tributyltin compounds are extremely immunotoxic to rats (Snoeij *et al*., 1985; Vos and Krajnc, 1983). Both triphenyltin and tributyltin have been shown to induce thymus atrophy and suppression of T cell–induced immune responses (Seinen *et al*., 1979; Snoeij *et al*., 1985). Triphenyltin intraperitoneally injected into mice for 14 days inhibited the T-cell–dependent humoral and cellular (induction of cytotoxic T-cell or induction of delayed hypersensitivity) immune response (Nishida *et al*., 1990). The lower molecular weight diethyltin and dipropyltin produced less pronounced effects, whereas octyltin and dioctadecyltin did not cause immunotoxic effects.

7.2.2.2 Human

There are no human studies available on chronic low-level exposure to organotin compounds. However, some case reports describe various health effects after accidental exposure to tributyltin and triphenyltin compounds. Patients, who had been exposed mainly to cutaneous absorption, developed acute nephropathy and disorders of the central nervous system (Colosio *et al*., 1991; Manzo *et al*., 1981; Prull and Rompel, 1970; Wax and Dockstader, 1995). Exposure to triphenyltin causes spontaneous involuntary movement of hands, facial twitching, and crying. Some of the patients experienced diplopia, drowsiness, giddiness, vertigo, bidirectional nystagmus, impairment of calculation ability, and disorientation in time and place (Lin *et al*., 1998). In addition to CNS abnormalities, delayed peripheral neuropathy, hepatitis, and leukopenia have been monitored 6 and 9 days after consumption of triphenyltin. Two case reports of triphenyltin poisoning indicated leukopenia, a decrease in responsiveness of neutrophils, elevated hepatic enzymes, hepatomegaly, and central nervous system effects (encephalopathy and delayed neuropathy) (Colosio *et al*., 1991; Lin *et al*., 1998). Liver damage has been reported in people using triphenyltin acetate as a spray (Horacek and Demcik, 1970). More than 100 deaths and more than 200 cases of illness occurred in France in 1954 because of ingestion of a preparation containing diethyltin diiodide and triethyltin monoiodide. The doses of diethyltin were estimated to be from 45–675 mg in nonfatal cases and from 380–675 mg in fatal cases (Barnes and Stoner, 1959). Severe headache, vertigo, visual abnormalities, paralysis, and convulsions have been reported after only a few days of exposure. Death occurred from coma, respiratory, or cardiac failure. A pronounced edema of white matter of the brain had been seen in fatal cases.

7.3 Mechanism of Action

Because of lipophilicity, organotin compounds can be regarded as membrane active, and the cell membrane is an initial site of activation (Tobin and Cooney, 1999). Indeed, it has been shown that tributyltin alters the plasma membrane/cytoplasm complex, decreases plasma membrane potential of the murine erythroleukemic cell, and causes cell death (Zucker *et al*., 1988). Organotins may affect lipid bilayers by altering membrane fluidity (Ambrosini *et al*., 1996). In a number of studies, the mitochondrion has been identified as a target of triorganotins. The major action of organotins is associated with interference with mitochondrial energy production, including interruption of oxidative phosphorylation, changes in permeability of outer mitochondrial membrane, and suppression of enzyme activity (Boyer, 1989; Stridh *et al*., 2001). Moreover, triorganotins increase calcium flux in mitochondria attributed to organotin toxicity (Miura *et al*., 1997; Viviani *et al*., 1995). It has been shown that triphenyltin affects calcium homeostasis through impairment of calcium influx through voltage-gated calcium channels, inhibition of Ca^{2+}-ATPase activity, and calcium uptake of sarcoplasmic reticulum (Miura *et al*., 1997; Viviani *et al*., 1995). Furthermore, tributyltin induced caspase activation and apoptosis in human peripheral blood lymphocytes without affecting the CD8+ T-cell population (Stridh *et al*., 2001). Triphenyltin at 0.1–1 µmol/L, as well as tributyltin at 0.5–5 µmol/L, also triggers caspase activation and induces apoptosis in Jurkart T lymphocytes. Both compounds at 1 µmol/L can induce phosphorylation and activation of MAPK kinases, which transmit extracellular signals into the nucleus and result in cell death (Yu *et al*., 2000). However, at high concentrations, triphenyltin and tributyltin inhibited caspase activity causing necrotic cell death (Stridh *et al*., 1999). Genotoxicity of a number of organotin compounds has been reported by various methods (Ghosh *et al*., 1990; Hamasaki *et al*., 1993). Organotin compounds inhibit microtubule assembly through direct interaction with tubulin (Jensen *et al*., 1991).

The mitotic activity of these compounds increases with increasing their lipophilicity. The highest mutagenicity has been shown for tributyltin compounds, whereas no evidence of mutagenicity has been found for dimethyltin and dibutyltin in the SOS chromotest with *E. coli* PQ37 and in PHA-stimulated human lymphocytes (Hamasaki *et al*., 1992). Triphenyltin compounds induce micronuclei in cultured Chinese hamster ovary cells (Chao *et al*., 1999). The increase in sister chromatid exchange level has been seen in CHO cells exposed to organotins by adding S9 microsomal fractions. Triphenyltins given orally at doses 2.5–25 mg/kg to mice induce an increase in micronucleated reticulocytes. Triphenyltin has been reported positive in both the Ames test and the mouse lymphoma TK+/mutation assay (Oshiro *et al*., 1991). These data indicate that these chemicals are potential chromosome mutagens. The carcinogenic potential of some butyltin compounds includes inhibition of the ability of natural killer cells to bind to tumor cells, thereby predisposing to malignancy (Whalen *et al*., 1999). The toxic effects of butyltin compounds include immune suppression in rodents (Penninks, 1993). The immunotoxicity of tributyltin may be due to the primary action of dibutyltin on the maturation of immature thymocytes. The toxic effect of triphenyltin is also explained by depolymerization of thymocyte F-actin (Chow and Orrenius, 1994). Moreover, immunotoxic effects of organotin compounds may involve cytoskeletal modification in addition to the perturbation of thymocyte calcium homeostasis.

However, despite several studies on organotin compound toxicity, the molecular targets of organotins have not yet been identified.

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CHAPTER

43

Titanium

TAIYI JIN AND MATHS BERLIN

ABSTRACT

There is no evidence indicating that titanium is an essential element for humans or other animals. Titanium belongs to the first transition group; its chemical behavior is similar to that of silica and zirconium. Although titanium compounds are, in general, absorbed poorly through ingestion and inhalation, titanium still can be detected in the blood, brain, and parenchymatous organs of individuals in the general population, with the highest concentrations being found in the hilar lymph nodes and the lungs. Titanium is excreted in urine; information on other routes of excretion is lacking.

Studies on titanium alloys used in implants and titanium compounds—such as its salicylate, oxide, and tannate derivatives—used in cosmetics and pharmaceuticals have not indicated any significant local effects on tissues, but, under certain circumstances, cases of inflammatory reactions and systemic effects have been observed. Thus, titanium cannot always be considered as being inert and biocompatible, as was once believed. Titanium tetrachloride, a strong irritant to mucous membranes and the eyes, can cause skin burns through accidental exposure. Experimental animal studies, clinical studies, and some epidemiological surveys have indicated that titanium dioxide is generally biologically inert, but cases of adverse reactions have been reported. In experimental studies on animals, titanium carbide, hydride, nitride, and boride have exhibited slight fibrogenic activity. Artificial fibers—such as potassium octatitanate and titanium phosphate fibers—possess fibrogenic properties.

Bronchoalveolar lavage cells from rats instilled intratracheally with TiO_2 particles induce hrpt-gen mutations in rat alveolar cells *in vitro*. Intramuscular injections of powdered titanium metal have induced fibrosarcomas and lymphosarcomas in rats; similarly, the organotitanium compound titanocene has induced fibrosarcomas in rats. Several epidemiological studies have suggested that TiO_2 dust has no carcinogenic effect on human lungs. IARC has listed titanium dioxide within Group 2B. There is sufficient evidence from experimental animal studies for the carcinogenicity of TiO2, but no adequate evidence exists for the carcinogenicity of titanium oxide in humans. A soluble titanate given to rats in their drinking water was found to disturb their reproduction in a three-generation study on rats.

1 PHYSICAL AND CHEMICAL PROPERTIES

Titanium (Ti): atomic weight, 47.9; atomic number, 22; density, 4.5 g/cm^3 at 20°C; melting point, 1660 ± 10°C; boiling point, 3287°C; silver-grey; water-insoluble. Titanium is one of the most common components of the earth's crust (ninth in abundance). It occurs naturally as ilmenite (iron titanate) and rutile (titanium dioxide). Titanium exhibits both metallic and nonmetallic characteristics; metallic compounds include titanium chloride, phosphate, sulfate, and nitrate; nonmetallic properties are exemplified by the calcium, iron, and sodium titanates. Titanium forms four distinct oxides: titanium monoxide (TiO), dititanium trioxide (Ti_2O_3), titanium dioxide (TiO_2), and titanium trioxide (TiO_3).

The most common oxidation states are +4 (TiO_2) and +3 (titanous, Ti_2O_3); compounds having oxidation state +2 (TiO) are less stable, as are oxycompounds such as titanyl chloride ($TiOCl_2$). A number of organometallic compounds of titanium, such as titanocene, are known; the most common are the alkyl- and aryltitanates of the general formula $Ti(OR)_4$. Complex titanium compounds possessing organic ligands are also known.

Titanium metal displays excellent corrosion resistance: it is as resistant as platinum against many agents, including concentrated nitric acid, moist chlorine gas, and common salt solution. An important property of titanium is that, on exposure to air or various liquids, it rapidly develops a layer of oxide, which reduces its reactivity (Kasemo, 1983). No metal or alloy is, however, completely inert *in vivo*. Elevated titanium concentrations have been reported in the blood, urine, and nails of humans having titanium-containing implants (Berglund and Carlmark, 1999; Bianco *et al*., 1996). Titanium dioxide exists in three crystalline forms: anatase, brookite, and rutile (WHO, 1982). Titanium tetrachloride occurs as a colorless to light-yellow liquid that is soluble in water but decomposes in hot water (US Department of Health and Human Sciences, 1993).

2 METHODS AND PROBLEMS OF ANALYSIS

Methods applied to the detection of titanium in water and food include spectrography and photometry. Although various atomic absorption techniques have also been reported, the use of a high-temperature reducing flame is desirable, because of its poor atomization and its tendency to form refractory oxides. Inductively coupled plasma optical emission spectrometry (ICPOES) techniques have been developed for the rapid determination of titanium in foods after acid digestion (Lomer *et al*., 2000). TiO_2 is usually measured as the metal. Inductively coupled argon plasma atomic emission spectroscopy (ICP-AES) is an alternative method for determining titanium dioxide in air, with detection limits in the parts per billion range and excellent recovery (96%; NIOSH, 1994d). This method can also be used to assay titanium in urine and tissues, with a detection level of 20 ppb and a good level of recovery (86%; NIOSH, 1994a).

Proton-induced X-ray emission spectroscopy has also been used with practical detection limits of approximately 0.001 µg for titanium in air and <0.001 µg of titanium per drop of water (Johansson *et al*., 1975).

X-ray fluorescence has been used widely for the determination of titanium in air, water, and biological samples; the detection limit for titanium in air is 0.011 $\mu g/m^3$ at a sensitivity of 0.01 $\mu g/m^3$ (Kalbasi *et al*., 1995).

Neutron activation analysis has been used for the determination of titanium in air, whereas spark source mass spectrography has been used for the analysis of water, food, and biological samples. The gravimetric filter weight (G/FW) is the most common method used for determining titanium and titanium dioxide as particulate matter in air (NIOSH, 1980; 1994b,c); spectroscopic methods are most commonly used for detecting the metal associated with particulates in air (NIOSH, 1994d).

3 PRODUCTION AND USES

3.1 Production

Titanium is one of the most common components in the earth's crust (ninth in abundance, 0.6% by mass); it occurs in a number of minerals as well as in living systems and natural bodies of water. Among the various mined titanium minerals, ilmenite is of great commercial significance. The world production of ilmenite in 2002 (including leucoxene) and rutile were approximately 5.64 million tons and approximately 0.35 million tons, respectively (*http://www.indexmundi.com/en/commodities/minerals/titanium/titanium_t15.html*). The recovery of titanium from secondary sources probably does not exceed 1% of the total production. Two main production processes are used for preparing commercial titanium dioxide pigments: sulfate and chloride processes.

3.2 Uses

Titanium metal finds an extensive number of applications in the aircraft and spacecraft industries because of its very high tensile strength, light weight, extraordinary corrosion resistance, and its ability to withstand extreme temperatures. When used for these purposes, titanium is usually used in the form of alloys, which are stronger and more resistant against corrosion than is the metal itself. Because of its resistance to corrosion and its chemical inertness, titanium is used widely in the chemical industry; for example, as tubing and for the lining of vessels used in the production of nitric acid and acetaldehyde. Its resistance to corrosion means that titanium is also useful in the paper pulp industry. Titanium is the newest metallic biomaterial among the most popular metallic alloys (Niinomi, 2002). Titanium and its alloys continue to receive much attention in medical and dental fields because of the balance between their excellent mechanical properties and corrosion resistance; they are used mainly

for implant devices that replace failed hard tissue, for example, artificial hip or knee joints, bone plate, and dental implants. Titanium and its alloys are also used in dental products, such as crowns, bridges, and dentures.

Titanium dioxide (TiO_2) is the most common titanium compound; it constitutes almost 95% of all titanium consumed. Titanium dioxide is the most common white synthetic pigment used in the paint industry. It has many favorable properties: ready availability, a comparatively cheap price, extreme whiteness and brightness, and a high index of refraction. On the basis of these characteristics, titanium dioxide is used extensively as a white pigment in paints, lacquers, enamels, paper coatings, and plastics. Because of its effectiveness as a sunscreen toward short-wave ultraviolet radiation, titanium oxide is included within a variety of drugs and cosmetics. It is also used as a color additive in confections, dairy products, and bread flours as a replacement flour-bleaching agent. Titanium dioxide serves as a clouding agent incorporated into dry beverage mixes and is used in tobacco wrappings and tobacco substitutes. Titanium dioxide is also used in the pharmaceutical industry as a constituent of tablets.

Titanium tetrachloride ($TiCl_4$) is another commercially important titanium derivative that is used as an intermediate in the production of titanium metal and titanium pigments and as a component and catalyst in the chemical industry. Titanium chloride ($TiCl_3$), which is prepared by the reduction of titanium tetrachloride, serves as a coloring agent and as a catalyst in various chemical processes.

4 ENVIRONMENTAL LEVELS AND EXPOSURES

4.1 General Environment

4.1.1 Food and Daily Intake

Titanium is poorly absorbed by plants and animals and is retained to only a certain extent. High levels of titanium in food products can be detected, however, when soil is contaminated by fly-ash fallout or titanium-containing sewage residues (used as fertilizers) and when titanium dioxide is used as a food whitener. High concentrations of titanium in certain types of cheese result from the addition of titanium dioxide as a whitener and as an aging accelerator. Food, which is considered to be the most important source of exposure to titanium, contributes >99% of the daily intake of the element. There is no relevant tolerable intake for titanium against which to compare estimated dietary intake (Joint Food Safety and Standards Group, 2000). Typical diets may contain approximately 0.3–0.5 mg titanium (Poole and Johnston, 1969; Schroeder *et al.*, 1963; Tipton and Stewart, 1969).

4.1.2 Water, Soil, and Ambient Air

It has been reported that titanium concentrations range from 2–107 μg/L in freshwater in Canada and the United States (Durum and Haffty, 1961) and 0.5–15 μg/L (mostly approximately 2 μg/L) in drinking water in the United States (Durfor, 1963). Titanium concentrations in seawater are mostly approximately 0.6–1 μg/L, but values up to 9 μg/L have been reported (Bowen, 1966; Ishibashi, 1968; Silvey, 1967).

The titanium content of soil generally ranges from 0.3–6%, with high levels found in the vicinity of power plants because of the combustion of coal (Klein and Russell, 1973).

Titanium concentrations in the atmosphere are comparatively low. Annual average concentrations in urban air are mostly <0.1 μg/m^3, and they are lower still in rural air (EPA, 1973). Air concentrations up to 0.5 μg/m^3 have been reported in urban and industrialized areas (EPA, 1973; Giauque *et al.*, 1974; Japan Environmental Sanitation Centre, 1971). The daily intake from urban air is approximately 1–5 μg; it is likely to be <1 μg in rural areas (Schroeder *et al.*, 1963; Woolrich, 1973). Compared with the amount of titanium absorbed from food, the intake of titanium from the air is negligible: under most conditions, <1% to the total daily intake.

4.2 Working Environment

Occupational exposure to titanium occurs principally in the form of dust; inhalation is the common route of exposure. High exposure levels have been reported from mining, from the production of the metal, and from the production and processing of titanium dioxide, carbide, and hydride. Typical dusty operations include the crushing, grinding, mixing, and sieving of rutile concentrates. High exposure to titanium compounds in the air has also been reported from the manual handling, screening, and packaging of titanium hydride powder (Skurko and Brahnova, 1973). Exposure to fumes and vapors occurs in the handling of titanium tetrachloride. Exposure to titanium tetrachloride may also occur in the reduction process, where workers are exposed to titanium tetrachloride and titanium oxychloride vapors and to titanium dioxide particulates (Garabrant *et al.*, 1987). Exposure may occur at any stage during the mining of ores and the preparation of titanium dioxide and in any of the industries in which the powder is stored and used. The American Conference of Governmental Industrial

Hygienists (ACGIH, 2005) suggested a threshold limit value/time-weighted average (TLV-TWA) for TiO_2 (CAS 13463-67-7) of 10 mg/m^3.

5 METABOLISM

An important property of titanium is that, on exposure to air or various liquids, it rapidly develops a layer of oxide, which reduces its reactivity (Olmedo *et al*., 2003).

5.1 Absorption

A number of studies have indicated that titanium is absorbed mainly through ingestion, but different conclusions have been drawn as to the extent. One study undertaken by Schroeder *et al*. (1964) found that titanium levels in the organs of mice given potassium titanium oxalate at a concentration of 5 mg/L in their drinking water during their entire life span were five times higher than those observed in control animals. Translocation of TiO_2 (rutile) particles to intestinal tract–associated tissues and other organs was observed in rats administered 12.5 mg/kg/day TiO_2 by gavage for 10 days (Jani *et al*., 1994). In another study that used mice, a very low degree of absorption from the gastrointestinal tract was observed: the whole-body count of experimental animals given ^{44}Ti intragastrically did not exceed the background level after 24 hours (Thomas and Archuleta, 1980). Blood titanium levels were found to increase after oral administration of TiO_2 (anatase) to human volunteers (Böckmann *et al*., 2000).

Many data indicate that titanium is absorbed poorly from the gastrointestinal tract in human beings. It is likely that transferrin may act as a specific carrier of titanium ions and may play a central role during the transporting and biodistribution of soluble titanium species throughout the organism (Ishiwata *et al*., 1991). Titanium concentrations found generally in urine (approximately 10 µg/L) suggest an absorption of <5%, assuming a daily intake of at least 300 µg. Although this estimate agrees with that reported by Schroeder *et al*. (1963), it may be erroneous because other possible routes of excretion, especially intestinal, were not been taken into consideration.

The deposition of titanium dioxide dust in the lungs of rats is similar to that observed for other particles (Ferin *et al*., 1983). Titanium dioxide is found in the lymphocytes and regional nodes in the lungs, indicating that a slow rate of removal occurs by this process. Clearance is also significantly decreased, or even ceases, at high exposure over a period of time because of overload (Bermudez *et al*., 2002; 2004). It is suggested that small amounts of titanium dioxide can enter the general circulation from the lungs (Lee *et al*., 1985). The absorption and transference of titanium to the liver and spleen in rats exposed to 10 mg/m^3 titanium tetrachloride aerosol for 6 hours per day, 5 days per week, for 2 years, have been reported (Lee *et al*., 1986).

5.2 Distribution

Several transport mechanisms have been described for titanium, including systemic dissemination by the vascular system in solution or as particles (Edel *et al*., 1985; Meachin and Williams, 1973; Merritt and Brown, 1995) and lymphatic dissemination as free particles or as phagocytosed particles within macrophages (Bianco *et al*., 1996).

Titanium concentrations of 2–9 mg/kg wet weight were found in the parenchymatous organs—such as the heart, lungs, spleen, liver, and kidneys—of mice that had been given titanium oxalate in their drinking water at a level of 5 mg/L throughout their lifetime. The concentrations were similar to those observed in wild mice, but were five times higher than the concentrations found in untreated animals (Schroeder *et al*., 1964). Six hours after titanium dioxide was administered to rats through intravenous injection at 250 mg/kg body weight, the highest concentration appeared in the liver; after 24 hours, the highest concentration was detected in the celiac lymph nodes, which filter the lymph from the liver (Huggins and Froehlich, 1966).

In the general population, titanium has been detected in various parenchymatous organs, with the highest concentrations consistently being found in the lungs (Hamilton *et al*., 1972/1973), probably as a result of inhalation of titanium-containing dust particles. Teraoka (1980) reported even higher concentrations in the hilar lymph nodes than in the lungs. In coal miners, lung concentrations have been reported to be several times than those found in the general population (Crable *et al*., 1967; 1968). Titanium was demonstrated in the lymphatic systems of three workers engaged in the processing of titanium dioxide pigments (Elo *et al*., 1972).

5.3 Biological Half-Time

At present, there is still an insufficient amount of data for estimating the biological half-time of titanium in humans or animals. The ICRP calculated a half-life of 320 days (ICRP, 1959). Although a half-life in mice was reported to be 640 days, the authors speculated that in man the half-life might be even longer (Thomas and Archuleta, 1980).

5.4 Excretion

Titanium taken orally remains mostly unabsorbed and is eliminated in the feces. In man, the excretion rate of titanium is approximately 10 µg/L urine (Schroeder *et al*., 1963). Tipton *et al*. (1966) reported, however, urinary excretion rates as high as 0.41 and 0.46 mg/day (30-day mean), respectively, from two adults. The mechanism of excretion and the possible amount of titanium excreted by the intestinal route remain unknown.

6 LEVELS IN TISSUES AND BIOLOGICAL FLUIDS

It has been reported that the blood concentration of titanium ranged from 0.03–0.15 mg/kg (Hamilton *et al*., 1972/1973). Titanium concentrations of 0.8 mg/kg wet weight in the brain, 1.3 mg/kg in the kidney cortex, 1.3 mg/kg in the liver, 3.7 mg/kg in the lungs, and 0.2 mg/kg in muscle have been reported (Hamilton *et al*. 1972/1973).

Titanium tends to accumulate with age in lung tissue, but not in other organs (Schroeder *et al*., 1964). Titanium concentrations of 33 mg/kg dry weight in the lungs and 150 mg/kg in hilar lymph nodes have been reported (Teraoka, 1980). No analytical methods have been described for the measurement of titanium dioxide in urine.

It has been observed that serum titanium concentrations were approximately 50 times greater, because of abrasion and wear of the titanium base material, in patients with failed patellar components of titanium-based joint implants (average concentration of serum titanium, 135.57 ng/mL) compared with those of a control group. There were, however, no significant differences in the levels of urinary titanium. Elevated serum titanium may, therefore, serve as a marker of failed patellar components in subjects who have undergone total knee replacements with titanium alloy bearings (Jacobs *et al*., 1999).

7 EFFECTS AND DOSE-RESPONSE RELATIONSHIPS

No evidence exists that titanium is an essential element for humans or other animals.

7.1 Local Effects and Dose-Response Relationships

7.1.1 Animals

7.1.1.1 Lungs

The effects of inhalation or intratracheal instillation of TiO_2 particles have been studied in rats, mice, rabbits (Dale, 1973), and hamsters. At levels of exposure causing little accumulation of particles in the lungs and with an effective clearance of deposited particles, no effects were found on the viability of alveolar macrophages (Maatta and Arstila, 1975) or any stimulation of fibroblasts (Heppleston, 1971). At heavier exposure, causing an overload of the rat lung clearance function and with accumulation of particles in the lungs, an inflammatory response was induced with increased numbers of neutrophils and fibrosis. The lung clearance of TiO_2 particles is less efficient in rats and mice than in hamsters (Hext *et al*., 2005). Thus, rat lungs are overloaded at lower particle loads than are those in other tested animals; they also exhibit a more pronounced tendency to undergo inflammatory and fibrogenic reactions. Lee *et al*. (1986) found that exposure to 250 mg/m^3 pigment-grade TiO_2particles for 1 year caused retention of Ti particles in the alveoles and alveolar ducts and impaired clearance with progressive tissue changes. Similar observations were made in studies designed to compare the development and possible progression of the lung responses of rats, mice, and hamsters exposed to a range of concentrations of pigment-grade TiO_2 and ultrafine-grade TiO_2 particles over a period of 90 days with subsequent retention of some exposed animals for up to 1 year. The ultrafine particles seemed to be more toxic than the pigment-grade particles when measured in terms of mg TiO_2/m^3; when the lung burden was measured in terms of the total particle surface area, however, a better correlation existed between the lung load and the histopathological response and the inflammatory reaction, in terms of an increased number of neutrophils in the lung lavage liquid (Baggs *et al*., 1997; Bermudez *et al*., 2002; 2004; Hext *et al*., 2002). Watanabe *et al*. (2002) compared the *in vitro* toxicities of particulate and fibrous titanium dioxide samples toward rat alveolar macrophages and found that the fibrous material was more toxic than the particles.

The intratracheal administration of 50 mg (in 1 mL saline) of titanium carbide, boride, or nitride caused mild fibroses in the lungs of rats (Brahnova, 1969; Brahnova and Samsonov, 1970); the administration of 50 mg of titanium hydride to rats led to a weak fibrogenic effect being observed 6 and 12 months later (Brahnova and Skurko, 1972).

The ability of titanium fibers to cause fibrosis is determined by their dimensions. A dose-related fibrosis was found in inhalation studies of rats, hamsters, and guinea pigs when using potassium octatitanate fibers having an average length of 6.7 µm and a diameter of 0.2 µm that were administered for 6 hours daily for 3 months in doses ranging from $2.9–41.8 \times 10^6$ fibers/L. The fibrosis was registered 15–24 months after treatment (Lee *et al*., 1981). Similarly, a dose-related fibrosis

was observed in rats after the administration of man-made titanium phosphate fibers having lengths of 10–20 μm and diameters of 0.2–0.3 μm (Gross *et al*., 1977).

Administration of a total dose of 75 mg of barium titanate, given in three weekly doses as a 5% suspension, through intratracheal instillation, did not produce any fibrotic changes in guinea pigs for up to 12 months (Pratt *et al*., 1953). Inhaling titanium hydride for 16 months, however, produced weak fibrogenic effects (Skurko and Brahnova, 1973).

7.1.1.2 Other Sites and Implants

Studies on rats given single intraperitoneal injections of 25 mg of titanium dioxide (Sethi *et al*., 1973) or an intravenous injection of 250 mg/kg body weight (Huggins and Froehlich, 1966) indicated that the compound is biologically inert.

Most of the medical reports regarding titanium in the early 1980s focused on whether titanium alloys were suitable implantation materials (e.g., Brunette *et al*., 1983; Kallus and Hensten-Pettersen, 1983; Kasemo, 1983; Schroeder *et al*., 1981). The general inertness of titanium has been demonstrated convincingly in several studies. The lack of irritation, the good degree of wound healing, and the encapsulation of the metal by fibrous tissues after the implantation of titanium metal in dogs all suggest that soft tissue has a high tolerance for titanium metal (Shpak and Margolin, 1971).

7.1.2 Humans

7.1.2.1 Lungs

Elo *et al*. (1972) studied lung specimens from three factory workers exposed for 9 years to the processing of titanium dioxide pigments; they found deposits in the pulmonary interstitium with cell destruction and slight fibrosis. Clearance of titanium dioxide through the lymphatic system was demonstrated by the observation of particles in the lymph nodes. A subsequent report (Maatta and Arstila, 1975) revealed the presence of aluminum and silica in the macrophages, in addition to titanium particles. It was suggested that the simultaneous exposure to silica compounds was responsible for the fibrotic changes, rather than the exposure to titanium dioxide. Clinical and epidemiological studies have generally confirmed the experimentally demonstrated lack of fibrogenicity of titanium dioxide. Through an autopsy study, extensive titanium dioxide deposits were discovered in two heavily exposed workers, but fibrosis was not detected (Ophus *et al*., 1979).

There are several epidemiological studies describing the effects of titanium dioxide. Studies on men exposed to titanium dioxide for prolonged periods did not reveal any signs of clinical or radiological abnormality. A study of 136 workers exposed to ilmenite and rutile in Sri Lanka did not find any significant difference in the incidence of radiological lesions of the chest compared with that of the general population (Uragoda and Pinto, 1972). A study of 207 workers engaged in the production of titanium dioxide from ilmenite, with an exposure time of 20 years for 90% of the workers, suggested that 47% of the subjects had the obstruction discovered by spirometry, which the authors suggested may have been caused by irritant effects associated with the sulfate process; no fibrotic effects attributable to the titanium dioxide exposure were noted, however (Daum *et al*., 1977). Chen and Fayerweather (1988) studied the rate of mortality among 1576 male employees who had been exposed to TiO_2 for more than 1 year in two TiO_2-producing plants in the United States. The mortality of the members of the group was monitored from 1935 to 1983. No excess mortality from nonmalignant respiratory disease was observed. Members of part of the group, 336 workers, were examined with X-rays; no cases of fibrosis were detected. A European multicenter epidemiological study monitored workers employed in 11 plants producing TiO_2 in six European countries. The mortality over a 30-year period from nonmalignant respirator disease was less than expected (Bofetta, 2004). A similar multicenter study in the United States monitored 4241 workers exposed in TiO_2-manufacturing plants. A mortality follow-up study, covering 40 years, did not reveal any adverse effects related to TiO_2 exposure (Fryzek *et al*., 2003).

Acute (short-term) exposure of humans to titanium tetrachloride may result in marked congestion of mucous membranes of the pharynx, vocal cords, and trachea and stenosis (constriction) of the larynx, trachea, and upper bronchi. Workers producing titanium tetrachloride have developed hyperemia and thinning of the mucosa of the respiratory tract, as well as bronchitis, possibly because of the production of hydrochloric acid on the rapid hydrolysis of titanium tetrachloride by water (EPA, 1985). Chronic inhalation exposure may produce upper respiratory tract irritation, chronic bronchitis, coughing, bronchoconstriction, wheezing, chemical pneumonitis, and pulmonary edema in humans. Pleural diseases of workers involved in titanium metal production have been linked with their chronic (long-term) occupational exposure to titanium tetrachloride. This finding suggests that chronic exposure to titanium tetrachloride may result in restrictive pulmonary changes (ATSDR, 1997).

7.1.2.2 Other Sites and Implants

Titanium is used widely as an implant material in orthopedics, oral surgery, and neurosurgery, because

it exhibits high biocompatibility (Schroeder *et al*., 1981; Williams and Adams, 1976). It has been shown *in vitro* that titanium particles can cause the osteogenic differentiation of human bone marrow, stroma-derived mesenchymal stem cells, to be suppressed; the bone marrow cells decreased and became apoptotic, while, at the same time, the levels of tumor suppressor proteins increased (Wang *et al*., 2003).

The *in vitro* cytotoxicity of titanium particles toward human neutrophils depends on the titanium particle size: the smaller the size, the greater the toxicity (Kumazawa, 2002). Titanium and its oxides may not be as inert and biocompatible as was once believed. Further studies are necessary to demonstrate the adverse effects caused by titanium and its oxides.

Titanium tetrachloride is highly irritating to the skin, eyes, and mucous membranes in humans; indeed, it may result in surface skin burns, whereas acute exposure may also damage the cornea. Conjunctivitis and keratitis have been discovered after contact with titanium tetrachloride (Mogilevskaya, 1983).

7.2 Systemic Effects and Dose-Response Relationships

7.2.1 Animals

Recent scientific evidence has indicated that titanium, or its corrosion by-products, may cause harmful reactions after traveling through the circulatory or lymphatic system. These harmful reactions usually take place in the blood, fibrotic tissue, and osteogenic cells.

In a comparative study of metallic biomaterials toxicity, assessing histochemical and immunohistochemical changes, the spleens of mice were observed to display irregular features within the capsule and medulla (depletion of T4 and B cells) after subcutaneous administration of a titanium solution (0.4 mg/mL) every 72 hours over a period of 30 days. Such alterations in the functioning of T4 and B cells may indicate hampered functioning of the immune system (Ferreira *et al*., 2003).

Rats administrated titanium dioxide at 25–250 mg/kg body weight through parenteral injections (Huggins and Froehlich, 1966; Sethi *et al*., 1973) did not exhibit any special changes other than those that could be expected from the injection of inert particles. Liver dystrophy was reported in rats after the intratracheal administration of 50 mg of titanium nitride, boride, or carbide (Brahnova, 1969; Brahnova and Samsonov, 1970). Administration of titanium hydride dust through intratracheal instillation caused similar effects (Skurko and Brahnova, 1973). Moreover, swelling of tubular epithelium in the kidneys occurred after administration of the nitride, whereas dystrophic changes were detected in the kidneys and hearts of the test animals after the absorption of the hydride.

7.2.2 Humans

Adverse effects have not been found from the small amounts of titanium occasionally released from implants into adjacent tissues (Brun and Hunziker, 1980; Lyell, 1979), but five cases of yellow nail syndrome were found for subjects with titanium implants who had high levels of titanium in their nails (up to 120 µg/g). Each of these subjects had undergone dental restoration with gold. The authors suggested that the high difference in redox potential between titanium and gold caused the migration of titanium into the tissues (Berglund and Carlmark, 1999).

There are no data available for the dose-response or dose-effect relationships related to the systemic changes caused by titanium compounds in human subjects.

Although titanium dioxide is regarded as being biologically inert, on the basis of experimental as well as clinical and epidemiological studies (Stokinger, 1981; WHO, 1982), cases of systemic side effects have been reported in patients taking drugs in the form of tablets containing titanium oxide. The symptoms ceased when the active substance was delivered in tablets lacking titanium dioxide (Berglund, 2004). Several metals, including titanium, may impair the immune response and cytokine release, suggesting that patients who experience extensive exposure to the metals may develop immune dysfunctions (Wang *et al*., 1996).

No significant information is available to demonstrate the cardiovascular, gastrointestinal, hepatic, renal, endocrine, and immunological effects of titanium tetrachloride (ATSDR, 1997).

7.3 Mutagenicity, Carcinogenicity, Teratogenicity, and Effects on Reproduction

7.3.1 Animals

Various titanium compounds have been tested with the rec-assay with *Bacillus subtilis* (Kada *et al*., 1980). The following compounds produced negative results: titanium trichloride, tetrachloride, boride, carbide, fluoride, and dioxide. Titanium tetrachloride has also been regarded as being nonmutagenic (Kanematsu *et al*., 1980, Ogawa *et al*., 1987). Bronchoalveolar lavage (BAL) cells from rats instilled intratracheally with TiO_2 particles, however, induced hrpt-gen mutations in rat alveolar cells *in vitro*. This induction can be prevented if catalase is added, suggesting that free oxygen radicals may be intermediates in the induction

of mutations (Driscoll *et al*., 1997). In a study of exposure to TiO_2 particles through inhalation, rats exposed to 250 mg/m^3 developed lung adenomas and keratinizing squamous metaplasia, suggestive of squamous cell carcinoma (Lee *et al*., 1985, 1986). Intratracheal instillation of a high dose of TiO_2 particles in female rats caused an increased incidence of malignant lung tumors (IARC, 2006). Other rodents, such as mice and hamsters, that had been instilled intratracheally with TiO_2 particles did not exhibit any increases in the incidence of tumors. The International Agency for Research on Cancer (IARC) has listed titanium dioxide within Group 2B; there is sufficient evidence from experimental animal studies for the carcinogenicity of TiO2.

It is commonly believed that titanium belongs to a group of metals that display low carcinogenicity (Sunderman, 1978). There is no evidence that TiO_2-coated mica produced either toxicologic or carcinogenic effects when fed to Fischer 344 rats at dietary concentrations as high as 5.0% (Bernard *et al*., 1990). The organic compound titanocene is carcinogenic when suspended in trioctanoin and administered by intramuscular injection, once a month, to rats and mice. Fibrosarcomas occurred at the injection sites and hepatomas and malignant lymphomas of the spleen developed in the animals (Furst, 1971; Furst and Haro, 1969).

Metallocene dichlorides $(C_5H_5)_2MCl_2$, where M is titanium, vanadium, molybdenum, or niobium, displayed cancerostatic activity against the Ehrlich ascites tumor system in mice. Treatment with such compounds led to a total cure. The survival times without tumor manifestation were longer in animals treated intraperitoneally with titanocene dihalides at doses ranging from 10–240 mg/kg body weight than they were in control animals (Köpf-Maier and Krahl, 1981; Köpf-Maier *et al*., 1980).

In reproduction studies, an adverse effect was discovered in the reproduction of rats exposed to soluble titanate (5 mg/L) in drinking water (Köpf-Maier and Erkenswick, 1984; Schroeder and Mitchener, 1971). Each group was followed through three generations. A significant reduction occurred in the number of animals surviving up to the third generation, and the male/female ratio reduced progressively. The controls continued to breed for four generations at the normal rate.

7.3.2 Human

Several epidemiological mortality studies suggest that TiO_2 dust exerts no carcinogenic effect on human lungs (Boffetta *et al*., 2001; 2004; Fayerweather *et al*., 1992; Fryzek *et al*., 2003); all of these studies considered cancer mortality. No studies have revealed any increase in cancer mortality that is related to TiO_2 exposure. According to the IARC, "there is inadequate evidence in humans for the carcinogenicity of titanium oxide" (IARC, 2006).

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CHAPTER

44

Tungsten

GEORGE KAZANTZIS AND PER LEFFLER

ABSTRACT

Almost one half of an ingested dose of tungsten is in the form of a soluble salt, and one third of a deposited inhaled aerosol of tungstic oxide was rapidly absorbed in those animal models that have been studied. Most of the absorbed tungsten was rapidly excreted in the urine. The small amount of tungsten retained was transferred in part from plasma to red blood cells and distributed mainly to spleen, kidney, and bone. Approximately 3 months after ingestion and 6 months after inhalation, the largest proportion of the body burden of tungsten was found in bone, although this was only a very small part of the administered dose.

The metabolism of tungsten is related to that of molybdenum, which it closely resembles in chemical properties.

Little is known about the toxicity of tungsten compounds, although the LD_{50} of soluble salts in the rat is relatively high. After occupational exposure to tungsten carbide dust by inhalation, cases of pulmonary fibrosis have been reported, but this "hard-metal disease" as it is often called is more likely to be caused by cobalt, with which tungsten carbide is fused. Recent studies have shown a risk of cancer induction after exposure to heavy metal tungsten alloys that requires further investigation. A reliable biokinetic model for tungsten in humans is required.

Apart from occupational sources, small quantities of tungsten are present in food and water; trace quantities, related to industrial emissions, have been found in the general atmosphere. Tungsten is also present in trace amounts in human serum and in urine and feces, elimination approximately balancing the intake of the metal in the few nonindustrially exposed subjects studied. The sparse data on the biological effects of tungsten have been previously reviewed by Browning (1969) and Stokinger (1981), and the chemistry and uses of tungsten have been reviewed by Rieck (1967) and Rose (1994). Industrial, medical, and military uses of tungsten have been expanding rapidly, as has the potential for the spread of tungsten to the environment (Leggett, 1997).

1 PHYSICAL AND CHEMICAL PROPERTIES

Tungsten (W): atomic weight, 183.9; atomic number, 74; melting point, 3410°C; boiling point, 5660°C; specific gravity, 19.3; oxidation states, +2, +3, +4, +5, +6; crystalline form, grey-black, cubic.

Tungsten, known also as wolfram, is a transitional element that belongs to group VIb of the periodic system, together with molybdenum and chromium. Tungsten is not oxidized in air at ordinary temperatures and is highly resistant to acids. Its chemical properties resemble those of molybdenum. Among the more common compounds are the canary yellow trioxide, tungstic acid, sodium tungstate, ammonium paratungstate, and tungsten carbide.

2 METHODS AND PROBLEMS OF ANALYSIS

Environmental samples can be analyzed for tungsten by atomic absorption spectrophotometry (AAS) with a detection limit of 3μg/mL. The addition of

sodium sulfate eliminates interference from molybdenum and other metals (NIOSH, 1977). A spectrophotometric method for the quantitative determination of tungsten and molybdenum in the presence of each other in the trace amounts expected in biological samples has been described by Cardenas and Mortenson (1974). The method involves the selective formation and extraction of the complexes of the metals with a dithiol reagent (toluene-3,4-dithiol) under specific conditions of acidity and temperature. Interference from other components found in biological samples has been found to be negligible. Estimation of trace concentrations of tungsten occurring in air and in water can be performed by instrumental neutron activation analysis (NAA) by use of automatic γ-ray spectroscopy (Cawse, 1974; Salmon, 1974). The detection limit of these authors' method, using a thermal neutron flux up to 10^{14} n/cm^2/sec for activation, was 3 ng/m^3 for industrial air and 10 μg/kg for rainwater. The precision of the method was estimated at 5–10%. NAA can also be used for the determination of trace amounts of tungsten in biological material (Wester, 1974). Inductively coupled plasma mass spectrometry (ICP-MS), atomic emission spectrometry (ICP-AES), and high-resolution ICP-MS (HR-ICP-MS) have been developed to meet new demands on monitoring tungsten levels in air (Rose, 1994) and solutions (Wang, 1999).

3 PRODUCTION AND USES

3.1 Production

Wolframite and scheelite are the common, naturally occurring sources of tungsten, the richest deposits being found in China, Alaska, and Mexico. The world production is in the order of 80,000 metric tons per year. The ore is crushed and ground, concentrated by various physical processes, converted to the oxide, and reduced to the metal. Tungsten carbide is produced by heating the finely powdered metal intimately mixed with carbon in an atmosphere of hydrogen in an electric furnace. In the production of tungsten carbide tools, the carbide is sintered with cobalt, which acts as a binder. The sintered material is then ground to its final shape. Other metals may be added, such as chromium, nickel, titanium, and tantalum, depending on the properties required for the final product.

3.2 Uses

Tungsten is a valuable metal because it has the highest melting point of all metals, it has great strength at high temperatures, and it has good conductivity for electricity and heat. It is used to increase the hardness and tensile strength of steel; it plays a vital role in the production of a number of other alloys noted for their hardness, such as chromium, cobalt, and tungsten alloy used for tipping and facing lathe tools. Many drills and cutting edges of tools are tipped with tungsten carbide, which gives them a hardness comparable to that of diamond. The metal is used for making filaments for incandescent lamps, and tungstates are used in X-ray tubes, fluorescent lamps, and lasers, and as pigments in dyes and inks. Tungsten has acquired importance in nuclear and space technology in the nozzles of rocket motors and protecting shields for spacecraft (Rieck, 1967), as well as in heat-resistant coatings (Matejicek *et al*., 2005). The use of heavy metal tungsten alloys in weapons has been introduced in small-caliber ammunition ("green bullets") as a replacement for lead (ORNL, 1998) and in kinetic-energy penetrators as a replacement for depleted uranium (ORNL, 1996). An increasing interest has recently been shown in WO_3 thin films, with grain sizes of approximately 60 nm, for a variety of applications such as optoelectronics, microelectronics, selective catalysis, and environmental engineering (Ramana *et al*., 2006).

4 ENVIRONMENTAL LEVELS AND EXPOSURES

4.1 General Environment

There are few documentations of anthropogenic release of tungsten into the general environment. Historical mining activities in Cornwall have resulted in metal-polluted estuarine sediments that contain high levels of tungsten (up to ≈650 mg/kg) (Yim, 1976). A clear link from such sites to human exposures remains to be established.

Inhalation of air and consumption of food are the dominating pathways of tungsten exposures to the general population. Air pollution resulting from industrial activities or coal power plants may span 15–19 ng/m^3 in air (Germani *et al*., 1981; Ondov *et al*., 1989). Background levels were reported as <10 ng/m^3 in air and (Jagielak and Mamont-Ciesla, 1979) and in certain vegetables, 6.3–39 μg/kg fresh weight (in onions) (Bibak *et al*, 1998). However, because of incomplete data, the total tungsten intake has been difficult to estimate accurately (ATSDR, 2005).

The dietary intake of tungsten, estimated by NAA in four subjects, ranged from 8.0–13.0 μg/day over a total of eight total diet estimations (Wester, 1974). Drinking water sampled in the three largest Swedish cities varied in tungsten concentration from 0.03–0.1 μg/L, depending on the sampling site. NAA was used (Boström and Wester, 1967). In a survey of atmospheric trace elements

in the United Kingdom performed during 1972–1973, again by NAA, tungsten was included in the "very low" category. The concentration in rainwater was found to be <1 µg/L in five of seven sampling stations. Total deposition of tungsten was found to be <2 µg/cm^2 per year at all seven sampling stations and <0.1 µg/cm^2 per year at six of these. Only very small concentrations of tungsten have been found in the atmosphere, and these have been related to industrial emissions or to nuclear fallout. Levels have usually been <1.5 ng/m^3 in air (Cawse, 1974). Tungsten radioisotopes have been produced in nuclear explosions, on some occasions in large amounts (Lane, 1963). These have been widely distributed in the environment, primarily as tungstic oxide (Romney and Childress, 1963).

4.2 Working Environment

A large amount of dust can be released from the crushing and milling of the ores wolframite and scheelite. In the loading and emptying from furnaces of graphite boats containing the reduced metal, air concentrations of tungsten and tungsten trioxide of 10 and 46 mg/m^3, respectively, were obtained (Mezentseva, 1963). In the various processes associated with tungsten carbide manufacture, air concentrations as high as 47 mg/m^3 were measured (Mezentseva, 1963). Dust may be produced during the mixing of the components and also in the shaping and grinding of the products. Coates and Watson (1971) described metal particles in the atmosphere with a mean diameter of 1.2–1.9 µm. In the tool-cutting industry, there is not only exposure to the dust of tungsten carbide but also to cobalt fume and dust and to the carbides of nickel, titanium, and tantalum. Although the number of workers employed in the manufacture of hard metal is small, the number exposed to hard-metal dust in tool rooms is relatively large (Bech, 1974).

5 METABOLISM

The tungstate ion, WO_4^{2-}, is the most soluble and frequently occurring form of tungsten in biological systems. Apart from the study by Kinard and Aull (1945), most observations on the absorption, distribution, and excretion of tungsten have been performed in animal models with radioisotopes (Aamodt, 1973; 1975; Ballou, 1960; Fleishman *et al*., 1966; Hamilton, 1951; Kaye, 1968; Scott, 1952; Wase, 1956). Built on animal data, attempts to develop a human metabolic model for tungsten have been made by the ICRP (1981; 1995). As discussed by Legget (1997), important gaps in data are identified concerning tungsten biokinetics in certain soft tissues such as brain, testes, uterus, and fetus.

5.1 Absorption

In the rat, after the ingestion of ^{185}W, 40% of the dose had been excreted in the urine after 24 hours, and approximately 58% had been eliminated in the feces or had remained unabsorbed in the gut, with only 2% remaining in the tissues (Ballou, 1960). Kaye (1968) followed the rate in rats of ^{187}W over a 3-day period, and of ^{185}W for up to 254 days, after administration of tungstate solution at pH 8 by gavage. The rapid absorption of tungsten from the gut could be demonstrated by the peak value of 17% of the administered dose in the carcass 1 hour after administration. Taking into account the total amount of tungsten excreted in the urine together with an estimate of the amount transferred from blood to feces, Kaye estimated the fraction of radionuclide passing from the gastrointestinal tract to the blood to be 0.47.

After inhalation of a ^{181}W-labeled tungstic oxide aerosol by beagle dogs, 60% of the inhaled activity was deposited in the respiratory tract. As much as one third of the deposited activity was cleared into the systemic circulation mostly within 10 days of inhalation, the remaining two-thirds were cleared into the gastrointestinal tract (Aamodt, 1975). Tungsten powder ($CaWO_4$) was sprayed into a single lung lobe to study particle translocation kinetics in dog lungs. After 24 hours, tungsten accumulated in macrophages of regional lymph nodes and peaked at day 7 (Grande *et al*., 1990). Studies by Edel *et al*. (1990) indicate that tungsten, inhaled as hard metal particles, may be released for systemic distribution.

5.2 Distribution

Rats were fed for a period of 100 days on diets in which tungsten had been incorporated either as the finely ground metal, as tungsten oxide, sodium tungstate, or as ammonium paratungstate (Kinard and Aull, 1945). The principal sites of deposition were bone and spleen with trace quantities (<1.0 mg percent as specified by the authors) in kidney and liver, and in some animals in blood, lung, muscle, and testes. No marked difference in distribution of tungsten was found with the different compounds ingested.

Ballou (1960), after oral administration of sodium tungstate, found, after 1 day, the greatest concentration in spleen, followed by kidney, pelt, bone, and liver. Again after administration of sodium tungstate by gastric intubation in rats, Fleishman *et al*. (1966) found the highest concentration in kidney followed by bone and spleen. The long-term study in rats of ^{185}W administered by gavage (Kaye, 1968) showed that after 100 days, more than 99% of the total body burden (approximately 0.4% of the administered dose) had been

retained in bone. In soft tissues, the highest concentration was found in spleen, thought likely to be because of the sequestering action in red blood cells, and, in decreasing order, in hair—although external contamination could not be excluded—kidney, pelt, and liver. The relatively high concentration in the kidney on the third day correlated well with the large fraction of the administered dose eliminated in the urine; the shape of the kidney retention curve reflected this. Over the first 2 days, the concentration in plasma was approximately equal to that in whole blood, after which period, activity associated with the red cells predominated. After intravenous administration of ^{181}W sodium tungstate in two beagle dogs, most of the activity was found in the plasma during the first 24 hours, with an average plasma/red cell ratio of 3:1, suggesting binding of tungsten to the cell surface rather than penetration of the cell membrane (Aamodt, 1973). It was further suggested that tungsten incorporation in red blood cells occurs later at sites of hemopoiesis.

In the inhalation study by Aamodt (1975) described previously, the highest concentration of activity 165 days after exposure was found in lung and kidney, whereas bone, gallbladder, liver, and spleen had concentrations almost one order of magnitude less than lung and kidney, although the bone concentrations may have been erroneously low because of the method of estimation. However, in terms of organ burden, most of the total activity was found in bone (37% of the body burden at sacrifice), lung (31%), kidney (15%), and liver (9.7%). At 165 days, average body burden calculated from tissue measurements was 0.017 of the inhaled activity.

5.3 Excretion

All reports agree that in animal experiments, much of the absorbed tungsten is rapidly excreted in the urine. In Kaye's (1968) study detailed previously, approximately 40% of the administered dose of ^{187}W was excreted by the kidney in the first 24 hours, but very little was excreted in the urine subsequently. A further 40% of the administered dose recovered from the feces by 24 hours is likely to have been accounted for by unabsorbed tungsten together with tungsten excreted into the gut with intestinal secretions and bile. By the end of 3 days, fecal elimination had accounted for 52% of the administered dose.

After intravenous administration of sodium (^{181}W) tungstate in beagle dogs, 91% of the injected activity was excreted in the urine within 24 hours (Aamodt, 1973). The ratio of activity excreted in the urine to that eliminated in the feces averaged 38. Although loss from the blood during the first 24 hours was very rapid, it was calculated that some of the tungsten, perhaps that bound to red blood cells or to plasma proteins, was either not filtered from the plasma or else some was being reabsorbed from the glomerular filtrate. The rate of decrease in blood activity after inhalation of tungstic oxide aerosol was slower than that for injected sodium tungstate, but this could be accounted for by activity entering the blood from the lung and the gut over an extended period after exposure (Aamodt, 1975). The ratio of cumulative urinary excretion to cumulative fecal elimination for 165 days ranged from 0.57–1.8, the variation being related to differences in clearance patterns of individual dogs.

In man, trace quantities of tungsten are excreted in urine and eliminated in feces (see Section 6). In a limited study on four normal young adults without specific exposure, the elimination by these two routes over 24-hour periods balanced the tungsten intake in food, with only small positive and negative variations (Wester, 1974). In a group of 10 workers engaged in the production of hard metals in the form of tungsten carbide, the daily excretion of tungsten in the urine averaged 490 μg W per 24 hours (Barborik, 1972).

5.4 Biological Half-Time

In the study by Kaye (1968), elimination of gastrically administered tungsten from the whole rat was very rapid, with a biological half-time of approximately 10 hours for the initial fast component of the elimination curve. Elimination from soft tissues was relatively rapid, but with a biological half-time of 44 days for the spleen. The biological half-time for ^{185}W in bone was calculated at 1100 days for the slowest component of a three-component elimination curve.

In Aamodt's experiments (1973; 1975), injected sodium (^{181}W) tungstate was removed from the rat body as if 82% was cleared with an 86-minute biological half-time, 15% with an 8.8-hour half-time, 2% with a 3.65-day, and 1% with a 99-day half-time. Inhaled (^{181}W) tungstic oxide in dogs was removed with a biological half-time of a little less than 9 hours for 94% of the activity in the visceral area, with the longest half-time of 139 days for 1.6% of the activity.

6 BIOLOGICAL MONITORING

Information on this aspect is scanty. Kinard and Aull (1945) were, in their oral dosing experiments referred to in Section 5.2, unable to detect tungsten in any of the tissues of their control group of animals.

In man, the mean serum concentration of tungsten, estimated by NAA, was found to be 5.8 ng/mL in eight

healthy subjects, with a standard deviation (SD) of 3.5. In 11 patients with hypertension, the mean serum level was 11 ± 13 ng/ mL before treatment and 16 ± 18 ng/mL after treatment with chlorthalidone. None of these differences were significant (Wester, 1973).

The urinary excretion of tungsten, again measured by NAA, ranged from 2.0–13.0 µg per 24 hours in four subjects in eight estimations. Fecal elimination in the same subjects ranged from 1.6–5.7 µg W per 24 hours. The subjects were healthy, young male and female adults in whom tungsten excretion did not seem to be affected by calcium intake in the diet in this small series of observations (Wester, 1974). In a group of 16 hypertensive patients, the mean tungsten excretion in the urine was 32 ± 63 (SD) µg per 24 hours before treatment and 36 ± 85 (SD) µg per 24 hours during treatment with chlorthalidone. The levels showed great variability between individuals and were not influenced by diuretic therapy (Wester, 1973). The presence of tungsten in blood, liver, or feces serves as a biomarker of exposure to tungsten or tungsten compounds.

7 EFFECTS AND DOSE-RESPONSE RELATIONSHIPS

7.1 Local Effects and Dose-Response Relationships

7.1.1 Animals

In a small number of experimental observations, tungsten metal and its compounds have not seemed to exert a significant effect on the respiratory system. Harding (1950) injected rats intratracheally with 5% suspensions in saline of metallic tungsten powder and of tungsten carbide, but no acute effects were produced, whereas rats similarly injected with metallic cobalt died within a few hours with hemorrhagic, edematous lungs. Similarly, Miller *et al*. (1953) observed no cellular reaction other than that caused by an inert dust with an intratracheal instillation of tungsten carbide in rats followed over a period of 18 weeks. Delahant (1955) found tungsten metal dust and also tungsten carbide with carbon to be relatively inert when instilled as an intratracheal suspension of 150 mg divided in three equal doses in guinea pigs followed up for a period of 30 days to 1 year. Schepers (1955), amplifying the observations made by Delahant, described focal interstitial pneumonitis and bronchiolitis as an acute reaction, with almost complete recovery after 1 year, but with some focal interstitial infiltration, a minor degree of atrophic emphysema, and some peribronchial and periarterial fibrocellular reaction with accompanying endarteritis obliterans. However, an intense inflammatory reaction resulted if cobalt was an ingredient of the injected material. Mezentseva (1963) gave white rats a single intratracheal dose of 50 mg metallic tungsten, tungsten trioxide, or tungsten carbide in 0.5 mL saline and sacrificed the animals after 4, 6, and 8 months. Histological changes were limited to the lungs and consisted of a proliferative reaction of the lymphoid and histiocytic elements, particularly at the accumulation sites of the administered dust, with subsequent mild fibrosis. The walls of small vessels were thickened and their endothelia swollen. Acute pulmonary edema developed in rats given an intratracheal instillation of tungsten carbide and cobalt alloy—hard metal—but not in rats exposed to tungsten carbide or cobalt alone (Lasfargues *et al*., 1992).

In a further study of repeated intratracheal instillation, tungsten carbide and cobalt in combination, but not alone, induced interstitial pulmonary edema (Lasfargues *et al*., 1995).

Because of an increasing use in munition, tungsten alloy was tested for carcinogenicity in rats receiving four pellets (low dose) or 20 pellets (high dose) of weapons-grade tungsten alloy embedded in muscle tissue at surgery. Results revealed a dose-dependent metastatic rhabdomyosarcoma within 6 months with a higher incidence compared with the positive control group exposed to nickel (Kalinich *et al*., 2005).

7.1.2 Humans

A hazard exists in the tungsten carbide industry, which may take the form of "hard-metal disease." The agent responsible for this disorder is believed to be cobalt, present as a constituent of the hard-metal alloy, but this has not been proven. Studies in a number of factories manufacturing tungsten carbide–based products have revealed the presence of respiratory symptoms associated with impaired respiratory function and radiographic abnormality in a proportion of those exposed. Moschinski *et al*. (1959) investigated 331 hard-metal workers and found evidence of pulmonary fibrosis in 59. The characteristic radiographic appearances were increased lung markings in the lower lung fields with nodular opacities in the more advanced cases. Ahlmark *et al*. (1961) described four cases in men who had not been exposed for more than 8 years. Bech *et al*. (1962) described the clinical and radiological findings in six cases of hard-metal disease collected over a period of 15 years. Complaints included cough, expectoration, shortness of breath, and tightness in the chest. In two of six cases, pulmonary function tests showed mild alveolar diffusion defects, and in one worker who died, diffuse pulmonary interstitial fibrosis was found. There was evidence of pulmonary fibrosis on the chest radiographs. In addition, a radiological survey was

performed on 255 workers exposed to hard-metal dust. This showed only slight changes in several of the workers and yielded one of the six cases described previously. Airborne dust concentrations at the time of the survey were of the order of 0.30 mg/m^3 for particles less than 5-μm projected diameter. Gravimetric samples taken in the sieving room showed 90% tungsten and 6% cobalt in the incombustible fraction. It was concluded that the cause of hard-metal disease was the inhalation of dust from the working environment, but that the responsible component was likely to be cobalt rather than tungsten. The absence of cobalt from the lungs of hard-metal workers, which contained both tungsten and titanium, was attributed to the high solubility of cobalt in plasma. In a later report, Bech (1974) described a total of 12 cases of hard-metal disease. Interstitial fibrosis was present in the lungs of all seven who died; emphysema was found in six of these. It was suggested that hypersensitivity to cobalt was involved in the development of the disease and that in early cases the disease may be arrested by removal from exposure. Although hard-metal disease was considered to be related to cobalt exposure, Bech (1974) suggested that tungsten carbide might enhance the solubility of cobalt in protein-containing fluid.

Coates and Watson (1971) described 12 cases, including 8 deaths, of diffuse interstitial pulmonary fibrosis in workers processing tungsten carbide. The clinical picture was one of nonproductive cough, exertional dyspnea, and weight loss.

Respiratory function tests showed a restrictive pattern with an abnormality of gas transfer. The radiological appearances were characterized by progressive, bilateral nodular, and linear shadowing involving major portions of both lungs. Detailed histological examination of three cases (Coates and Watson, 1973) showed deposits of collagen and elastic tissue in the septal areas and alteration of the alveolar lining cells (modified type-l pneumocytes) with swelling and formation of microvilli. Unidentified, hard, multifaceted crystals were present in the affected areas of the lung. These authors, too, attributed the condition to the inhalation of cobalt-containing dust.

A cohort of male tungsten miners was included in a Chinese study. The risk of silicosis at comparable silica dust exposures, was clearly higher among tungsten miners (cumulative risk, CR = 0.964) compared with cohorts of tin miners (CR = 0.901) and pottery workers (CR = 0.225) (Chen *et al*., 2005).

An increased mortality from lung cancer was observed among the hard-metal workers in three Swedish factories (Hogstedt and Alexandersson, 1990) and one French factory (Lasfargues *et al*., 1994). Statistically significant increased lung cancer mortality was observed in workers manufacturing hard metals exposed simultaneously to tungsten carbide and cobalt, confounding by smoking was considered to be unlikely (Moulin *et al*., 1998).

This excess mortality occurred mostly in workers exposed to unsintered hard-metal dust (Wild *et al*., 2000). Tungsten carbide is bioavailable despite its low solubility; the bioavailability of tungsten increased in the order tungsten metal, tungsten carbide, and tungstenate, where wet grinding is carried out. In a biological monitoring study in hard-metal workers, the highest tungsten concentrations in urine were found in grinders exposed to tungstenate, with high concentrations also in departments producing tungsten carbide and heavy alloys (Kraus *et al*., 2001).

7.2 Systemic Effects and Dose-Response Relationships

Tungsten has not been shown to be an essential trace metal in either animal or plant metabolism.

7.2.1 Animals

In a series of feeding experiments, young rats were fed for a period of 70 days with different concentrations of sodium tungstate, tungstic oxide, and ammonium paratungstate mixed in Purina dog chow (Kinard and Van de Erve, 1941). Sodium tungstate, equivalent to 2% W, tungstic oxide equivalent to 3.96% W, and ammonium paratungstate equivalent to 5% W were markedly toxic, causing initial weight loss followed by the deaths of all animals in each group within 10 days. In diets having an equivalent of 0.5% W, sodium tungstate and tungstic oxide produced death in three quarters of the rats, whereas ammonium paratungstate produced no fatalities. The same authors (1943) in similarly designed feeding experiments administered 2, 5, and 10% tungsten metal powder over 70 days and found no effect on weight gain in male rats, but a 15% reduction in females.

In an extensive live-term study, Schroeder and Mitchener (1975) added 5 mg/L as sodium tungstate to the drinking water of rats. At this dose level, there was little detectable effect as measured by serum cholesterol, glucose, uric acid, and incidence of tumor formation. However, a slight enhancement of growth was seen in rats of both sexes, and a small but significant shortening of longevity was noticed in the tungsten-dosed male rats.

Tungstic acid has been used to produce experimental epilepsy in laboratory animals (Kusske *et al*., 1974). In a series of cats, 0.02 mL of tungstic acid gel applied to the surface of the cortex gave rise to abnormal EEG

activity after a 20–30 minute interval, which increased and resulted in sustained ictal activity. The effect produced was so consistent that tungsten has been used to produce model systems of experimental epilepsy.

After the intraperitoneal injection of tungsten oxide in rats, no cellular reaction has been observed (Frederick and Bradley, 1946). Later studies by Lison *et al*. (1990; 1991; 1992) clearly indicate that cell reactions do occur in mouse peritoneal and rat alveolar macrophages related to the combined exposure to tungsten carbide and cobalt.

7.2.2 Humans

There are no data available on occupational exposures to compounds of tungsten that incriminate these as toxic or as hazardous agents to other parts than the direct local contact sites (i.e., in the lung). Kruger (1912) observed no ill effects in patients given 25–80 g powdered tungsten metal by mouth as a substitute for barium in radiological examinations. However, the occurrence of the interstitial lung disease termed "hard-metal disease" led to findings that the combined exposure to cobalt and tungsten particles may be an important explanatory factor (Lison *et al*., 1996). Recent findings on human cell cultures *in vitro* and on rats *in vivo* indicate that tungsten may have a synergistic effect with cobalt or nickel leading to tumorigenicity (Kalinich *et al*., 2005; Miller *et al*., 2001).

Recent studies by Gatti *et al*. (2004) have documented nanometer-sized particles containing toxic metals (i.e., tungsten, aluminium, antimony, copper, gold, iron, lead, mercury, nickel, silver, titanium, zinc) captured in the bloodstream of humans. Because of the suspicion of particles being a trigger of blood coagulation, this finding addresses the emerging problem of the lack of risk evaluation of nanoparticles in general.

7.2.2.1 Human Cell Culture

Studies on human peripheral lymphocytes showed that genotoxic effects (DNA single strand breaks) were evident after the exposure to a combination of tungsten and cobalt (Anard *et al*., 1997; De Boeck *et al*., 1998).

The use of tungsten in some diagnostic equipment such as radiopaque catheters has called for an increased testing of biomaterials. Tungsten acid as a pure substance added to cultivation medium resulted in a stimulated metabolic activity in human urothelial cells (Pariente *et al*., 1999). Other studies using tungsten in water solution, support the view that tungsten itself will produce toxicity at such high concentrations that may not be exceeded in human serum (Peuster *et al*., 2003).

However, a metal powder of tungsten in combination with cobalt and nickel resulted in a synergistic increase in DNA breakage and chromosomal aberrations when exposed to human osteoblast cells (Miller *et al*., 2001). Transformed cells showed alterations in *ras* oncogene expression and induced tumors when transplanted to nude mice, which was interpreted as a neoplastic transformation to a tumorigenic phenotype of the osteoblast cells.

7.3 Interaction with Molybdenum

Sodium tungstate antagonizes the normal metabolic action of molybdate in its role as metal carrier for the enzymes xanthine dehydrogenase (Higgins *et al*., 1956), sulfite oxidase and aldehyde oxidase (Johnson and Rajagopalan, 1974), and nitrate reductase (Notton and Hewitt, 1971). It is of interest in this respect that the tungstate ion WO_4^{2-} is isomorphic with the molybdate ion MoO_4^{2-}. Higgins *et al*. (1956) showed that sodium tungstate added to the diet inhibited the intestinal deposition of xanthine oxidase in the rat and reduced both xanthine dehydrogenase and molybdenum concentrations in the liver of the chicken. Owen and Proudfoot (1968) fed sodium tungstate to goats and cows and showed a reduction in xanthine oxidase secreted in milk, in some cases to an undetectable level. They postulated that tungsten could preferentially occupy enzyme sites normally occupied by molybdate.

Cohen *et al*. (1973) administered tungstate to rats maintained on a low-molybdenum diet and demonstrated a loss of both xanthine oxidase and sulfite oxidase activities. The tungsten-treated rats seemed healthy but were more susceptible to bisulfite toxicity. On exposure to high levels of sulfur dioxide, the tungsten-treated, sulfite oxidase–deficient animals showed evidence of systemic sulfite toxicity and had much shorter survival times than the controls. Sulfite oxidase seems to be involved in the oxidative metabolism and thus in the detoxification of sulfur dioxide as well as bisulfite.

Sulfite oxidase activity in rat liver is negligible at birth but increases rapidly between the fifth and eleventh day after birth. Activity is considerably impaired by administration of tungsten for 20 days before delivery (Cohen *et al*., 1974).

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CHAPTER

45

Uranium

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ABSTRACT

Uranium is an element with chemical and radiological properties that have made it useful in industry and commerce but toxic at sufficiently high levels to humans and the environment. Various analytical methods are available to determine the presence, concentration, or quantity of uranium in a range of media. These confirm its presence in ambient air, water, and soil, so human exposure is assured. At least seven of its more than 100 mineral forms are found at mineable levels in various parts of the world, and the primary producers are in Canada, Russia, Ukraine, Australia, and Central Africa. Uranium is mined primarily for the ^{235}U isotope. The process of enrichment adjusts the ratio of the three natural isotopes ($^{234,\ 235,\ \text{and}\ 238}U$) to produce two fractions. The one with an increased proportion of ^{235}U is enriched uranium and the source of energy production for nuclear reactors and weapons. The remaining portion is depleted in ^{235}U and termed depleted uranium (DU). Apart from energy production, uranium is used in a range of products that include glass tinting agents, ceramic glazes, gyroscope wheels, chemical catalysts, shields for high-intensity radioactive sources, X-ray tube targets, and military kinetic penetrator munitions. Uranium is such a ubiquitous substance that individuals are continually exposed to it in air, water, and food, as well as to the radiation it emits. Intake from water and food each approximate 0.9–1.5 μg/day, with this being variable on the basis of the source of water (higher in ground water) or type of diet (higher in beef, beef kidney, onions, parley, and salt, but lower in poultry, fruit juices, canned vegetables, and dairy products. Air concentrations are normally low. The highest human exposures are in the uranium milling and production industries, with the greatest environmental remediation challenge coming from large volumes of mill tailings. Human exposure involves inhalation and ingestion (or more recently, military fragment wounds). Uranium absorption is low (0.2–<6%) by the typical routes of exposure. Once absorbed to blood, its distribution and elimination kinetics are primarily a function of solubility and oxidation state. Uranium entering body fluid in the tetravalent state is converted to hexavalent as the uranyl ion, which complexes with available citrate or bicarbonate in blood, or proteins in plasma. Distribution is throughout the body, with primary long-term concentration in lung (for heavy occupational exposure), bone, liver, and kidney. Initial elimination is rapid, with at least two time-phase components for excretion. Computer programs are available to estimate the timeframe concentrations in and radiation doses to the various organs and tissues. Federal Guidance Report No. 9 (EPA, 1988) and the computerized version of Federal Guidance Report No. 13 (EPA, 2005) provide Sv/Bq conversion factors for estimating the radiation dose (committed dose equivalent) received by the body and selected organs from inhaled or ingested uranium isotopes. Uranium has been found to cross the placenta and be excreted in breast milk. Overexposure can affect human and animal health. Although uranium is both a chemical and a radioactive material, it has been determined that its adverse health effects are primarily a result of its chemical rather than radiological toxicity (ATSDR, 1999). The damage mechanism for uranium retained in the lung involves irritation to the deep lung with the long-term potential for fibrosis and emphysema. The mechanism

for renal toxicity involves the accumulation of uranium in the tubular epithelium and subsequent irritation that progresses to damage and necrosis on the basis of exposure level and duration. This may be associated with increased cellular oxidative stress, altered expression of genes involved in cell signaling, and inhibited sodium-dependent phosphate and glucose transport systems. The impact on liver enzymes and receptors might affect drug therapy regimens. Uranium can also interfere with bone remodeling and liver integrity. The health effects from exposure by normal routes are primarily to the kidney, and this damage has been shown to be reversible. Even before efforts began to produce uranium for nuclear weapons and energy production purposes, uranium was recognized for its ability to induce nephritis in laboratory animals. Less severe effects have been observed for the liver, lung, nervous, and reproductive system. Cancer is not expected, because it has not been observed in high-dose human and animal studies. If it should occur, bone sarcomas are regarded as the most likely to occur, because uranium and radium deposit long term in bone, and radium dial painters developed bone sarcomas.

1 PHYSICAL, CHEMICAL, AND RADIOLOGICAL PROPERTIES

Uranium (U) was discovered in 1789 by the German chemist Martin Heinrich Klaproth (1743–1817) and named after the planet Uranus. It occurs with a typical natural abundance of 2–4 ppm (mass fraction of $2–4 \times 10^{-6}$) in the crust. The highest concentrations in the United States are found in the four corners region (Arizona, Colorado, New Mexico, and Utah), whereas the highest on earth to date are in Canada (>25% uranium by mass). The most active mines are in Canada, Russia, that region's independent states (Kazakhstan, Ukraine, and Uzbekistan), Australia, and Central Africa (Namibia, Niger, and South Africa). A series of mining, milling (through acid leach or alkali roast), and purification steps produce a mixture of oxides that can be reduced to form a silvery white metal that is malleable, ductile, slightly paramagnetic, and almost as dense as tungsten. Three metallostructural configurations are known (orthorhombic, tetragonal, or body-centered cubic). Like aluminum, it reacts in air to produce a protective oxide coating. The metal powder is pyrophoric, igniting at elevated temperatures in air, CO_2, and N_2 to form the oxide, carbide, and nitride, respectively. The periodic table places uranium in the actinide series of elements, and similarly, it can exist in any of six oxidation states (0, +2, +3, +4, +5, and +6). The +4 state is relatively stable and is associated with hydroxides, phosphates, and fluorides. The +6 state is the most stable when present as octaoxide (U_3O_8 or yellow cake), but as hexafluoride it dissociates rapidly on contact with liquid water or water vapor in air. These last two states are the most relevant to human exposure and commercial efforts. Table 1 summarizes the chemical

TABLE 1 Chemical and Physical Properties of Uranium and Compounds

Property	Uranium	Uranium dioxide	Uranium trioxide	Uranium octa-oxide	Uranium tetra fluoride	Uranyl hexafluoride	Uranyl fluoride	Uranyl nitrate
At./Mol weight	238.029	270.03	286.03	842.08	314.02	352.02	308.03	502.13
Formula	U	UO_2	UO_3	U_3O_8	UF_4	UF_6	UO_2F_2	$UO_2(NO_3)_2$
Color	Silvery	Brown-Black	Yellow-red	Dk olive green	Green	Colorless	Pale yellow	Yellow
Melting Pt (°C)	1135	2878	Dec[a]	Dec[a] at 1300	960	Sublimes at NTP[b]	Dec[a] at 300	Dec[a] at 100
Boiling Pt (°C)	4131	No data	NR[c]	NR[c]	No data	56.2	NR[c]	NR[c]
Solubility in water	Insol	Insol	Insol	Insol	Vss[d]	Dec[a]	Sol	Miscible
Solubility other	Acids	HNO_3	HNO_3, HCI	HNO_3, H_2SO_4	Conc base/acid	$CC1_4$, chloroform	Ethanol	Ethanol
Density (g/cm^3)	18.95	10.96	7.29	8.30	6.70	4.68 at 21°C	6.37	2.81 at 13°C
Conv[e](pCi/μg)	0.67	0.59	0.56	0.57	0.45	0.45	0.52	0.32

[a]dec, Decomposes.
[b]NTP, Normal temperature and pressure.
[c]NR, Not relevant.
[d]Vss, Very slightly soluble.
[e]Conv, Mass to radioactivity conversion (based on isotopic ratio in undisturbed crustal rock).
Adapted from ATSDR (1999).

and physical properties of uranium and several of its most important compounds.

The renowned French physicist, Antoine Henri Becquerel (1852–1908), was awarded the 1903 Nobel Prize in Physics for discovering the radioactive nature of uranium in 1896. All 22 of its currently recognized isotopes ($^{217–219, 222–240, 242}$U) are radioactive. The three that occur naturally ($^{234, 235, \text{and}238}$U) are part of two decay chains, headed by the latter two. In these, an atom of ^{235}U transforms 11 times into different nuclides and finally becomes ^{207}Pb, whereas ^{238}U transforms 14 times (one of which produces ^{234}U) and eventually becomes ^{206}Pb. Table 2 shows the order by which a uranium atom transforms through intermediate species (including radium and radon) to become an atom of lead. The ratio of $^{234, 235, \text{and}238}$U in the undisturbed crust is 0.000055:0.0072:0.99275 by mass and 0.489:0.022:0.489 by radioactivity. These ratios change imperceptibly over a lifetime and slowly over millennia because of differences in the long radioactive half-lives of the ^{235}U (7.04×10^8 years) and ^{238}U (4.468×10^9 years) parents of the radioactive decay chains. Despite the relatively short half-life of ^{234}U (2.457×10^5 years), this isotope and ^{238}U contribute equally to the radioactivity ratio because they are in secular equilibrium. These ratios can change significantly in either direction when the rock (or especially soil) is in contact with water, as in an aquifer. $^{234 \text{ and } 238}$U are in secular equilibrium in undisturbed rock, but their activity equivalence can vary from 0.8–10 in drinking water, 0.8–8 in precipitation, and 0.5–1.2 in soil (EPA, 1996; 2005a). The process has not been fully explained. It may be associated with an increased rate of thorium dissolution over uranium at the surface of rock or soil grains, as well as with the ejection of uranium atoms whose alpha emissions directed inward might recoil the atom from the surface and into interstitial water. Both processes increase ^{234}Th and its progeny ^{234}U in surrounding water relative to the solid, and environmental factors could further alter the ratio. The consequence of nonequilibrium ratios to conversions between mass and radioactivity units is addressed at the end of Section 2.

Some radiological information that distinguishes the natural uranium isotopes is shown in Table 3.

2 ANALYTICAL METHODS

Uranium can be analyzed chemically, physically, or radiologically, depending on the media, analytical equipment, regulatory requirement, and other needs. Conversion between radiological and mass units requires knowledge of the relative proportions of the three natural isotopes. The standard 0.67 pCi/μg (0.025 Bq/μg) conversion factor for undisturbed crustal rock may not be appropriate for a given sample because of a normal disequilibrium between ^{234}U and ^{238}U, for which the ratio has been found to have a broad range (e.g., 1.2–40 for drinking water). As a result, the convention used in this chapter is to first display the units used in the source document followed by the conversion value when considered appropriate.

Several analytical methods are available for determining the presence, concentration, or quantity of uranium or its compounds in a range of media. The methods are generally characterized as photometric (fluorometry or kinetic phosphorescence analysis [KPA]), radiometric (alpha spectroscopy, gamma spectroscopy, liquid scintillation spectroscopy, gas flow proportional counting, X-ray fluorescence, or neutron activation analysis), and mass spectroscopic (MS). Any of these methods can be used for *in vitro* analyses of biological samples (urine, feces, blood, hair/nails, or tissues) or environmental samples (water, soil, sediment, air, flora, or fauna). *In vivo* analysis requires the detection of emitted gamma radiation by gamma spectroscopy, because available alpha and beta particles are absorbed within the organism. The goals (regulatory and detection limits, gross versus isotopic analysis, and cost) determine the suitable methods, required sample preparation steps, and counting time. Detection limits have been published for KPA as 0.05 μg/L for urine to 0.6 μg/L for serum (Ejnik *et al.*, 2000), and for mass spectroscopy may currently be <3 ng/L of urine for individual uranium isotopes.

TABLE 2 Order by Which a Uranium Atom Transforms into Lead

1[a]	2	3	4	5[b]	6	7	8	9[b]	10	11[b]	12	13	14[b]	15
^{238}U (parent)	^{234}Th	^{234}Pa	^{234}U	^{230}Th	^{226}Ra	^{222}Rn	^{218}Po	^{218}At ^{214}Pb	^{214}Bi	^{214}Po ^{210}Tl	^{210}Pb	^{210}Bi	^{210}Po ^{206}Tl	^{206}Pb (final)
^{235}U (parent)	^{231}Th	^{231}Pa	^{227}Ac	^{227}Th ^{223}Fr	^{223}Ra	^{219}Rn	^{215}Po	^{215}At ^{211}Pb	^{211}Bi	^{211}Po ^{207}Tl	^{207}Pb (final)			

[a]The parent nucleus transforms sequentially to the right until it becomes the final stable nuclide.
[b]Double entries in a cell indicate predecessor nucleus exercises two transformation options.

TABLE 3 Radioactive Properties of Natural Uranium Isotopes

			Decay energy Mev, (% abundance)					
Isotope	Weight %	Radioactivity %	Total	Alpha	Electron/ Beta	Gamma	Half-life (years)	Specific activity (pCi/g)
^{234}U	0.0055	48.9	4.859	4.775 (71.4) 4.722 (28.4)	0.009 (11.0) 0.033 (20.9)	0.013 (10.0)	2.457E5	6.24
^{235}U	0.072	2.2	4.679	4.398 (55) 4.366 (17) 4.215 (5.7) 4.596 (5) Other (17.3)	0.009 (27) 0.021 (22) 0.031 (6.9)	0.003 (24) 0.093 (5.6) 0.144 (11) 0.186 (57)	7.04E8	0.00216
^{238}U	99.2745	48.9	4.274	4.151 (21) 4.198 (79)	0.029 (15.3) 0.044 (4.2)	0.013 (7.3)	4.468E9	0.000336

DOE, 2005. Decay radiation search. *http://www.nndc.bnl.gov/nudat2/dec_searchi.jsp June 2*, 2005.

Accurate ^{235}U/^{238}U ratios have been reported for small volumes of urine (2 mL) with concentrations less than 10 ng/L (Pappas *et al*., 2003).

Samples may be analyzed directly, but additional sample preparation steps are followed when the concentration of uranium is too high or low for the method or when self-absorption of emitted radiations or interference with other substances is significant. This sample preparation can range from simple dilution or concentration on an ion exchange column, to more complex prechemistry that can involve dry ashing, wet ashing (with acid and/or peroxide), tracer addition, dissolution, coprecipitation, and electrodeposition.

The photometric methods are used for gross uranium analysis. Fluorimetry is a standard method for analyzing uranium in drinking water samples. It is generally the least expensive of all techniques and involves adding sodium fluoride to a sample, illuminating it with ultraviolet radiation, and detecting the emitted yellow-green fluorescence. UV lights can be used to demonstrate the presence of uranium in glass (e.g., antique Vaseline glass). KPA is performed by exciting the aqueous solution with a laser, viewing the linearity of the decay profile as specific for uranium, and recording the initial intensity as proportional to the uranium concentration (Heyeda *et al*., 1997). Some elements (chlorine, thorium, cobalt, iron) can quench the luminescence, but efforts can be taken to account for this effect (Birkenfeld *et al*., 1995). A spectrophotometric method for analyzing uranium in water uses tri-*n*-octylphosphine oxide on an octadecyl-bonded silica membrane and achieves at limit of detection of 100 ng/L (Shamsipur *et al*., 1999).

Radiometric analytical methods rely on measuring radiation emitted by uranium or its progeny or secondary emissions produced when this radiation activates a liquid or solid scintillator. Alpha spectroscopy with a silicon diode detector is extremely useful for uranium isotopic analysis. It involves producing energy calibration and geometry efficiency curves with standard alpha emitters, counting a very thin sample in a vacuum, and relating the counts under individual photopeaks to identify individual isotopes and their activities in the sample. Gamma spectroscopy with sodium iodide, cesium iodide, or intrinsic germanium is limited for uranium isotopes; their gamma emissions are not intense and are close to those for radium, requiring care to ensure the quality of results. In liquid scintillation, the sample is mixed with a scintillation cocktail, the uranium alphas or betas activate the fluid to emit light pulses, the energy and intensity of which are used to determine the concentration of total uranium, or more difficultly, that of its individual isotopes. NAA is a trace quantity method that overcomes limitations of gamma spectroscopy by exposing the sample to neutrons in a nuclear reactor, converting ^{238}U to ^{239}U on the basis of neutron flux and time, and counting the resulting ^{239}U beta emissions to estimate the quantity of ^{238}U.

Physical analysis methods that use hyphenated mass spectrographic techniques, such as inductively coupled plasma mass spectrometry (ICP-MS), use sensitive, but expensive, equipment to more directly and quickly analyze samples (Caldwell *et al*., 2005; Ting *et al*., 1996). Samples are digested to remove organics or diluted to guard against problems with high solid concentration and internally standardized to correct for signal variation and blank correction with ^{233}U or a similar standard, if available. After such pretreatments, samples are injected into the plasma, where the samples are broken down into atoms and ionized for detection. The need

for digestion can be eliminated and sample preparation time reduced to <1 hour by use of an appropriate chelation or ion exchange column with sufficient prewash and postwash volume, and adjusting the mass offset to the high mass end of the spectral peak in magnetic sector instruments (Pappas *et al*., 2006). Quadrupole ICP-MS units can give accurate total uranium and isotopic ratios for urine concentrations above approximately 50 ng/L, whereas high-resolution, double-focusing magnet units resolve individual isotopes more accurately. The latter can achieve a limit of detection <3 ng/L in a 100-µL sample (Pappas *et al*., 2002). Both have some difficulty resolving isotopes whose masses are multiples of that for the carrier gas. ICP-QMS was used to determine that black rain fallout stains on concrete from the atomic bomb detonation over Hiroshima in 1945 contained a higher proportion of ^{235}U than natural uranium, confirming that the fission was not complete (Fujikawa *et al*., 2003).

Measuring the relative ratios of the natural uranium isotopes and anthropogenic uranium isotopes (e.g., ^{236}U) along with trace radionuclides is a method that can be used as part of a forensic evaluation to identify potential sources of uranium in a sample. Because of the wide natural variability in uranium isotopic ratios, the reliability of this method will depend in part on the ability to find and accurately measure the isotopic ratios in uncontaminated reference samples.

Examples for demonstrating the consequence of converting between mass and radioactivity without considering the isotopic ratios can be found in U.S. EPA Environmental Radiation Data Reports. Report 78 for April–June 1994, gives Albany, NY, precipitation concentrations for ^{234}U, ^{235}U, and ^{238}U of 0.016 pCi/L (0.00059 Bq/L), 0.001 pCi/L (0.000037 Bq/L), and 0.002 pCi/L (0.000074 Bq/L), which totals 0.019 pCi/L (0.00068 Bq/L) (EPA, 1996). The use of 0.67 pCi/µg (0.025 Bq/µg) as a generic conversion factor results in a mass concentration of 0.028 µg/L. Alternately, the use of respective isotopic conversion factors of 6240 pCi/µg (231 Bq/µg), 2.16 pCi/µg (0.080 Bq/µg), and 0.336 pCi/µg (0.012 Bq/µg) yields mass concentrations of 0.0000026 µg/L, 0.00046 µg/L, and 0.0060 µg/L, which total 0.0065 µg/L, or a factor of 4 less than by the other method.

3 PRODUCTION AND USES

3.1 Production

There are more than 100 minerals known to contain uranium, but the bulk of uranium production comes from seven (autunite, carnotite, comminite, pitchblende, torbernite, tyuyamunite, and uraninite). Once mined, uranium ore is milled by grinding the ore into fine sand, the U is removed by acid leach or alkaline roast, and its crude oxide is formed through chemical separation and precipitation as U_30_8. Low extraction efficiencies (5–15%) in the early 1940s quickly increased during World War II as the understanding of uranium chemistry improved and can now approach 90%. Uranium can also be recovered from unconsolidated deposits or sufficiently rich mill tailings through the process of *in situ* leach mining that involves creating underground barrier curtains, injecting chemicals to dissolve the uranium, vacuum recovering the liquor, and treating it to remove uranium.

Most uranium oxide is directed toward the nuclear energy industry for which it is separated into enriched and depleted U fractions. The most effective methods are ultracentrifugation followed by gaseous diffusion, with laser separation being studied. The United States uses gaseous diffusion that involves converting uranium oxide to uranium hexafluoride (that sublimes near room temperature) and passing the gas through a long series of membrane filters. Diffusion rates are higher for ^{234}UF and $^{235}UF_6$ than $^{238}UF_6$, causing incremental separation of the mixture into enriched and depleted fractions that have different ratios of the three natural isotopes. Enriched U contains higher than natural proportions of ^{235}U and ^{234}U (i.e., greater than 0.72 and 0.0055%, respectively), making it fissionable and more radioactive, whereas the depleted U (DU) contains less of these isotopes and is suitable for more conventional industrial uses. The degree of enrichment is determined by the desired use, with 3% being suitable for commercial power reactors, and >95–97% used for warship propulsion systems and weapons. European producers achieve this separation to produce enriched uranium by ultracentrifugation. For this method, each fraction (enriched uranium or depleted uranium) can be stored as the hexafluoride or converted to oxide or metal. The oxide is often prepared for use as power reactor fuel by shaping and sintering the material to form cylindrical fuel pellets, which are stacked in rods and arrayed in bundles that are placed inside the reactor vessel. Uranium metal has industrial applications (e.g., counterweights), as well as military uses. Natural, enriched, and depleted U are chemically and biologically the same, but the shift in ^{234}U (the most radioactive isotope) makes enriched U the most and DU the least radioactive.

Uranium production (as U_3O_8) peaked in the United States at 2×10^{10} g in 1980 and then decreased to 9×10^9 g (2 million pounds) in 2003. Imports have remained relatively flat at $9–13 \times 10^9$ g/year. There has been a global shift by production location and a price increase as

French and Japanese programs expanded slightly and China entered the market. In 2003, the primary producers were Canada (1.23×10^{10} g), the former Soviet Union (1.03×10^{10} g), Australia (8.97×10^{9} g), and Central Africa (3.71×10^{9} g), with lesser amounts in other countries, for a world total of 4.17×10^{10} g. Worldwide demand for energy and uranium production increased significantly over the following 3 years, during which the market price increased fourfold to approximately US $100/kg. The consequent increase in mining and milling activities can be done with limited impact on environmental and public health, but acids associated with such operations can destabilize tailings and induce acidification and salinization in the surrounding environment (Carvalho *et al*., 2005).

3.2 Uses

Uranium and its different colored compounds have art, industrial, commercial, and militarily uses. Uranium glass has an apple green tint, whereas ammonium diruanate ceramic glaze is orange. High density makes it suitable for gyroscope wheels in guidance systems, counterbalances for helicopter rotor blades, and weights for airplane control surfaces. Catalysis makes it useful to the chemical industry, and its potential for multielectron reduction of CO_2 has been studied (Castro-Rodriguez *et al*., 2004). High electron density makes it useful as a gamma radiation shield for intense industrial radiography sources. High density and self-sharpening properties make it the most effective metal for armor and bunker-piercing weapons. UO_2 extends the life of high-intensity bulb filaments and is used for photographic toning. U stains and dyes are used in the leather, wood, and silk industries.

Low enriched uranium (~2–5% ^{235}U) can be used as the fuel for civilian nuclear power plants, whereas high enriched uranium (>90% ^{235}U) can be used to fuel nuclear-powered warships or nuclear weapons.

4 ENVIRONMENTAL LEVELS AND EXPOSURES

4.1 Environmental Levels and Human Exposure

Uranium is present in food, water, and air because it is a natural component of soil. Environmental concentrations are functions of its differential distribution in minerals and its redistribution by natural processes (dissolution, wind and water erosion, precipitation, volcanic action) and human activities (mining of any heavy metal, processing of uranium ore for power and military applications, processing of phosphate ore into phosphoric acid and fertilizer, burning coal, and processing oil shale). Human exposure is normally highest from food and water, in equal amounts, and lowest from ambient air. The Centers for Disease Control and Prevention established and maintains a U.S. national database for human exposure to environmental chemicals that is available on the Internet (CDC, 2005). The uranium data in Table 4 are based on ICP-MS analysis of urine samples (Caldwell *et al*., 2005; Ting *et al*., 1999) and are summarized in Section 8.1.

Computer programs are available to estimate the timeframe concentrations in and radiation doses to the various organs and tissues. Federal Guidance Report No. 11 (EPA, 1988) and the computerized version of Federal Guidance Report No. 13 (EPA, 2005) provide factors for estimating the radiation dose in Sievert (committed dose equivalent) received by the body and selected organs per Bq of inhaled or ingested uranium isotopes.

4.1.1 Food and Daily Intake

Most uranium in foods is thought to be from residual dirt, because most plants and animals do not bioconcentrate uranium. This is supported by the observation that uranium concentrations decrease at higher trophic levels.

Uranium intake from food has been estimated as 0.02–0.04 Bq/day (0.9–1.5 µg/day) on the basis of extensive food studies. The highest concentrations were found in scallops (690–1046 ng/g) (Bettinelli *et al*., 2004), beef kidney (70 ng/g), onion (69 ng/g), parsley (60 ng/g), table salt (40 ng/g), and beef (26 ng/g). The lowest were in fruit juices (0.04–0.12 ng/g), canned vegetables (0.09–0.18 ng/g), dairy products (0.08–0.31 ng/g), and poultry (0.14–0.42 ng/g) (EPA, 1985; NCRP 1984). Potatoes (2.7–2.9 ng/g [NCRP, 1984]; 15–18 ng/g [EPA, 1985]) constitute the highest dietary intake of U because of combined concentration and consumption (EPA, 1985). Uranium intake averaged 0.71 µg/day for Japanese males from 31 prefectures (Shiraishi *et al*., 1992).

Large differences between reported values have been associated with the extent to which food preparation (cleaning and removal of outer vegetable surfaces) is accomplished. These washing steps reduce the residual uranium-containing dirt, thus reducing the amount of uranium in the final food product. A decision to analyze the food either raw or prepared affects the reported results, with uranium concentrations being potentially higher in raw vegetables than in prepared food.

4.1.2 Water

Uranium intake from water and its associated radiation dose is typically in the same range as food,

TABLE 4 Uranium Concentration of Urine

	Geometric mean(95% conf. interval)	Selected percentiles (95% confidence interval)[a]				Sample size
		50th	75th	90th	95th	
CREATININE CORRECTED (µgU/g creatinine)						
Total, age 6 and older	.008 (.007–.010)	.007 (.006–.009)	.014 (.011–.018)	.026 (.020–.033)	.040 (.028–.054)	2689
Age group						
6–11 years	**.010** (.008–.011)	**.010** (.008–.012)	**.015** (.013–.019)	**.027** (.018–.032)	**.033**(.027–.048)	368
12–19 years	**.007** (.006–.008)	**.007** (.006–.008)	**.012** (.009–.016)	**.020** (.014–.026)	**.026** (.020–.042)	762
20 yr and older	**.008** (.007–.010)	**.007**(.006–.009)	**.014** (.011–.019)	**.027** (.020–.039)	**.043** (.030–.063)	1559
Gender						
Males	**.007** (.006–.008)	**.007** (.006–.008)	**.012** (.010–.015)	**.022** (.018–.028)	**.033** (.025–.047)	1334
Females	**.009** (.008–.011)	**.009** (.007–.011)	**.016** (.012–.021)	**.029** (.021–.042)	**.045** (.031–.067)	1355
Race/ethnicity						
Mexican Americans	**.012** (.010–.016)	**.012** (.009–.016)	**.021** (.015–.028)	**.033** (.024–.053)	**.049** (.033–.077)	682
Non-Hispanic blacks	**.005** (.005–.006)	**.005** (.005–.006)	**.008** (.007–.010)	**.013** (.011–.014)	**.017** (.014–.029)	667
Non-Hispanic whites	**.008** (.007–.009)	**.007** (.006–.009)	**.013** (.011–.016)	**.025** (.018–.032)	**.034** (.025–.051)	1132
UNCORRECTED (µgU/L urine)						
Total, age 6 and older	.009 (.007–.010)	.008 (.006–.009)	.014 (.011–.016)	.029 (.022–.037)	.046 (.034–.062)	2690
Age group						
6–11 years	**.008** (.007–.010)	**.008** (.006–.010)	**.014** (.010–.020)	**.025** (.020–.036)	**.037** (.025–.049)	368
12–19 years	**.010** (.008–.012)	**.009** (.008–.012)	**.017** (.012–.023)	**.030** (.021–.042)	**.041** (.027–.088)	762
20 yr and older	**.009** (.007–.010)	**.007** (.006–.009)	**.014** (.011–.016)	**.030** (.022–.039)	**.046** (.034–.059)	1560
Gender						
Males	**.009** (.008–.011)	**.008** (.007–.010)	**.014**(.012–.019)	**.033** (.023–.043)	**.046** (.035–.065)	1335
Females	**.008** (.007–.010)	**.008** (.006–.009)	**.013** (.011–.016)	**.027** (.018–.037)	**.040** (.029–.062)	1355
Race/ethnicity						
Mexican Americans	**.013** (.010–.016)	**.011** (.009–.015)	**.022** (.016–.027)	**.039** (.031–.054)	**.054** (.045–.067)	683
Non-Hispanic blacks	**.008** (.007–.009)	**.007**(.007–.009)	**.012** (.010–.013)	**.020** (.017–.027)	**.030**(.023–.037)	667
Non-Hispanic whites	**.008** (.007–.009)	**.006** (.006–.008)	**.012** (.010–.014)	**.026** (.018–.034)	**.036**(.028–.049)	1132

[a]Data from 2001–2002 survey.
Adapted from CDC (2005).

but this is highly dependent on the source. Uranium concentrations tend to be lower in surface than in ground water, with surface water concentrations rarely exceeding the EPA limit of 30 µg/L and groundwater being potentially higher and more variable over time (ATSDR, 1999). A 90,000-sample survey found these to respectively average 0.04 and 0.12 Bq/L (Drury, 1981). Ohanian (1989) reported a population-weighted average of 0.01–0.07 Bq/L, and ATSDR (1999) estimated an average intake of 0.02–0.04 Bq/day. Where indicated, recommended drinking water treatment methods include ion exchange, lime softening, reverse osmosis, and coagulation/filtration (EPA, 2005b), but these may not be efficient for uranium.

Uranium distributes to surface and ground water primarily through dissolution and from the natural erosion of rock and soil. Factors that control the mobility to water are the concentration of uranium in soil, oxidation-reduction potential, pH, characteristics of complexing agents, and nature of sorbing materials. Uranium is likely to be in solution as a carbonate complex in highly alkaline and oxygenated water (Herczeg *et al.*, 1988); however, in acidic waters (pH < 6) containing low concentrations of inorganic ions and

high concentrations of dissolved organic matter, the uranium is in solution as the soluble organic complex (Brunskill and Wilkinson, 1987).

Typical sources of elevated uranium water concentrations include well water, spring water, and effluent or leachate from various types of mines and mills. Well water tends to contain higher concentrations than surface water in the same area because of the increased surface area of and contact time with the rock and soil particles through which aquifer water flows. This difference may not be recognized in areas where regulators require distributors but not individual well owners to analyze drinking water. The population-weighted average for U.S. surface water was reported as 0.8 µg/L, with a range of 0.08–7 µg/L (Lowry *et al.*, 1987). This can be compared with higher levels reported for a European bottled water (~1000 pCi/L [~37 Bq/L]) and for Finland (up to 150 Bq/L). An example of an area where differences occur between surface and groundwater is the state of South Carolina. Most drinking water in that state derives from surface sources for which levels are below the EPA MCL, but sampling of wells in the city of Simpsonville revealed such elevated levels that a health study of residents was conducted (ATSDR, 2002). Several months after consumption from these wells ended, urine from 105 residents was found to be elevated (avg, 0.481 µgU/g creatinine; range, ND–2.659 µgU/g creatinine) and 85% exceeded the 95th percentile concentration of a national reference population. Six months later, the average concentration had decreased, but 87% remained above the national 95th percentile, indicating that retention of uranium from long-term exposure is detectable in urine long after exposure ends (Orloff *et al.*, 2004). Differences between potable surface and ground water can be magnified when the well water derives from aquifers flowing through uranium-rich deposits. Well water concentrations in some Canadian provinces were so high as to prompt Health Canada to develop public health recommendations that individual provinces can adopt. Mining areas throughout the world are sources of elevated uranium that could lead to high drinking water concentrations. Examples include the Uravan site in Canada (1500–16,000 pCi/L [~56–590 Bq/L]) and the tailings pond of the United Nuclear site in the state of New Mexico (as high as 140 Bq/L). A break in the United Nuclear tailings pond dam in 1979 sent 352 million liters of tailings liquid into the Rio Puerco (EPA, 1988).

4.1.3 Soil and Rock

Uranium is present in most rocks and soil as a mineral with an average mass fraction of approximately 2×10^{-6} (0.05 Bq/g). UNSCEAR has determined that the median world concentration is 35 Bq ^{238}U/kg, with a population-weighted average of 33 Bq/kg (UNSCEAR, 2000). Concentrations are not uniformly distributed, tending to be lower in basic rocks (e.g., basalt) and higher in acidic rocks (e.g., salic) and granite. Areas suitable for mining uranium, silver, and phosphate can exceed a mass fraction of 0.1. Canada is the world's largest producer of uranium. As of 2005, its McArthur River mine was considered to have the largest high-grade ore deposit at 2×10^{11} g U_3O_8 with an average ore grade of 25% (Cameco, 2005).

4.1.4 Air

The source of atmospheric uranium is resuspended surface soil on the basis of air particles and soil from the same area having similar uranium concentrations. Uranium in ambient air is typically in the attogram/m^3 (nBq/m^3) range, with ^{234}U/^{238}U activity ratios ranging from 1 to 7, differing significantly from the equal proportions found in crystal rock (EPA, 1999).

Levels measured in 37 U.S. cities for 1993 gave results ranging from 13–265 aCi/m^3 (4×10^{-7}–1×10^{-5} Bq/m^3; 1.7×10^{-11}–2.9×10^{-10} g/m^3) for Honolulu, Hawaii, and Las Vegas, Nevada, respectively. This last value was partially attributed to enrichment in ^{234}U based on a ^{234}U/^{238}U ratio of 1.64. This survey was repeated in 52 U.S. cities for 2003, and values ranged from 7.6–202 aCi/m^3 (2.8×10^{-7}–7.5×10^{-6} Bq/m^3; 1.0×10^{-11}–2.9×10^{-10} g/m^3) for Barnwell, South Carolina, and El Paso, Texas, respectively, with an average of 42.5 aCi/m^3 (1.6×10^{-6} Bq/m^3; 5.6×10^{-11} g/m^3) (EPA, 2004). The ^{234}U/^{238}U ratio was lower for Las Vegas than in the earlier survey. Care should be taken in assessing the presence of either depleted or enriched uranium in the sample on the basis of isotopic uranium ratios because of their variability in nature. Examples of areas expected to be unaffected but having ratios different from unity include Fairbanks, Alaska (ratio, 1.63), Lansing, Michigan (ratio, 2.02), Hartford, Connecticut (ratio, 0.79), and Albany, New York (ratio, 0.73). These mass values were calculated on the basis of reported concentrations for the three natural isotopes and their half-lives.

Two estimates of annual intake were 0.3 pCi/y (0.01 Bq/y, ~0.4 µg/y) (UNSCEAR, 1988) and 3 pCi/y (0.1 Bq/y, ~4 µg/y) (Cothern, 1987), showing that uranium intake varies with location. Levels incrementally increase downwind of industries (coal power plants, heavy metal mines or mills) or natural events (erupting volcanoes) that release uranium. For example, those near a Canadian refinery were found to range from 2–200 ng/m^3 (Tracy and Meyerhof, 1987).

Reports of uranium concentrations downwind of a nuclear detonation were not found, but even well-designed bombs may only be 20% efficient, leaving at least 80% of the original uranium (Alt *et al.*, 1989) to

enter the fireball and be environmentally distributed. This should be a small contributor to the overall radiation dose that is dominated by the fission products produced in such an event. On the basis of the preceding data, a 50-kg bomb might leave an amount of unfissioned uranium found in approximately 10^{14} m^3 of natural air or 10^4 m^3 of natural soil.

4.1.5 Other

Uranium is ubiquitous and is present in many items, including the paper used to make books. It has been estimated that the range of radiation dose rates to the eyes from reading books is $0.29–4.19 \times 10^{-4}$ μSv/h from the ^{238}U. That same paper contained other radionuclides such that the dose rate from ^{137}Cs was roughly half, from ^{232}Th was comparable, and from ^{40}K was approximately 50 times higher (Imtiaz *et al.*, 2005).

4.2 Working Environment

Occupational exposure to uranium primarily involves inhalation of mixtures of uranium compounds with solubilities in water ranging from <1 mgU/L (for oxides) to >300,000 mgU/L (for uranyl nitrate) (Harrington and Rhuele, 1959). Particle size is a key feature for initial lung retention. The most highly exposed workers have typically been engaged in extraction and processing operations of the uranium fuel cycle or weapons production (BEIR IV, 1988). Uranium concentrations in occupational settings are regulated to prevent overexposures. Other groups with relatively unregulated and potentially high exposure include those who produce or use phosphate fertilizers (Tadmor, 1986) or make glass or glazed pottery (Rossol, 1997). The regulation of such technologically enhanced naturally occurring radioactive material (TENORM) is increasing.

4.3 Remediation

Sites contaminated with uranium can be controlled and remediated, but this has proven costly. Because of the large volumes involved, regulators are considering allowing disposal in landfills as an option to radiological repositories (EPA, 2003). Most remediation has involved drying contaminated water, physically removing contaminated soil, or immobilization in place by surrounding the tailings with a clay, composite, or capillary barrier. The focus is on limiting uranium migration and radon emission by selecting the appropriate barrier, for which computer modeling has been used (Leoni *et al.*, 2004). An environmental impact statement was prepared to address potential options for dealing with 12 million tons of tailings piles along the Colorado River to eliminate the potential for flood erosion of these tailings into a significant drinking water supply. The least expensive estimate was for stabilizing the tailings in place (US $249M). Significantly higher cost estimates were reported for relocating the tailing to each of three distant sites by each of three modes (truck, rail, or pipeline). Costs ranged from a low of US $407M for a truck haul to the closest site to US $543M for shipment by pipeline to the most distant site (DOE, 2005a). A Record of Decision was signed to move the tailings by truck 30 miles away (DOE, 2005b).

Uranium can be removed from tailings and deposits by use of chemicals. One of these is Fenton's reagent, which is a mixture of iron and hydrogen peroxide. When the liquid solution is blended or injected into soil, it produces hydroxyl radicals that nonselectively oxidixe metals and produce solubilized metal radicals that are extracted with the liquid through suction or pumping production wells. This method has been demonstrated to be practical for uranium and other environmental contaminants (e.g., pentachlorophenol and polychlorinated biphenyls) (Lin and Luong, 2004). Hydrogen peroxide was found to remove 60–80% of depleted uranium from soils at U.S. Army sites and was more effective than either citric acid (≤40%) or sodium bicarbonate (≤60%) (Choy *et al.*, 2005).

Another chemical extractant is D2EHPA (di[2- ethylhexyl] phosphoric acid), which has reportedly been used to commercially recover environmental metals (beryllium, cobalt, iron, uranium, zinc, and rare earth elements). The molecule is prepared in an organic phase (e.g., toluene) in which it deprotonates. When the organic solution mixes with an aqueous phase containing metals, those metals are chelated into the organic phase with a pH-dependent efficiency, and the organic phase is retrieved. The efficiency is enhanced through the addition of other extractants (dialkylphosphorus extractants plus neutral phosphorus extractants) (IAEA, 2001). Tri-*n*-butyl phosphate has been used to precipitate uranium from acid mine wastewater (Thomas and Macaskie, 1998).

Plants being studied for their potential to concentrate uranium and other heavy metals by phytoextraction and hydroponic rhizofiltration include Indian mustard (*Brassica juncea*), Russian thistle (*Salsola tragus*), and purple amaranth (*Amaranthus blitum*). Uptake rates have been enhanced 14-fold over controls by treating the soil with citric acid (Ebbs *et al.*, 2001; Frey *et al.*, 2004) or chelating agents, or by increasing stromatal transpiration rate (Gleba *et al.*, 1999).

5 TOXICOKINETICS

The absorption, distribution, and excretion kinetics of uranium and its compounds depend on the chemical form, and for inhaled particles, also their physical aerodynamic and thermodynamic properties.

5.1 Absorption

Absorption of uranium is low by all exposure routes (inhalation, oral, and dermal). Once uranium is absorbed, its distribution and elimination are considered to be independent of the route of exposure and a function of solubility. The solubilities of some common uranium compounds are given in Table 1. In addition, the solubility is high for uranium tetrachloride and uranyl acetate and low for ammonium diuranate. Although uranium tetrachloride is highly soluble, it is easily hydrolyzed to less-soluble uranyl chloride and oxidized to insoluble uranium dioxide, which reduces absorption of the uranium (ATSDR, 1999).

5.1.1 Inhalation

The deposition of inhalable uranium dust particles in the lungs depends on the particle size, and absorption increases with solubility in biological fluids. Particles larger than 10-µm atmospheric median aerodynamic diameter (AMAD) preferentially deposit in the upper respiratory tract and are likely to be transported promptly (with half-times of 10–100 minutes [ICRP, 1994]) out of the tracheobronchial region by mucociliary action and swallowed. Smaller particles and vapors travel more deeply into the lung and can reach the alveoli. The high density of uranium typically causes particles of its dust to have large AMAD values. This is supported by findings that uranium workers exposed to high levels of uranium dust had very low lung burdens, so only a small fraction penetrated into the alveolar region (West and Scott, 1966; 1969). That fraction was estimated to be 1–5% (Harris, 1961). The uranium dust produced when uranium metal penetrators were fired against hard targets contained a range of particle sizes that would enable it to distribute significantly through the pulmonary system (average, 2.5 µm AMAD; GSD, 6.1 µm; range, 0.2–40 µm). The size distribution decreased rapidly with time as larger particles settled out more quickly (DOD, 2004).

ICRP (1994) developed a deposition model for aerosols and vapors that applies to uranium. It includes three levels of particle solubility and a wide range of particle sizes, with selectable parameters (for gender, age, and level of physical exertion), five compartments (representing the portions of the respiratory tract), and clearance to blood.

Estimates of systemic absorption from inhaled uranium-containing dusts in occupational settings on the basis of urinary excretion of uranium range from 0.76% for mill workers (Wrenn *et al*., 1985) to 5% for crushermen (Fisher *et al*., 1983). Pharmacokinetic assessments indicate that lung absorption factors are 2–4% for 3-month-old children and 0.2–2% for adults, on the basis of compound absorbability (ICRP, 1996). The more soluble uranium compounds (acetate, fluoride, hexafluoride, nitrate, and tetrachloride) absorb into blood from the alveoli within days. Less soluble compounds (tetrafluoride, trioxide, and some dioxide and octaoxide) remain in the lung and associated lymph node glands for weeks. The insoluble compounds (other dioxide and octaoxide) can remain in the lungs for years. The dust after a metal penetrator impacted a hard target contained several oxides with moderate to low solubility in simulated lung fluid; up to 36% dissolved with <10 day half-time and >58% dissolved with >100 day half-time (DOD, 2004). The degree of insolubility for any sample of uranium dioxide or uranium octaoxide depends on the temperature and conditions under which it was formed, with high-fired, sintered samples being less soluble than those produced at lower temperatures and pressures.

Animal data support that uranium absorption is a function of particle size and solubility, but when aerosols of purified uranium compounds were tested, greater absorption was observed. Animal data on deposition and absorption in the lung indicate large species differences (Spoor and Hursh, 1973; Voegtlin and Hodge, 1949). Absorption of uranium hexafluoride was 18–40% in rats and 20–31% in guinea pigs (Leach *et al*., 1984), and of uranium trioxide was 23% in dogs (Morrow *et al*., 1972). These higher absorption values could be artificialities if ingestion also occurred. Aerosols produced for high-dose uranium studies have been observed to settle onto the fur of the animals, and ingestion occurred through grooming (Roberts, 1998).

5.1.2 Ingestion

Human gastrointestinal absorption of uranium has been shown to vary from <0.1–6%, depending on the solubility of the uranium compound. Absorption was reported as 0.5–5% in a group of four males ingesting 10.8 mg uranium in a soft drink (Hursh *et al*., 1969), <0.25–0.40% in a group of 12 volunteers given drinking water high in uranium (Wrenn *et al*., 1989), and 0.5–5% in another drinking water study (Harduin *et al*., 1994). Similar results were obtained in dietary balance studies (Leggett and Harrison, 1995; Spencer *et al*., 1990; Wrenn *et al*., 1989). ICRP (1995) recommends the use of 0.2% for insoluble compounds and 2% for soluble hexavalent compounds for modeling the kinetics of dietary uranium in humans unless specific additional information is known. Limited data on infants suggest

that age has little effect on absorption (Leggett and Harrison, 1995; Limson-Zamora *et al*., 1992), but animal studies are not supportive on this point.

Animals absorb comparable percentages of ingested uranium, with a central value of 1%. A 2-year feeding study in rats found factors of 0.05–0.5% for uranyl fluoride and 0.5–2% for uranyl nitrate, with a rate that was independent of uranium concentration (Wrenn *et al*., 1985). The rates in rats were increased a factor of 2 by fasting (Sullivan *et al.*, 1986), 3.4 by iron deficiency (Sullivan and Ruemmler, 1988), and 3.6 when neonates were compared with adults (Sullivan, 1980b). Fasting showed that these effects occur in mice and baboons (Bhattacharyya *et al*., 1989). Also, the fractional absorption for uranyl nitrate was found to be up to a factor of 3 higher in 2-day-old rats (1–7%) than for adults (Sullivan and Gorham, 1982), but comparable to that for other animals (0.038–0.078 for rats; Wrenn *et al*., 1995).

5.1.3 Dermal Exposure

Dermal absorption has not been quantified in humans, but toxicity experiments in animals (mice, rats, rabbits, and guinea pigs) indicate that water-soluble uranium compounds are the most easily absorbed through the skin and eye (Orcutt, 1949). Uranyl nitrate applied to the skin penetrated the stratum corneum within 15 minutes, accumulated initially in the skin, and then distributed over the next 48 hours producing renal effects. No penetration was observed for the more insoluble dioxide, acetate, or ammonium diuranate (De Rey *et al*., 1983). Once systemic, the uranium can distribute in the same manner as with oral exposure.

5.2 Metabolism and Distribution

Uranium in body fluids generally exists as a uranyl ion UO_2^{2+} complex. For tetravalent uranium, this occurs by oxidation to the hexavalent form, and hexavalent uranium is converted to the uranyl ion. Metallic uranium (e.g., as embedded fragments from military wounds) dissolves slowly and is similarly converted. The uranyl ion then generally complexes with citrate or bicarbonate anions or with plasma proteins (Cooper *et al*., 1982; Dounce and Flagg, 1949; Stevens *et al*., 1980).

For all routes of exposure, uranium distributes through the blood throughout the body and is rapidly taken up by the tissues or excreted in the urine. Fisenne and Perry (1985) found whole blood and red cells of New York City residents averaged 0.14 mgU/kg compared with values ranging from <0.04–86 mgU/kg globally. When uranium was intravenously injected (as uranyl nitrate), 25% remained in the blood after 5 minutes, 5% after 5 hours, 1% after 20 hours, and <0.5% after 100 hours (Bassett *et al*., 1948; Bernard and Struxness, 1957). There is an initial preferential concentration in liver and kidney, but this shifts quickly to include bone. The concentration in tracheobronchial lymph nodes (for insoluble forms) and bone rise over time because of limited turnover.

Bone eventually becomes the largest long-term repository of uranium for all exposure scenarios other than heavy inhalation exposure in the workplace. Occupational inhalation exposure adds to the long-term lung burden, increases the lung/bone ratio, and can cause the lung to be the largest repository. ICRP (1996) considers that the typical human body burden of uranium from ambient exposure is 90 µg, with 66% estimated to be in the skeleton, 16% in the liver, 8% in the kidneys, and 10% in other tissues (ICRP, 1996). Fisenne (1993) ranked the organs as bone (57%) > muscle > fat > blood > lungs > liver > kidney (0.36%). This is the same order as found in a study of nonoccupational Asian adults in China, India, Philippines, and the Republic of Korea. The content of various organs (5.23 µg in skeleton, 1.09 µg in lungs, 0.20 µg in liver, and 0.19 µg in kidney) was reportedly 40 times lower for the kidney and 10 times lower for the skeleton than would be predicted by the ICRP Reference Man model. The skeletal burden for the Asians was 4 times lower than for Chinese and comparable to Indians, whereas the kidney burden was comparable across the groups (Iyergar *et al*., 2004). For a long-term, highly exposed worker whose primary route of exposure was inhalation and whose highest urinalysis value was 30 µg/L, a full body autopsy at age 83 found the highest uranium concentrations in tracheobronchial lymph nodes and the rank order of organ content was lung > skeleton > liver > kidney (Russell and Kathren, 2004). In a comprehensive study of tissues from two long-time residents of an area with high well water concentrations who had no known occupational exposure, the skeleton was the primary depot for uranium (Kathren, 1997). It is not known whether maternal bone stores of uranium (like those of calcium and lead) are mobilized during pregnancy and lactation.

Animal studies have shown similar relative distributions. Inhalation of the most soluble compounds showed no long-term accumulation unless the dose was very high (>0.25 mgU/m^3). When insoluble uranium dioxide was inhaled for up to 5 years, accumulation did occur (in the tracheobronchial lymph nodes, lungs, bones, and kidneys of rats, dogs, and monkeys), but most was cleared over a period of a few years after exposure ended (Leach *et al*., 1973). For ingested uranium in the rat, doses >20 ppm (a mass fraction of 2×10^{-5}) caused a rapid increase in organ content, indicating that this may be a threshold for renal effects (Arruda-Neto *et al*., 2004). The distribution of uranium from metal implanted in

muscle was investigated in the rat and found to be highest in kidney and bone, at times ranging from 12 days to 18 months (Pellmar *et al*., 1999).

ICRP (1979) developed a uranium compartmental model for uranium distribution and excretion, which Fisher *et al*. (1991) later adjusted on the basis of exposure events. ICRP (1995) subsequently developed the current biokinetic model that applies to uranium after its uptake to blood, regardless of exposure route. It is patterned after composite human and animal data and recognition that uranyl and calcium ions have similar skeletal kinetics. Its features include five major compartments (for blood, kidney, liver, skeleton, and soft tissues) with multiple subcompartments and bidirectional transfer rates for three solubility types.

5.3 Elimination and Excretion

Most inhaled uranium returns to the throat through the mucociliary elevator and joins ingested uranium, and more than 95% of this is excreted unabsorbed in the feces. Fecal excretion accounted for 99% of the uranium excreted by uranium ore crushermen who inhaled ore dust (Fisher *et al.*, 1983).

Uranium remaining in the lung is eliminated on the basis of solubility. A worker who inhaled uranium tetrafluoride for 5 minutes excreted 0.2% of the uranium on day 1, and this gradually increased for 60 days and then returned to normal after approximately 1000 days (Zhao and Zhao, 1990). The long-term half-time for human lung on the basis of uranium dioxide exposure of German workers was estimated to be 109 days (Schieferdecker *et al*., 1985). In studies of animals involving more soluble compounds, 60% of the retained uranium as nitrate (Ballou *et al*., 1986), hexafluoride (Leach *et al*., 1984), and trioxide (Morrow *et al*., 1982) was excreted in urine within 1 day for the rat, dog, and guinea pig.

Once uranium reaches the bloodstream, by whatever route of exposure, there is an initial rapid excretion in which renal and fecal excretion, respectively. account for >98% and <2% of the total (Spencer *et al*., 1990). Urinary elimination is facilitated by the ultrafiltrable nature of the uranyl-bicarbonate and uranyl-citrate complexes, which are filtered in the glomerulus and tubules. Tetravalent uranium attached to plasma protein is less filterable and is eliminated over a longer period of time. Once the uranyl ion is split from its complex in the kidney, the uranium readily transfers to urine if the pH is high. At low pH, the excretion can be delayed.

The pattern of excretion tends to follow a two-phase model for which the half-times in kidneys have been reported as 1–6 days for 99% of the uranium and 1500 days for the remainder (ICRP, 1979). More recent estimates of the biological half-times for the compartments are 2 and 50–60 days (Diamond *et al*., 1989), 2 and 13 days (Bentley *et al*., 1985), or 3 and 103 days (Wrenn *et al*., 1986). The initial half-time for retention in bone has been estimated to be 11 days. The amount excreted is expected to be a function of intake route, because ingested uranium is excreted mainly unabsorbed in feces, whereas the absorbed amount (e.g., from inhalation or dermal absorption) is excreted primarily through the kidneys. This was observed in an Italian population compared with one in Germany and indicated the importance of having appropriate local reference levels (Bagatti *et al*., 2002). Because the rate of uranium excretion in urine can be used to approximate the long-term average rate of systemic uptake (discounting the small retained fraction), the values in Table 4 can be used as comparators where suitable local reference levels are unavailable.

Uranium has been shown to transfer to human breast milk, with a transfer factor of 21.3 (ratio of concentrations in food [μg/kg] to milk [μg/L]), although significant individual differences were observed (Wappelhorst *et al*., 2002). This indicated to the authors that 5% of the oral intake could be excreted in breast milk. A much smaller value would be anticipated considering that <0.1–6% of oral intake becomes systemic (Section 5.1.2), and most of that is excreted in urine. In animals, uranium has been found to cross the placenta after parenteral administration.

6 MECHANISMS OF ACTION

The mechanistic theories of uranium toxicity have been probed primarily by traditional means. They have looked at temporal changes in urinary levels of several analytes (e.g., protein, β_2-microglobulin, amino acids, alkaline phosphatase, ALT, and glucose) as a function of uranium intake. But, these are nonspecific indicators of uranium damage that can be caused by other agents. The database has been supplemented more recently with genetic tools primarily involving *in vitro* exposures of cells as well as *in vivo* studies after acute injections or more prolonged exposures through drinking water or inhalation. The *in vitro* immersion and *in vivo* injection studies can deliver higher concentrations of uranium to cells than is possible with traditional methods or relevant to environmental exposures (Muller *et al*., 2006). This facilitates more robust expression of subtle effects or observation of effects that may not occur otherwise after inhalation, oral, or dermal exposure. These studies have examined mechanisms of cellular uptake of uranium and toxicity to a number

of subcellular systems. The effects of uranium on the kidney have received particular attention, because this organ system is the major target for toxicity.

This section identifies the rationale for focusing on the chemistry of uranium rather than on the radiation its atoms emit. Then, it addresses the mechanisms of action by route of exposure (inhalation, oral, and dermal) and by target (kidney, liver, lungs, and immune system).

6.1 Chemical versus Radiological

Uranium is both a chemical and a radioactive material whose adverse health effects involve potential contributions from both chemical and radiological mechanisms. However, a critical review of the significant uranium studies led the Agency for Toxic Substances and Disease Registry to conclude that natural uranium and depleted uranium are essentially chemical toxicants when exposure occurs by the inhalation, ingestion, and dermal routes (ATSDR, 1999). The contribution from radiation was evaluated in a study in which albino rats were exposed by inhalation to high concentrations of UO_2, and after a week, one group received 600 Gy of neutron radiation equivalent to approximately 300 times the alpha radiation dose from the UO_2. No significant difference in pulmonary cellular reactions (number and size of macrophages, type I, and type II cells) were observed between the two groups, indicating that the effects were from uranium metallotoxicity and not its radiation (Morris *et al*., 1989). This is supported by Miller *et al*. (2002), who calculated that the chemical generation of hydroxyl radicals relative to uranium oxidative damage of DNA is 6 orders of magnitude greater than for the radiolytic generation of these radicals. Although radiation from uranium can damage cells and theoretically cause cancer, the results of inhalation, oral, and dermal studies in both humans and animals have not associated uranium exposure with cancer. Highly enriched uranium is ~50 times more radioactive than natural uranium and up to 100 times more radioactive than depleted uranium, and exposure to this form is more likely to include a radiological component of damage.

6.2 Route of Exposure

Inhalation of uranium will result in a portion of the material (even if it is soluble) being retained for years. High exposure to particles that reach the deep lung irritates the alveolar epithelium, and over time this can cause hyperplasia, hypertrophy, and metaplasia, leading to pulmonary fibrosis and emphysema. However, it is possible that the damage is from other irritants associated with the exposure (silica dust, diesel particles, or radon), because many inhalation studies are negative for such effects.

Ingested uranium (and even inhaled uranium of all forms) enters the bloodstream, where it binds with citrate, bicarbonate, or plasma protein, and potential mechanisms of damage have been offered. The primary toxic action is mediated by accumulation of uranium in the renal tubular epithelium. If damage occurs, it can involve a loss of cell processes (growth extensions in the epithelial cell membrane structure), a reduction in the density of endothelial fenestrae (the flat endothelial cells lining the diffusion membrane portion of the capillary walls), and a reduction in the surface area of mesangial cells (the extension of connective tissue into the glomerulus). This reduces reabsorption efficiency in the renal tubule, the glomerular filtration rate, and at sufficiently high doses, degenerates the glomerular and tubular walls through necrosis and atrophy. Heavy metal ions, such as uranyl ions, are also effective in delaying or blocking the cell division process, thereby magnifying the effects of cell necrosis.

Dermal exposure to sufficiently soluble forms of uranium (e.g., nitrate and acetate versus oxides) can result in systemic uptake. The toxicokinetics should be the same as for ingested uranium.

6.3 Hepatic Mechanisms

Souidi *et al*. (2005) evaluated the potential for uranium to enhance kidney, liver, lung, and brain toxicity associated with drug therapy because of adverse impact on liver enzymes involved in drug metabolism. The concern was that uranium might have an adverse impact on liver CYP enzymes (involved in drug metabolism) and PXR and CAR receptors (that mediate the effects of drugs on the regulation of CYP genes). They exposed rats to uranium in drinking water at a dose of 1 mgU/rat/day for 9 months and looked for both overt toxicity and increases in mRNA levels of several cytochrome P450 CYP enzymes and their nuclear receptors PXR, RXR, and CAR in the various tissues. Increases relative to controls for CYP1A1 mRNA were not found; for CYP3A1 mRNA were 900% in kidney, 300% in liver, and 200% in brain; for CYP3A2 mRNA were not observed in kidney but 300% in lung and 200% in liver; and for CYP2B1 mRNA were 300% in kidneys. PXR mRNA increased 200% in kidneys, CAR mRNA doubled in lung, and RXR mRNA was not altered. The authors found that this level of uranium exposure was not nephrotoxic. They concluded that this level increased the expression of PXR and CAR nuclear receptors involved in regulating CYP2B and CYP3A drug-metabolizing enzymes, thus potentially affecting drug therapy regimens.

6.4 Immunological Mechanisms

Wan *et al*. (2006) exposed mouse peritoneal macrophages and spleen CD4+ T cells to uranium *in vitro* over a wide concentration range, studied a number of markers of apoptosis/necrosis, and conducted lymphoproliferation assay and microarray RTPCR studies of a number of genes. They found dose-related apoptosis/necrosis with thresholds between 50 and 100 µmol/L for macrophages and 100–500 µmol/L for T cells. Macrophage accessory cell function was also attenuated at 200 µmol/L after relatively short time periods. Microarray data from both lines demonstrated that 43 total genes were altered, two of which were observed for both macrophages and CD4+ cells, with one (Mdk) changing in the same direction. For macrophages, expression of 10/29 genes changed by a factor of 2, with most ($n=6$) involving signal transduction (as demonstrated by Prat *et al*. [2005]), and 1 each involving protein binding, development, neurotropism, and apoptosis. For CD4+ T cells, the expression for 2 of 14 genes doubled (involving chemokines and neurotropism), that for chemokines and cytokines varied inconsistently, and moderate up-regulated changes were seen for three interleukins (indicating a potential impact on T-cell differentiation and on immune response).

6.5 Pulmonary Mechanisms

Monleau *et al*. (2006) studied the genotoxic and inflammatory effects of nose-only inhaled depleted uranium (DU) particles as dioxide (UO_2) or peroxide (UO_4) on lung bronchoalveolar lavage (BAL) cells (primarily alveolar macrophages) of rats. Exposure concentrations were selected to be nonnephrotoxic and comparable to high human exposures, but renal failure occurred in one group of rats that was inconsistent with reports for highly exposed humans (McDiarmid *et al*., 2004). Results of these studies demonstrated DNA strand breaks, increased cytokine expression, and production of hydroperoxides in lung tissues. These data suggest oxidative stress as a major component of uranium lung toxicity. Repeated exposures seemed to demonstrate potential effects in both the BAL cells and kidney cells, rather than a protective effect reported by Tolson *et al*. (2005). Results of comet assay analysis at neutral pH demonstrated some double-strand DNA breaks in BAL cells, and the authors suggested that radiation might contribute to the genotoxicity of DU particles. The potential for radiation to be a significant contributor to uranium toxicity was discounted by both Morris *et al*. (1989) and Miller *et al*. (2002), as addressed previously.

6.6 Renal Mechanisms

One theory for the damage is that the bicarbonate complex releases uranium on reaching the kidney (possibly because of a pH shift), and the uranium combines with available phosphate and protein. Damage assessments show that the most sensitive damage site is the proximal tubule followed by the glomerulus. The epithelial damage is normally temporary, indicating that uranium binds weakly and releases within days. The healing process initially produces cells that are histologically different from the original, but this resolves with time, and the cells return to normal. Another theory involves uranium inhibiting the ATPase activity and sodium transport mechanisms in mitochondria, potentially wiping out the brush border, thus disrupting the epithelial cell's ability to function and repair damage (Brady *et al*., 1989).

Muller *et al*. (2005) conducted an *in vitro* study into the roles of sodium-dependent phosphate cotransporters on the uptake and cytotoxicity of uranium in kidney tubule cells and the impact of phosphate, calcium, and carbonate on toxicity. The cells included proximal tubule LLC-PK_1 cells that express and distal tubule MDCK/NaPi-2 cells that were transfected to express a sodium-dependent phosphate cotransport system. The authors found that uranium uptake and cytotoxicity were dependent on the type-IIa subfamily of the sodium phosphate cotransporter system, that uranium cell concentrations below 500 µmol/L were not cytotoxic, and that toxicity could be enhanced (by treatment with phosphate) or reduced (by treatment with calcium or carbonate, which offsets the phosphate effect). More importantly, they proposed, with the help of computer-assisted speciation modeling, that it may be one of the uranium-phosphate complexes (probably $UO_2PO_4^-$) that produces renal toxicity rather than the widely accepted belief that uranium carbonate and citrate complexes are the most renally cytotoxic forms. Goldman *et al*. (2006) studied the *in vitro* effects of uranium on glucose transport in rat kidney brush border membrane vesicles and found that uranium exposure inhibited glucose transport in these vesicles without altering the equilibrium volume of the brush border membrane. This indicated that uranium slows the sodium-coupled glucose transport system without affecting the lipid matrix. The authors suggested that it may be due partially to a reduction in number of membrane transporters. The magnitude of the inhibition was pH dependent, with greater effects observed at pH 7.0 and lesser effects observed at pH 7.6.

Prat *et al*. (2005) studied human embryonic renal (HEK293) cells exposed to uranium (at concentrations producing 20–50% cell lethality [CI20–CI50]) *in vitro* by use of toxicogenomic and 2-D gel electrophoretic proteomic techniques to study the response of this cell line. Depending on the uranium level, between 45 and 208 genes exhibited a greater than twofold deregulation either up or down. The largest group of these (40%)

has no recognized function. Of the remainder, 24% are involved with uranium stress response (signal transduction/trafficking or calcium handling), whereas fewer are associated with cell development, differentiation, or proliferation, followed by cell defense, structure, or metabolism. Currently, there are no biomarkers of effect specific to uranium, but new candidates could include either a set of 18 deregulated genes that were common among the exposure groups or a set that were down-regulated at all doses with a corresponding loss of proteins (e.g., HSP90, which prevents irreversible protein aggregation in stressed microtubules). The temporal profiles of expression for selected genes (3 up-regulated and 3 down-regulated) as a function of uranium concentration seemed to suggest a concentration threshold. This occurred between toxic adaptation (at 0.25 nmol/L U, producing 30% lethality and a complex sinusoidal gene response that returned to normal at 24 hours) and overt toxicity (at 0.5 nmol/L U, causing 50% lethality and similar but diverging sinusoidal response). The profile shapes indicate that cytotoxic doses of uranium elicit a complex response from renal cells.

Tolson *et al*. (2005) studied alterations in the heat shock protein (Hsp) response as an adaptive mechanism for attenuating the renal toxicity of uranium. They pretreated rats with a dose of 5 mgU/kg for 10 days before a challenge dose of 10 mgU/kg and observed decreased necrosis of proximal tubule cells and azotemia in some animals (called responders) but not in others (termed nonresponders). Kidney homogenates from these animals were found to contain increased amounts of Hsp25, Hsp32, and Hsp70i but not the constitutively expressed Hsc70. Correlative studies of the proliferating cell nuclear antigen demonstrated that the cells expressing increased amounts of the Hsps were regenerating, and that only those animals expressing such regeneration were protected against subsequent exposure. Complimentary *in vitro* studies that used RK3E rat and LLC-PK1 porcine epithelial cells showed that uranium pretreatment was protective, but Hsps were not induced by exposure to uranium (as they are for other metals), and that Hsps induced by thermal stress are cytoprotective against subsequent uranium exposure. The authors concluded that the Hsps are not responsible for protection against uranium toxicity *in vivo* but are involved in renal tubular epithelial regeneration that subsequently protects the kidney from uranium. Whether or not the protection offered during the epithelial repair period remains after repair was not evaluated. The expression of Hsps in liver was also evaluated, but uranium did not alter their levels, indicating they may have no role in hepatic protection. However, liver is perhaps an order of magnitude less sensitive than kidney (ATSDR, 1999), and because renal protection seems to require passing an injury threshold, the liver doses may have been insufficient to have caused the damage needed to elicited a protective response.

Taulan *et al*. (2004) studied renal toxicogenomic responses (serial analysis of gene expression) of mice exposed to uranium in drinking water for 4 months at high dose levels (80 and 160 mgU/L). They observed alterations in the expression levels of 200 genes, most of which were up-regulated. Many of these genes were related to oxidative stress responses, cellular metabolism, ribosomes, signal transduction, and transport systems. The authors found that uranium caused the dose-dependent production of H_2O_2, which can induce oxidative stress, and suggested that oxidative stress plays an important role in the observed genomic responses.

6.7 Skeletal Mechanism

The presence of uranium can interfere with the initial deposition of calcium in the bone matrix or with bone remodeling (Guglielmotti *et al*., 1985). Kurttio *et al*. (2005) conducted a drinking water study among individuals who consumed elevated concentrations of uranium in well water. A slight statistical association was found between uranium exposure and CTx (an indicator of bone resorption, serum type I collagen carboxyl-terminal telopeptide) for men ($P = 0.05$) but not for women.

6.8 Summary on Mechanisms

Overall, the results of the preceding studies indicate that the adverse health effects associated with uranium exposure are from its metallotoxicity and not from its radiation. Uranium exposures produce cellular toxicity primarily on renal proximal tubule epithelial cells and on a number of other cell types through oxidative stress. The most apparent and noninvasively measurable result is an increase in the urinary concentration of several substances that are a direct result or indirect consequence of tissue damage. There are effects on a number of molecular systems involved in cell signaling pathways, drug metabolism, gene expression, and apoptosis/necrosis. Some of these effects seem to be of a primary nature with others more related to cell adaptation or regeneration. The exact sequence of events leading up to target cell death is the subject of ongoing research in a number of laboratories.

7 EFFECTS AND DOSE-RESPONSE RELATIONSHIPS

The health effects from exposure to uranium by normal routes (inhalation, oral, and dermal) is primarily to the kidney. Less severe effects occur to the liver, lung, and nervous system, and no reproductive or developmental effects are expected to occur except at extreme

or lethal doses. Uranium is also less likely to cause adverse cardiovascular, gastrointestinal, hematological, musculoskeletal, or ocular effects. Cancer has not been associated with uranium exposure. When exposure occurs in a manner than can produce extreme local concentrations, such as in studies where metal pieces were implanted to simulate fragment wounds or in *in vitro* assessments, additional effects, including cancer, may be possible (Miller *et al*., 2000; 2005). Such potential effects have not been apparent in soldiers with embedded uranium fragments from wounds received a decade earlier (McDiarmid *et al*., 2004).

7.1 Organ and Tissue Effects

7.1.1 Cancer

Human and animal studies have not drawn any association between uranium exposure by normal routes and cancer of any type. The International Agency for Research on Cancer (IARC), the U.S. Department of Health and Human Services, and the National Toxicology Program have not classified uranium as to its carcinogenicity (ATSDR, 1999). IARC (2006), for example, assigned it to Group 3, which means it is not classifiable as to carcinogenicity to humans. IARC assessed implants of depleted uranium and concluded that there is inadequate evidence for its carcinogenicity (IARC, 1999).

Lung cancer in miners of various types (uranium, silver, coal) has been attributed to other known or suspected carcinogens (radon gas, diesel exhaust particles, or freshly cracked silica dust). The National Academy of Sciences has determined that the most likely cancer to occur after oral exposure to uranium is osteosarcoma, but the report also concluded that exposure to natural uranium may not have a measurable effect (BEIR IV, 1988). This potential has been studied *in vitro*, and it was found that soluble and insoluble DU compounds caused human osteoblast cells to change to the tumorigenic phenotype (Miller *et al*., 2000). This information is currently not transportable to an assessment of cancer in organs of individuals. In an intramuscular implantation study designed to mimic battlefield shrapnel wounds, pellets of DU and tantalum as a control (2×1 mm cylinders, 2.5 ± 2.5 mm squares, and 5×5 mm squares) were implanted in the hindquarters of rats. Tumor rates were determined at the end of the lifespan. Implantation site sarcomas were significantly increased in the large DU square group, slightly increased in the large Ta and small DU groups, and unaffected by the DU pellets, indicating the shape of DU fragments is a factor in shape-related carcinogenesis (Hahn *et al*., 2002). Miller *et al*. (2005) implanted leukemia-prone mice with DU pellets, injected them with murine hematopoietic cells, and later found an elevated rate of myeloid leukemia compared with unexposed mice as long as a sufficient number of pellets had been implanted.

7.1.2 Dermal Effects

Skin contact with uranium or its compounds has not been found to cause an adverse effect in humans. This may be the result of good tolerance or that humans have not been exposed to high enough levels long enough for an effect to be observed.

High-dose studies in the rabbit and rat that used highly soluble uranium compounds placed in contact with shaved skin for many days have demonstrated that uranium can cause cells to swell and vacuoles to form, as if the cell is trying to compartmentalize and isolate itself from the excess uranium. Manifestations include skin irritation and inflammation, which can lead to damage of hair follicles and sebaceous glands. Continued high-level exposure can produce ulceration and necrosis, so damage can be permanent. The potential exists for an immune system response leading to future contact dermatitis, such as occurs with aluminum.

Uranyl nitrate applied to the shaved backs of rabbits eventually caused moderate erythema (at 1.4 mgU/kg), ulceration (at 4.2 mgU/kg), or superficial necrosis (at 56 mgU/kg). Such effects were not observed at much higher doses for uranium octaoxide (147 mgU/kg), uranium dioxide (458 mgU/kg), uranyl fluoride (618 mgU/kg), uranium trioxide (666 mgU/kg), or uranyl acetate (3,929 mgU/kg) (De Rey *et al*., 1983; Orcutt, 1949).

7.1.3 Developmental Effects

Humans have not shown any developmental effects from uranium exposure at levels thus far received. Animal results are negative except for high oral doses (Domingo *et al*., 1989; Paternain *et al*., 1989). Then there is a decrease in viability index (number of pups viable at day 21/number born), lactation index (number of pups viable at 21 days/number retained at day 4), and growth. Those that survive longer can have skeletal malformations (bipartite sternebrae, reduced or delayed ossification at >14 mgU/kg/day), cleft palate, and renal underdevelopment. This shows the propensity of uranium to affect the kidney and deposit in bone.

7.1.4 Hepatic Effects

The liver is probably an order of magnitude less sensitive to damage than is the kidney, and many animal studies are negative, even at high doses. When damage does occur, it involves the disruption of cell function and permeability, but the etiology is not known.

Histological findings include vesiculation (the formation of vesicles, or liquid filled sacs, causing swelling), nuclear pyknosis (a reduction in the size of the nucleus), and excessive vacuolation (void space). The liver disturbances are seen clinically as congestion, lesions, mild degeneration, and fatty infiltration, with urinary catalase being a biomarker of effect.

In one human case study, an individual who drank uranyl acetate and benzodiazepine demonstrated liver disfunction as increased serum levels of ALT, AST, and GGK. These signs subsided 6 months after exposure (Pavlakis *et al*., 1996).

In animal inhalation studies, the impact on the liver increased from no effect on rabbits exposed to 22 mgU/m^3 as high-grade ore to focal necrosis of the liver for rats exposed to 0.4 mgU/m^3 as uranium tetrafluoride (Dygert, 1949a,b). New Zealand rabbits exposed to uranium as uranyl nitrate in drinking water (1.4 and 41 mg/kg/day) for 91 days showed irregular accentuation of zonation in the liver, accompanied by increased variation in hepatocellular nuclear size, nuclear pyknosis, and extensive cytoplasmic vacuolization. These changes were found to be treatment-related but not dose-related (Gilman *et al*., 1998).

7.1.5 Neurological Effects

Uranium does not seem to cause nervous system problems in humans exposed by inhalation, ingestion, or dermal contact. However, this may not be the case for embedded fragments of uranium, such as in those who received DU shrapnel wounds during the Persian Gulf War. Some of these individuals have been followed for more than a decade, and testing has indicated that the uranium might cause a lowering of performance efficiency scores with electrophysiological changes in the hippocampus. A follow-up study determined that no statistically significant differences existed between low- and high-exposure groups for the neurocognitive parameters measured. A potential decrease of accuracy impairment index for the two most highly exposed individuals may have been associated with complications of combat injuries (McDiarmid *et al*., 2004).

Animals do not seem sensitive to uranium at low doses, and results are equivocal at high doses. Rats fed large doses of uranium had difficulty walking, with an unstable gait, weak muscles, and rigid limbs. Additional effects included piloerection, tremors, hypothermia (lowered body temperature), decreased pupil size, and exophthalmia (bulging of the eyeballs, comparable to that which occurs with hyperthyroidism). In addition, they showed irritability, hyperactivity or lassitude, and some respiratory arrest. Complicating this picture are findings that much larger doses did not cause such effects in rabbits, which are generally thought to be a more sensitive species to the adverse effects of uranium, and the apparent absence of effects on brain histopathology of both the rat and rabbit.

7.1.6 Pulmonary Effects

The toxicity of inhaled uranium compounds on the lung is a function of particle size and solubility. Higher lung toxicity is associated with smaller particles (<5 µm are well retained) and lower solubility (dioxide, octaoxide, and metal). The inhalation or ingestion of pure uranium compounds has not been found to cause significant harm in human or animal studies unless it involved uranium hexafluoride or was injected. High-dose animal studies did find rhinitis and slight degenerative changes (in rats and dogs exposed to 16 mg U/m^3 as uranium trioxide and dogs exposed to 9.5 mg U/m^3 as uranyl nitrate, but not when exposed to uranium dioxide and triuranium octaoxide).

At extreme doses or when mixed with other toxic agents (such as radon, silica, diesel particles, and other fumes), the result can be interstitial inflammation, which for prolonged elevated exposure can lead to pulmonary fibrosis, pulmonary edema, and eventually emphysema. A mixture of 1% U with 34% SiO_2 has been found to induce tumor necrosis factor (TNF) and the production of fibroblasts and collagen (Zhou *et al.*, 1999). These effects are comparable to those that have been associated with inhaling diverse inorganic dust. The 11th Report on carcinogens issued by the National Institute of Environmental Health Sciences (NTP, 2005) lists silica dust as a known human carcinogen and diesel particles as reasonably anticipated to be human carcinogens. This seems to be especially true for freshly cracked silica dust present in mine air.

7.1.7 Renal Effects

Uranium can be toxic to the kidneys and is considered less potent than lead, cadmium, and mercury (Goodman, 1985). Sufficiently high doses irritate, degenerate, or damage the kidney, with the most sensitive portions being the proximal convoluted tubule followed by the glomerulus. Low-dose irritation appears as cell hyperplasia and metaplasia, which at higher doses results in cellular necrosis. The shedded necrotic cells are captured as hyaline casks that are found in all portions of the tubular system. Glomerular damage is revealed as a thickened glomerular capsular wall, shrinkage of the glomerular capillary network, and decreased glomerular filtration rates. This results in increased urinary levels of amino acids, proteins, glucose, alkaline phosphatase, and β_2-microglobulin relative to creatinine, which are not specific to uranium toxicity. If the dose is not lethal, regeneration of

the injured epithelium begins within 2–3 days and is essentially complete in 2–3 weeks. Some cells in the new epithelial lining initially can be morphologically different than the original tissue, but complete recovery has been observed for humans. The body shows a level of accommodation such that doses found to be protective at intermediate durations are also protective for chronic exposure (i.e., the severity of lesions does not seem to be increased by continued exposure) (ATSDR, 1999; WHO, 2004).

Unlike animal studies, few human data are available that adequately describe the dose-response toxicity of uranium after exposure. Renal effects in humans have only been seen in high chronic dose and certain poisoning incidents, but not in highly internally exposed soldiers. U.S. servicemen with embedded DU fragments and followed for 10 years have shown elevated steady state levels of urine uranium rivaling those of the mill workers studied by Thun *et al*. (1981; 1985), but the evidence indicates that no renal damage has occurred. Thun reported the average urine concentration for Cotter Uranium Mill workers in Colorado as 7.2 (95% confidence interval [CI], 4.0–14) μg/L in 1981, increasing annually for each previous year to 65.2 (95% CI, 7.0–120) μg/L in 1975. McDiarmid *et al*. (2004) reported a range of 0.1–78.125 μgU/g creatinine. The units are roughly interchangeable (i.e., 1 μgU/L ≈ 1 μgU/g creatinine) on the basis of average daily excretion in adult males of 1.6 L/day of urine (ICRP, 2003) and 1.6 g/day of creatinine (Braunwald *et al*., 2001). Because a retinol binding protein test gave results for the high-dose soldiers that approached statistical significance, plans are to continue studying the group for any future detriment (McDiarmid *et al*., 2004).

A worker who inhaled a more soluble and highly concentrated uranium tetrafluoride powder for 5 minutes showed a delayed kidney toxicity starting 30 days after exposure that slowly resolved after 1065 days (Zhao and Zhao, 1990). A man who deliberately ingested 15 g of uranyl acetate (131 mgU/kg assuming 70 kg body weight) along with an unknown amount of benzodiazepine developed renal toxicity (doubling of blood urea and 3.5-fold increase in creatinine levels) over a period of 16 hours. It required chelation therapy (with Ca-EDTA, sodium bicarbonate, and mannitol) and dialysis, with plasma uranium levels peaking at 3.24 μmol/L the day after chelation therapy started, and symptoms continuing for more than 6 months (Pavlakis *et al*., 1996). One study found a dose-response nephrotoxicity (indicated by the presence of urinary amino acids and β_2-microglobulin) in 39 male uranium mill workers exposed for more than a year to uranium oxide concentrations exceeding the then current occupational standard of 1.0×10^{-10} μCi/mL (3.7 Bq/m^3; 0.15 mg/m^3) by up to eightfold. The incidence and urinary concentrations correlated with the length of time spent in the yellow cake drying and packaging area (Thun *et al*., 1985), although individual exposure information was lacking compared with current standards. Reports of other studies either found no renal effects or did not report them, and such negative findings were for workers exposed to high levels of insoluble uranium compounds (Eisenbud and Quigley, 1955).

The renal damage from heavy occupational exposure has been shown to be completely reversible over time. A histological kidney study of 7 chronically exposed uranium workers at autopsy and 6 referents with no known exposure to uranium showed that the groups were indistinguishable by pathologists experienced in uranium-induced renal pathology (Russell *et al*., 1996). Epidemiological studies of uranium miners and mill workers have not demonstrated unusual rates of kidney disease, and negative findings regarding renal injury among current uranium miners and mill workers exposed to dusts of both soluble and insoluble uranium compounds are particularly significant in view of the high levels of exposure.

A drinking water study was conducted on 325 individuals in Finland who derived their drinking water from private wells. Measured levels included uranium in drinking water and excretion concentrations of β_2-microglobulin, glucose, calcium, and phosphorus. One conclusion was that uranium intake enhanced excretion of these other substances in a manner that indicated renal damage. Another was that no threshold exists for renal damage (Kurttio *et al*., 2002); however, the tabulated data seem to support the converse—that a threshold does exist. Either the analytical method was not sufficiently sensitive to reach any conclusions (β_2-microglobulin) or the measurements were not statistically significant for most dose categories. Also, drinking water concentrations of calcium and phosphorus (which can be coelevated with uranium) were not measured but rather interpreted on the basis of excretion levels.

Results of animal studies with high exposures to either soluble or insoluble uranium are sufficient to conclude that uranium has a low order of metallotoxicity in mammals. The data indicate that the amount of uranium required to damage the kidney depends on its solubility and oxidation state. Inhalation exposure to uranium oxides caused no renal effects in rats and guinea pigs exposed to up to 10 mg/m^3 for 1 year (Stokinger *et al*., 1953) or in dogs and monkeys exposed to 5 mg/m^3 for 5 years (Leach *et al*., 1970; 1973). Exposure of mice to more soluble uranium tetrachloride for 30 days produced renal necrosis at 11 mg/m^3 but not at 2.1 mg/m^3 (Rothermel, 1949). When exposure

involved very soluble uranyl nitrate, dogs exposed to 0.05 mg/m^3 for 1–5 years showed renal damage (Leach *et al*., 1970). This trend was seen in oral studies. Rats exposed to approximately 12,000 mgU/kg/day as either uranium dioxide or trioxide for 30 days showed no effect, whereas dogs exposed to soluble uranyl nitrate at 95 mgU/kg/day but not at 47 mgU/kg/day had elevated protein and glucose in the urine (Maynard and Hodge, 1949). A more detailed histological study that used nitrate on the rabbit found that the male was more sensitive. The lowest adverse effect levels (LOAELs) occurred at 0.20 mgU/kg/day (nuclear pyknosis and hyperchromicity), 0.88 mgU/kg/day (reticulin sclerosis), and 4.82 mgU/kg/day (tubular dilation, atrophy, protein casks, and collagen sclerosis) (Gilman *et al*., 1998). Dermal studies indicate that oxides are ineffective at inducing renal effects but that nitrates applied at 237 mgU/kg caused renal failure in the rat (De Rey *et al*., 1983).

7.1.8 Reproductive Effects

Uranium has not yet been shown to affect the human reproductive system. Service members with embedded shrapnel have shown elevated urine uranium levels, indicating a wide systemic distribution of the element. A number have been followed for several years with no apparent reproductive effects being observed. In a comparison of low- and high-uranium exposure groups, there were no significant differences observed regarding sperm count and motility characteristics. Neuroendocrine and thyroid hormone levels were within normal range. A difference was noted in free thyroxine levels in blood (1.08 vs. 1.66 ng/L for high- and low-exposed groups, respectively), but the values were within normal limits (McDiarmid *et al*., 2004).

Animals show no reproductive impact from uranium except from lethal doses. At those levels, the effects include reduced sperm counts, reduced implantation of fertilized egg and reduced litter size, increased rate of fetal resorption and mortality, and even testicular lesions. This has led to studies of sperm counts and motility in exposed veterans.

7.2 Health Guidance Values

ATDSR, WHO, and EPA evaluated the uranium health effects literature and determined the health guidance values in Table 5, which are expected to cause no adverse health effects in humans. These were based on animal studies, because human studies are not sufficiently robust for developing such guidance.

8 BIOMARKERS

8.1 Biomarkers Used to Assess Exposure

Biomarkers of uranium exposure include *in vitro* measurements of biological samples and *in vivo* measurements of gamma radiation emitted from the body. Urinalysis is the most common and least invasive method and is used to assess occupational worker exposure, because results correlate with average breathing air concentrations. Values >l00 µgU/L indicate a recent high exposure, whereas those <40 µgU/L could result from depot mobilization or normal bone remodeling in an unexposed individual (Butterworth, 1955). The

TABLE 5 Health Guidance Values for Uranium Exposure

Source	Exposure route	Form of uranium	Exposure duration	Health guidance value
ATSDR (1999)	Inhalation	Soluble compounds	Intermediate (14–364 days)	0.4 µg/m^3
ATSDR (1999)	Inhalation	Insoluble compounds	Intermediate (14–364 days)	8 µg/m^3
ATSDR (1999)	Inhalation	Soluble compounds	Chronic (365+ days)	0.3 µg/m^3
ATSDR (1999)	Oral total		Intermediate (14–354 days); applies to chronic	2 µg/kg/d
Canada	Oral (water)			20 µg/L IMAC[a]
EPA (2000b)	Oral (water)		Chronic	30 µg/L MCL[b]
	Oral (total)			0.6 µg/kg/d TDI[c] (chemical)
WHO (2004)	Oral (water)			15 µg/L (chemical provisional)
WHO (1984)	Oral (water)			140 µg/L (radiological)

[a]IMAC, Interim maximum acceptable concentration.
[b]MCL, Maximum contaminant level.
[c]TDI, Tolerable daily intake.

Centers for Disease Control and Prevention reported average urine concentrations for the U.S. population by age, gender, and ethnicity as measured by ICP-MS. At the 95th percentile, the average was 0.046 µg/L for all individuals age 6 and older, with a male/female ratio of 1.15, and Mexican American concentrations over twice that of non-Hispanic blacks and whites. When normalized to creatinine, the male–female difference disappeared, but the Mexican to non-Hispanic ratio increased to 3 for males. The mean population values for the last survey period were 0.007 µgU/L or 0.008 µgU/g creatinine, whereas the 95th percentile values useful for screening purposes were 0.046 µgU/L or 0.040 µgU/g creatinine (CDC, 2005). Urinalysis can be supplemented by gamma spectroscopy analysis of the whole body or chest area with a sodium iodide or, preferably, a hyperpure germanium detector. The United States Transuranium and Uranium Registries (USTUR) (Russell *et al*., 1996) has demonstrated through autopsy results of highly overexposed workers that uranium is present years after exposure and concentrates primarily in bone and lung tissue.

8.2 Biomarkers Used to Characterize Effect

No biomarkers of effect specific to uranium have been identified, but uranium overexposure is known to damage renal proximal tubules and reduce urine filtration, whereas higher levels damage the liver (Pavlakis *et al*., 1996; Stokinger *et al*., 1953; Zhao and Zhao, 1990). The urine of an individual adversely affected by uranium will show elevated concentrations of catalase, protein, glucose, β_2-microglobulin relative to creatinine, amino acids, and probably other substances not well studied. Assessing multiple parameters simultaneously is an approach for uniquely identifying uranium damage (Zamora *et al*., 1998), but an appropriate test array has not yet been developed.

The USTUR reported that autopsy results from a double-blind histological evaluation showed that kidney damage to a highly overexposed worker was completely repaired over time. The pathologists could not distinguishable slides of his tubules from those of an unexposed individual.

9 TREATMENT METHODS FOR REDUCING TOXIC EFFECTS

The kidney is the critical organ for inhaled or ingested uranium (ATSDR, 1999), and treatment has focused on reducing the uranium concentration in that organ, as well as in bone, because it represents a significant long-term storage depot. Uranium from typical air, food, and water produces a body burden that does not call for treatment. When overexposure occurs or is expected, samples should be collected of the contaminated material, urine, feces, vomitus, secretions from any wounds, and any other items that are indicated, and analyzed. Internal monitoring should be used, if available, to quantify the internalized uranium initially and during treatment to assess its effectiveness. Care should be taken to eliminate external contamination on skin or clothing that could be internalized or radiologically masquerade as a larger amount of internalized uranium.

The generally recommended treatment method is to alkalinize the urine to reduce the chance of causing permanent damage or necrosis of the kidney tubules. Sodium bicarbonate is suitable, because it complexes with uranium, mobilizes uranium from sensitive tissues or depot storage locations (kidney, liver, and bone), and facilitates its elimination (Cooper *et al*., 1982; Fisher *et al*., 1991). This is addressed in the following along with summaries for other substances previously evaluated with mixed reviews.

The treatment protocol recommended by the Radiation Emergency Assistance Center/Training Site (REAC/TS) is to dissolve 2 ampules of sodium bicarbonate (44.3 milliequivalents [mEq] each; 7.5%) in 1000 cm^3 normal saline and administer intravenously at 125 cm^3/hour. The alternate protocol is to orally administer two bicarbonate tablets every 4 hours until the urine reaches an alkaline pH of 8–9. Treatment can be continued as long as it is effective and medically indicated. Nonspecific treatment methods (induced emesis or pulmonary lavage within reasonable time frames) can also be conducted with medical oversight. The use of calcium DTPA should be avoided, because it may increase bone deposition of uranium (REAC/TS, 2002). Although *in vitro* studies indicate that DTPA may also enhance the cytotoxicity of uranium to renal tubular epithelial cells, an *in vivo* study on rats found no nephrotoxicity for a therapeutic regimen of 30 µmol Zn-DTPA per kg followed by the same dose of Ca-DTPA (Houpert *et al*., 2003).

Studies on animals have identified several chelating agents that can increase the elimination of internal uranium, but their limited effectiveness (<40%) when tested separately and in combination may marginalize their value in treating cases of uranium poisoning. These include gallic acid, DTPA (diethylamine tetraamine pentaacetic acid), EDHPA (ethylenediamine-N,N′-bis[2-hydroxyphenylacetic acid]), and Tiron® (sodium 4,5-dihydroxybenzene-1,3-disulphonate). The linear molecule, 3,4,3-LIHOPO, may be more effective than sodium bicarbonate at reducing organ burden in the rat if injected immediately on uptake

of uranium, but the advantage is lost if treatment is delayed 30 minutes. Compared with controls, 3,4,3-LIHOPO reduced the uranium concentration to 23% in kidney and 46% in bone versus respective values of 87% and 67% for sodium bicarbonate (Henge-Napoli *et al*., 1995). A 30-μmol/kg dose of either of the two LIHOPO decorporated 20% of injected uranyl nitrate (Ramounet-Le Gall *et al*., 2003). Studies have been conducted to assess the usefulness of various multidentate hydroxypyridine (HOPO), catecholamine (CAM), and terphthalamide (TAM) ligands for uranium treatment (Gordon *et al*., 2003).

Phenyl acetate was reported to suppress the neoplastic transformation of immortalized human osteoblast cells to a tumorigenic phenotype by uranium (Miller *et al*., 2001). Because animal studies have not identified cancer as a health outcome after inhalation, oral, or dermal exposure, the potential usefulness of this agent for normal protective purposes is unclear.

A protein-monoclonal antibody combination was found to increase the effectiveness of 2,9-dicarboxy-1,10-phenanthroline (DCP) at chelating the uranyl ion, while being selective for uranium over other metals. Blake *et al*. (2004) took a murine protein and developed three efficient rat antibodies. After binding DCP to the protein and allowing it to chelate uranyl ions from solution, the complex was intraperitoneally injected into rats. Subsequent injection of any of the antibodies demonstrated a significant affinity, specificity, and high rate of complex formation for uranium ions compared with other metals.

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CHAPTER

46

Vanadium

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ABSTRACT

Absorption of vanadium from the gastrointestinal tract is poor, not exceeding 2% in humans. Soluble compounds of vanadium are absorbed to a considerable extent after inhalation and concentrated in the lung, but available information is not adequate for a reliable estimation of dose-response relationships. Absorbed vanadium is widely distributed in the body. In animals the highest values are found in bone, kidney, liver, and spleen. Bone maintains essentially unchanged levels for several weeks. Low concentrations have been detected in brain and in animal placenta and testes. Urine is the dominating route of excretion of absorbed vanadium. Animal and human data indicate that excretion occurs in at least two phases. A three-compartment model for elimination is described in humans with half-times after intravenous injection of 1.2 hours, 26 hours, and 10–12 days. Vanadium is essential for certain bacteria and microorganisms. Some reports suggest that vanadium is essential for mammals, but no biochemical function has been defined in humans. The total dietary intake is estimated to be 6–30 and in some regions up to 50 μg/day. The use of vanadium salts as a supplement in athletes and body builders has been reported. Local effects in experimental animals are mainly seen in the respiratory tract. They may be acute and chronic, including bronchitis and pneumonia. Systemic effects have been observed in liver, kidney, nervous system, cardiovascular system, and blood-forming organs. Metabolic effects include interference with the biosynthesis of cystine and cholesterol, depression and stimulation of phospholipid synthesis and, at higher concentrations, inhibition of serotonin oxidation. Vanadate has been shown to inhibit Na^+-K^+-ATPase, phosphatases and several other enzyme systems. Vanadium compounds enhance the effects of insulin and have been shown to lower blood glucose in animal and human experiments in diabetic individuals. Both acute and chronic effects of occupational exposure to vanadium pentoxide (V_2O_5) and other vanadium compounds have been described. They are manifested mainly as delayed, but reversible irritation of the respiratory tract involving excess mucus production and prolonged coughing, accompanied by bronchospasm, wheezing, and diarrhea in cases of more severe exposure. Eye irritation and conjunctivitis have been reported in workers. Tracheobronchitis may result from heavy, long-term exposure. Changes in lung function indicating obstruction and an increase in inflammatory biomarkers have been demonstrated in boiler cleaners after prolonged exposure. Vanadium is not mutagenic in Ames test. However, pentavalent and tetravalent vanadium compounds have produced aneuploidy in somatic cells *in vitro* and *in vivo*. A clear evidence of carcinogenic activity has been shown in mice after inhalation of vanadium pentoxide. The International Agency for Research on Cancer (IARC) has classified vanadium as a possible carcinogen (Group 2B). Biological monitoring of vanadium in serum, blood, and urine is used to follow exposure to vanadium compounds in occupational and population studies. Urine analysis, being a noninvasive method, is suitable for monitoring of workers. Reviews on environmental, toxicological and occupational health aspects of vanadium have been published by IPCS (1988), ATSDR (1992), Domingo (1996), WHO (1996), IPCS (2001), Barceloux (1999), HSE (2002), and EFSA (2004).

1 PHYSICAL AND CHEMICAL PROPERTIES

Vanadium (V), CAS No 7440-62-2, occurs in two isotopic forms, the most abundant being ^{51}V (99.75%). The atomic weight is 50.94, the atomic number is 23, the density is 6.11 (at 18.1°C), the melting point is 1910 ± 10°C, and the boiling point is 3420°C. Elemental vanadium is a soft, corrosion-resistant, steel-gray solid. Vanadium is an element of group Vb of the periodic system and belongs to the first transition series. It forms compounds mainly in oxidation states +3, +4, or +5. The most stable oxidation state is +4, in which vanadium forms oxovanadium(IV) ion (vanadyl).

Vanadium halides include pentafluoride, tetrachloride, oxovanadium chloride, dichloride, and trichloride. The most common commercial form is vanadium pentoxide, V_2O_5, CAS No 1314-62-1 (IPCS, 2001). Vanadium pentoxide dissolves in acids giving dioxovanadium(V) ion, and with bases it forms vanadate (VO_4^{3-}), which is chemically similar to phosphate (PO_4^{3-}). Because of reducing conditions in tissues, V^{3+} and V^{4+} predominate bound to small peptides, whereas V^{5+} predominates in plasma usually bound to transferrin (Crans *et al*., 1998; IPCS, 2001). The so-called metavanadates are compounds of the type $NaVO_3$. Vanadium (III) oxide dissolves in acids giving a hexaaquo ion. Vanadium (III) salts are strongly reducing. Vanadium carbonyls may contain the element in all oxidation states. A more detailed discussion on the very complex chemistry of vanadium in the environment and in biological systems is found in Crans *et al*. (1998).

2 METHODS AND PROBLEMS OF ANALYSIS

Neutron activation analysis (NAA) is an accurate and sensitive method for the analysis of trace and ultratrace elements including vanadium (de Goeij, 2000; Moens and Dams, 1995; Pyrzyn'ska and Wierzbicki, 2004; Sabbioni *et al*., 1996). A major advantage of NAA is the possibility to analyze materials that are difficult to dissolve without sample preparation. Disadvantages with the method are that it is rather expensive and requires accessibility to a nuclear rector. Also the turnaround of NAA is long, and the sample throughput is slow.

From 1990 forward, atmospheric deposition of trace metals has been analyzed with instrumental neutron activation (INNA) in mosses in different countries in Europe (i.e., France, Poland, and Romania) (Culikov *et al*., 2002; Galsomiès *et al*., 1999; Grodzin'ska *et al*., 2003). Detection limits were approximately 1 µg/g dry weight for nontreated samples. INNA was also used to study vanadium levels in particulate matter (e.g., Cao *et al*., (2003). Lower detection limits can be reached with radiochemical NAA (RNAA) (Moens and Dams, 1995).

Inductively coupled plasma mass spectrometry (ICP-MS) is well suited for the analysis of solutions with low vanadium concentration (Moens and Dams, 1995). The detection limits for ICP-MS are lower than those for NAA. ICP-MS is a relative method requiring standards. Different techniques (e.g. pneumatic nebulization, electrothermal vaporization [ETV], or laser ablation) may be used for sample preparation. With ICP-MS, information on the isotopic composition of the element is provided as well. Spectral interference in serum and other biological material, because of polyatomic species formed from the sample matrix (e.g., $^{35}CL^{16}O^+$ and $^{34}S^{16}O^1H^+$ that overlap with the ^{51}V vanadium isotope), when using ICP-MS with a quadropole as a mass filter, can be dealt with in various ways (e.g., by coupling ETV with ICP-MS) (Moens and Dams, 1995; Pyrzyn'ska and Wierzbicki, 2004). Thereby most of the solvent and a large part of the matrix can be removed before introduction of the analyte (vanadium) into the plasma, thus reducing the concentration of potentially interfering species (Björn *et al*., 1998). Analysis with ETV-ICP-MS gives approximately an order of magnitude lower detection limits (0.1–0.3 µg V/L in serum and seawater) than pneumatic nebulization ICP-MS (Pyrzyn'ska and Wierzbicki, 2004; Yu *et al*., 1997). Matrix effects are corrected for when standard additions or isotope dilutions are used for standardization (Moens and Dams, 1995).

During the 1990s, commercially available magnetic sector ICP-MS instruments were introduced. By use of these instruments, sector field (SF) ICP-MS can be operated either in a low or a higher resolution mode (HR-ICP-MS) and has many advantages compared with quadropole ICP-MS. The detection limits are approximately 10 times lower, and most of the spectral interferences are eliminated (Moens and Dams, 1995). The detection limits were found to be 0.02 µg/L in urine and 0.1 µg/L in serum with medium resolution (SF) ICP-MS in a study by Begerov *et al*. (2000). Yang *et al*. (2002), using HR-ICP-MS, reported vanadium concentrations in the range of <0.01–1.5 µg/L in urine and <0.01–0.76 µg/L in serum.

Another technology to manage the problem with spectral interferences when using quadropole ICP-MS has been developed in the past 5 years. With the so-called dynamic reaction cell technique (DRC-ICP-MS) for elimination of spectral interferences, detection limits down to 0.006 µg/L have been reported with water (Liu and Shiuh-Jen, 2002).

Atomic absorption spectrometry (AAS) in the electrothermal (ET) or hydride generation mode is still

commonly used for measuring vanadium in environmental and biological samples (Begerov *et al*., 2000). Pyrzyn'ska and Wierzbicki (2004) reviewed problems that may occur in ET-AAS, such as tailing of the absorbance signal, carbide formation, and acid interferences. However, if vanadium levels are to be monitored in unpolluted areas or in nonexposed people, the detection levels of GF-AAS may not be sufficiently low. Neither are direct spectrophotometric methods sensitive and/or selective enough without sample pretreatment (Pyrzyn'ska and Wierzbicki, 2004). Solid-phase spectrometry has higher sensitivity and lower detection limits than direct methods.

As is the case with other trace metals, reliable analyses of low vanadium contents require good quality control programs, including careful contamination-free sample collecting and processing as well as a sensitive method of analyzing (Cornelis *et al*., 1980; 1981; Sabbioni *et al*., 1996).

3 PRODUCTION AND USES

3.1 Production

There are approximately 65 vanadium minerals, but commercial mining is restricted to carnotite (potassium uranyl vanadate), vanadinite, roscoelite, and patronite (ATSDR, 1997). Most vanadium is, however, produced from residues of iron and titanium extraction (Heslop and Jones, 1976). A significant source of vanadium is the extraction from the furnace (boiler) ash of power plants that are fueled with residual oils (Exley *et al*., 1966). The world production of vanadium has increased from approximately 29,000 tons in 1978 (U.S. Bureau of Mines, 1979) to 44,000 tons in 2004 (US Geological Mineral Survey Resources Program, 2004). The major producers are China, South Africa, and Russia.

The technology of vanadium production involves roasting of vanadium-bearing ores with a sodium salt, water extraction of the resulting sodium metavanadate ($NaVO_3$), and precipitation to obtain sodium hexavanadate, which on fusion yields technical grade vanadium pentoxide. Reduction of V_2O_5 with calcium or alumothermic reduction combined with electron-beam melting is used to obtain vanadium metal (Fisher, 1975).

3.2 Uses

Approximately 85% of vanadium is used in the production of special steels and alloys, some of which have a potential application for fuel cladding in nuclear power production. There is an increasing demand for vanadium in high-strength low-alloy (HSLA) steels. The aerospace market is a big user. Vanadium pentoxide and metavanadates are important catalysts in inorganic and organic chemical industries (e.g., in sulfuric acid and plastics production). Although catalytic uses of vanadium are of great technological value, they account for <1% of the vanadium consumption (e.g., approximately 0.3% of the vanadium consumption in the United States (Fisher, 1975; U.S. Bureau of Mines, 1994). Vanadium pentoxide is also used in some pigments and inks in the ceramic industry (IPCS, 2001).

4 ENVIRONMENTAL LEVELS AND EXPOSURES

4.1 General Environment

4.1.1 Food

Food is the major source of exposure of vanadium for the general population. Vanadium levels in the diet from five regions in the United States ranged from 30.9 ± 1.5 in the Southeast to 50.5 ± 1.5 µg/kg dry weight in the West (Harland and Harden-Williams, 1994). WHO (1996) reported daily intakes between 6 and 30 µg. Myron *et al*. (1977), Byrne and Kosta (1978), and Evans *et al*. (1985) reported vanadium concentrations in food ranging from 1–30 µg/kg fresh weight. Beverages, fats and oils, milk, fresh fruits, and vegetables had the lowest levels, ranging from 1–7 µg/kg. Higher levels, 7–30 µg/kg, were found in whole grains, seafood, and meats. The highest concentrations of vanadium were recorded in parsley, dry mushrooms, and oysters. Vanadium concentrations in French and Californian wines were reported to range from 7.0–90.0 µg/L in red, and from 6.6–43.9 µg/L in white wines (Teissedre *et al*., 1998).

4.1.2 Air

Combustion of fossil fuels, especially of crude oils, is the most dominant source of emissions of vanadium into the atmosphere (Mamane and Pirrone, 1998). The emissions from oil combustion are estimated to be 58,500 tons per year, which accounts for 91% of the total global emissions from both natural and anthropogenic sources (IPCS, 2001). The EPA (1977) and Schroeder *et al*. (1987) have reviewed vanadium concentrations in air up to the early 1980s. The range of concentrations was great and varied from 0.4–1460 ng V/m^3 in urban air in the United States and 11–73 ng/m^3 in Europe. The concentrations were lower in rural areas.

There is still a wide concentration range of levels of vanadium reported in air from 0.5–1230 ng/m^3 in urban locations worldwide (Mamane and Pirrone,

1998). There are indications that current vanadium concentrations in urban locations are lower now than they were in the 1960s and 1970s when less refined crude oils were used. The concentrations reported varied with location: from 1–15 ng V/m^3 on the West Coast of the United States to 10–40 ng/m^3 in the urban cites on the East Coast. Recent data from rural sites showed vanadium concentrations from 0.3–5 ng/m^3, with a typical value in most locations of approximately 1 ng V/m^3. A mean concentration of 0.72 ng V/m^3 has been reported from rural Northwest Canada (Pyrzynska and Wierzbicki, 2004).

It is difficult to calculate any health risks from these data, because they are based on different methods of sampling time and analysis, and the samples vary from fine particles (<2.5 μm [$PM_{2.5}$] to total suspended particulates [TSP]). Earlier, it was estimated that approximately 1 μg of vanadium could enter the respiratory tract per day if air concentrations were assumed to be about 50 ng/m^3 (Byrne and Kosta, 1978). In the past 15 years, new information on the ambient levels and chemical composition of airborne particles has been published: this information will enhance the estimations of exposure (Mamane and Pirrone, 1998). For instance, in Chicago, $PM_{2.5}$ levels were 0.7 ± 1.8 and those of $PM_{2.5–10}$ were 1.9 ± 22.2 ng/m^3, respectively, during June–July 1991. TSP and PM_{10} (particles with an aerodynamic diameter <10 μm) were collected in Pavia in Northern Italy during 1999 and analyzed for different trace metals including vanadium with INNA or ETAAS (Rizzio *et al*., 2001). Mean vanadium concentrations (ETAAS) of PM_{10} were 5.6 ng/m^3 during the summer and 18.8 ng/m^3 during the winter.

4.1.3 Mosses

Since the 1970s, atmospheric trace metal deposition is regularly monitored through moss analysis in Scandinavia and several other European countries (Rühling, 1994; Rühling and Steinness, 1998). During a 1995–1996 survey, the mean/median background concentrations of vanadium varied from 1.1 dry weight in Ukraine to <2 μg/g in northern Scandinavia and 3.0 μg/g in Italy. The highest levels, up to 42 μg/g dry weight, were found around local point sources like coal and oil boiling power plants and refineries in Lithuania. The Kaliningrad area also had high levels (i.e., >30 μg/g). A clear pattern of long-range transport and transboundary pollution was seen in many countries. There has been a general decrease in the moss concentrations of vanadium (and nickel) that are typical constituents of crude oil in Western Europe and in the former East Germany (DDR) since 1985. This is mainly because of better control legislation, less use of crude oils, better flue gas cleaning, and closing of old industrial plants.

4.1.4 Water

The concentrations of vanadium in water depend largely on the geographical location (Hamada, 1998). Surface fresh water normally contains < 3 μg V/L. Much higher levels have been reported in drinking water from volcanic areas (e.g., 50–130 μg/L around Mount Etna) (Tamasi and Cini 2004). Typical values of vanadium concentrations in drinking water are below the detection limit, 1 μg/L (IPCS, 1988). When detected, the concentration usually is 1–6 μg/L, with a maximum of 20 μg/L (Barceloux, 1999; IPCS, 1988). Recently, results from a drinking water survey were reported from Tuscany in Italy (Tamasi and Cini, 2004). The vanadium levels varied from 0.3–1.8 μg/L in half of the samples and were below the detection limits in the remaining. Concentrations in bottled mineral water in Canada and in Japan were reported to be low and well within drinking water guidelines (Dabeka *et al*., 2002; Suzuki *et al*., 2000). The average concentrations of vanadium in seawater were low (i.e., 0.1–2 μg/L) (Bengtsson and Tyler, 1976). In a recent review on seawater, vanadium in the open ocean was measured between 1–3 μg V/L (Miramand and Fowler, 1998).

4.1.5 Soil

Vanadium is widely distributed in the earth's crust as different vanadates of other compounds (Mamane and Pirrone, 1998). Vanadium concentrations in soils, reported by several authors, vary between approximately 5 and 140 mg/kg and may reach very high values (i.e., up to 400 mg/kg) when the soils are polluted by fly ash (Bengtsson and Tyler, 1976). Basic slug, a by-product from the steel industry, has been widely used as a fertilizer because of its high content of calcium oxide and phophate. However, it also contains impurities, including vanadium. In an incident with basic slag contaminated with 3% vanadium that was applied to a pasture, a herd of cattle was poisoned (Frank *et al*., 1996).

Vanadium levels in soils depend on the contents in the underlying mineral, but there may also be a transport from urban and industrial areas. Higher levels of vanadium in topsoil in areas with higher population density have been reported in the Nordic countries (Steinnes and Rühling, 2002). For instance, concentrations were <4 μg V/g dry weight in Northern Sweden and >15 μg/g in the coastal regions in the Southeast and West.

4.1.6 Coal and Oil

Some coal may have up to 1% vanadium (Borisenko, 1973); however, the weighted average for U.S. coal did not exceed 30 mg/kg (NAS, 1974). The highest concentrations of vanadium in oils have been found in the Middle East and Venezuela (Davies *et al*., 1971). Venezuelan residual oils were recently shown to bear 200–300 mg/kg V, Middle Eastern oils 10–20 mg/kg V, and North African oils 50–90 mg/kg V (Mamane and Pirrone, 1998). Residual fuel oils manufactured from US crude oils contained 25–50 mg V/kg.

4.2 Work Environment

Roshchin (1968) reported that levels of occupational exposure to vanadium and its compounds in vanadium pentoxide production were 0.1–30 mg/m^3 of V_2O_5 dust and 0.03–1.2 mg/m^3 of lower oxides. During vanadium metal production, the levels of condensation aerosols were 0.3 mg/m^3 (98% of all particles were <5 μm in diameter); in the production of vanadium catalyzers, the aerosol levels were 0.5–4.0 mg/m^3. In a review on occupational exposures from 1987–1995 in different vanadium industries, IPCS (2001) reported time-weighted averages (TWA) of vanadium in air from 0.04–88.7 mg/m^3. Corresponding mean concentrations in the urine of the exposed workers displayed a wide range (i.e., 0.1–762 μg/L, with a mean urine concentration of 2.3–92 μg/L).

In a recent British risk assessment document on vanadium, occupational exposure levels are reported to be high (up to 20 mg/m^3) in total inhalable fraction during the cleaning of boilers and furnaces fueled with vanadium-containing oils (HSE, 2002). Lower levels approximately 0.1 mg/m^3 were obtained if wet cleaning methods were used. The limited exposure data were all below the detection limit of 0.01 mg/m^3. Concentrations between 0.01 and 1.9 mg/m^3 (total inhalable vanadium) have been obtained in industrial surveys of chemical manufacturing plants handling vanadium catalysts. Exposure levels during the manufacture of vanadium-containing pigments in the ceramic industry were normally <0.2 mg/m^3 (as total inhalable fraction); the highest exposure during a worst-case scenario was 0.17 mg/m^3 (HSE, 2002). The 8-hour TWA limits for total inhalable and respirable V_2O_5 in the United Kingtdom are 0.5 mg/m^3 and 0.4 mg/m^3 (as V), respectively.

Stainless steel construction workers may be exposed to vanadium through welding fumes and airborne particulate matter (APM). In a recent Polish study, the vanadium concentrations in APM varied between 0.02 and 0.79 μg/ m^3, with median values from 0.07–0.24, depending on workplace (Kucera *et al*., 2001). These values were well below the maximum admissible Polish limit of 1 mg/m^3.

5 TOXICOKINETICS

5.1 Absorption

5.1.1 Inhalation

5.1.1.1 Animal Studies

A common finding in a number of animal experiments from the 1980s was a significant and rapid absorption of vanadium compounds instilled in the trachea of rodents (HSE, 2002). Dill *et al*. (2004) have reported inhalation studies on lung deposition and clearance of vanadium pentoxide in mice and rats. In this study, completed within the US National Toxicology Program on toxicity and carcinogenicity, the animals were exposed to V_2O_5 at three different concentrations (e.g., 0.5–2 mg/m^3 [rats], and 1–4 mg/m^3 [mice], for 6 hours/day, 5 days/week for up to 18 months). The results indicated that there was a species difference between mice and rats. The differences in blood vanadium concentration between exposed and control rats were generally small. In contrast, exposed mice had higher blood concentrations than controls and blood concentrations, integrated over time, increased in proportion to exposure concentration. For example, at the 26th day of exposure, mean blood vanadium was 0.28 ± 0.08 μg V/g in control mice, but 0.38 ± 0.05 in mice exposed to 1 mg/m^3, 0.42 ± 0.07 in mice exposed to 2 mg/m^3, and 1.1 ± 0.3 μg V/g at an exposure to 4 mg/m^3.

5.1.1.2 Human Studies

The extent of absorption of different vanadium compounds in the lungs has not been determined adequately, although there is an estimate that approximately 25% of soluble vanadium compounds is absorbed (ICRP, 1960). The absorption from the lung depends on the particle size and solubility of the compounds (Task Group on Lung Dynamics, 1966; see also Chapter 3).

Workers exposed to vanadium have higher levels of vanadium in urine and blood than controls (e.g., 3.02–769 μg/L in workers from a vanadium pentoxide production plant compared with 0.066–53.4 μg/L in controls) (Kucera *et al*., 1994). The vanadium concentrations in blood and urine dropped rapidly after the exposure had ceased, and no significant correlations between air and blood or urinary levels were found (Kiviluoto *et al*., 1981a; Thürauf *et al*., 1979). However,

Roshchin *et al*. (1980) and Maroni *et al*. (1984) reported a correlation between the concentrations of vanadium in the urine of workers and the levels of exposure.

Vanadium concentrations in urine were measured over an 8-day period in 20 boilermakers during the cleaning of a large oil-fired boiler (Hauser *et al*., 1998). Urine samples were collected in the morning (start of shift) and at the end of shift, as well as on the following Monday after a 38-hour time period without potential exposure. Air vanadium concentrations, estimated with personal sampling devices and work history diaries, ranged from 0.36–32.19 μg V/m^3, with a median of 18.5 μg V/m^3. The authors found an increase in vanadium urine concentrations from 0.83 mg–1.52 V/g creatinine during the first working day. There was no significant correlation between the concentration of vanadium in the air (as PM_{10}) and the concentration in urine. The authors concluded that this might be due to a misclassification because of lack of information on the use of personal protective devices, the use of spot urine samples versus cumulative urine samples, and incomplete vanadium clearance from each previous day's exposure.

5.1.2 Ingestion

5.1.2.1 Animal Studies

The uptake of radioactive V_2O_5 given orally to rats was 2.6% (Conklin *et al*., 1982). Parker and Sharma (1978) and Roshchin *et al*. (1980) obtained similar results. Later studies have confirmed a poor gastrointestinal (GI) absorption (approximately 3% of administered dose; Ramanadham *et al*., 1991). These authors reported that absorption of vanadate (V^{5+}) was higher than that of vanadyl (V^{4+}). Bismaltolato-oxovanadium (IV) (BMOV), a synthetic organic vanadium compound with insulin-mimetic action, was absorbed to a higher extent than vanadyl sulfate in rats. Tissue uptake was approximately 2–3 times higher after oral administration of BMOV than after vanadyl sulfate (Setyawati *et al*., 1998).

5.1.2.2 Human Studies

The daily dietary intake of vanadium is in the range of 10–60 μg (Harland *et al*., 1994). In general, ingested vanadium compounds are poorly absorbed. Curran *et al*. (1959) reported that approximately 0.1–1% of the very soluble oxytartarovanadate was absorbed from the human GI tract. The ICRP (1960) estimate for the absorption of soluble vanadium compounds was 2%.

5.1.3 Skin

According to Stokinger (1967), solutions of soluble vanadium compounds may be absorbed through the skin of rabbits. Roshchin *et al*. (1982) reported that a solution of 5% $VOSO_4$ had a sensitizing effect on guinea pigs' skin. Skin is, however, probably a minor route for vanadium absorption in man (EPA, 1977). According to IPCS (2001), there are no data relating to potential systemic toxicity through dermal exposure.

5.2 Distribution

Absorbed vanadate (VO_4^{2+}) is converted to vanadyl (VO^{2+}), although the vanadate form also exists (Fantus *et al*., 1995). Vanadate is transported in the serum mainly bound to transferrin (Harris *et al*., 1984; Sabbioni and Marafante, 1981), whereas vanadyl is carried by albumin. Extracellular vanadium will be in the vanadate (5+) state, and intracellular vanadium will most likely be in the vanadyl (4+) state (Rubinson, 1981).

The total body pool of vanadium has been estimated to be 100–200 μg, depending on the content in the lungs (Byrne and Kosta, 1978). Inhaled vanadium may concentrate in the lung tissue because of poor absorption, because it is most often in an insoluble form (Schroeder, 1970). Tipton and Shafer (1964) have reported that the vanadium content in the lungs seems to increase with age. Brown and Taylor (1975) estimated the lung concentration to be 0.01–1 mg/kg; Byrne and Kosta in 1978 found levels to be 19–140 ng/g fresh weight (NAA).

5.2.1 Animal Studies

The absorption, distribution, and retention of the radioactive isotope $^{48}V^{4+}$ administered by four different routes were studied in albino rats by Roshchin *et al*. (1980). There was a fast (30 minute) distribution to all internal organs after intratracheal, subcutaneous, or intraperitoneal administration. The greatest amount of vanadium accumulated in bone tissues after intratracheal administration (9.7% of administered dose), and there was a delay in postexposure increases of the kidney concentration of vanadium. The highest kidney concentration was found on days 8–16 and was approximately 4.5% of the dose given. Retention in the lungs occurred mainly after IT instillation.

In a number of other reports from the 1980s that were reviewed by the UK Health & Safety Executive (HSE, 2002), the translocation and clearance of intratracheally instilled vanadium compounds in rodents were analyzed. Common findings were an initial rapid clearance from the lungs into blood, liver, and bone, followed by a slower clearance. These studies also indicated that there was a significant absorption of vanadium from the lungs. Cohen *et al*. (1996) reported a tendency for vanadium to accumulate in the lung of rats in a time-dependent manner. Similar findings with a proportional increase in the lung burden of vanadium with time were reported by Dill *et al*. (2004).

Söremark *et al*. (1962) reported that vanadium concentrated in the fetuses of mice. Later studies reported that vanadium was preferentially accumulated in the placenta rather than in the fetus itself (Hackett and Kelman, 1983; Roshchin *et al*., 1980). Paternain (1990) found a dose-related increase of vanadium in the liver, kidney, spleen, in the whole fetus, and in the placenta of pregnant mice administered vanadyl sulfate pentahydrate on days 6–15 of pregnancy. The highest concentration was found in placenta (Paternain *et al*., 1990). Oberg *et al*. (1978) and Roshchin *et al*. (1980) reported a low-grade accumulation of vanadium in the testes of rats.

In conclusion, absorbed vanadium (as V^{5+} or V^{4+}) is distributed mainly to bone, liver, and kidney (HSE, 2002; Ramanadham *et al*., 1991; Roshchin *et al*., 1980; Setyawati *et al*., 1998; Sharma *et al*., 1980). Three days after administration, 10–25% of the administered dose was found in bone, 4–5% in liver and kidney, 0.1% in spleen, and 0.2% in the testes (IPCS, 2001). Brain levels of vanadium were found to be considerably lower than in the other organs, suggesting a blood–brain barrier for the tested vanadium salts. Vanadium compounds can pass the placenta, but the levels of the metal are most often higher in the placenta than in the fetus.

5.2.2 Human Studies

Vanadium is found in all body tissues. Byrne and Kosta (1978) reported concentrations of 3.3, 7.5, and 0.5 ng/g fresh weight in kidney, liver, and muscle, respectively (by NAA). Vanadium has also been found in the placenta at a concentration of 3 ng/g fresh weight (Thürauf *et al*., 1978). It has been suggested that there is no cumulative effect of vanadium in human tissue (Byrne and Kosta, 1978; Naylor *et al*., 1984; Schroeder, 1970).

More recent reports on tissue levels of vanadium are scarce. In a study on autopsy samples from 78 non-occupationally exposed subjects from NE Spain, the concentrations of vanadium in brain, bone, kidney, liver, and lung were all below the detection limit (i.e., <0.125 µg/g wet weight) (Garcia *et al* 2001). In a study from Mexico City, the concentrations of vanadium in lung tissues from the 1960s and 1990s were compared (Fortoul *et al*., 2002). During this period, the mean concentrations had increased from 1.04 ± 0.05 in the 1960s to 1.36 ± 0.08 µg/g dry weight, implying increasing vanadium concentration in ambient air, most likely caused by an increased number of motor vehicles.

5.3 Elimination and Biological Half-Time

5.3.1 Animal Studies

Parenterally administered vanadium compounds are rapidly excreted, mainly in urine. Rats and rabbits given intravenous and intraperitoneal injections of sodium metavanadate demonstrated a 5:1 ratio of urinary/fecal excretion; they excreted 61% of the dose through urine within 24 hours. Intestinal excretion amounted to 10–12% (Talvitie and Wagner, 1954). These results were confirmed in later dog and rabbit experiments (Roshchin, 1968). Conklin *et al*. (1982) found that 90% of intratracheally instilled $^{48}V_2O_5$ had cleared from the lung by day 3, and 40% had been excreted primarily in the urine. The main part of orally ingested vanadium is excreted mainly unabsorbed with feces in a couple of days. Setyawati *et al*. (1998) reported a fecal excretion 24 hours after an oral dose of vanadyl sulfate or BMOV of 75 and 62%, respectively.

Studies in rats and mice showed a three-compartment model for elimination with plasma half-times of 15 minutes, 14 hours, and 8.5 days (Parker *et al*., 1980; Sabbioni and Marafante, 1978). Liver, kidneys, bone, spleen, and testes had the longest half-times (Parker and Sharma, 1978; Parker *et al*., 1980; Sharma *et al*., 1980). Ramanadham *et al*. (1991) calculated the half-life to be longer, 12 days, for elimination of vanadium from the kidney in rats, fed $VOSO_4$ in the drinking water. According to IPCS (2001), the pattern of vanadium distribution and elimination in animals indicates that there is a potential for accumulation and retention of absorbed vanadium, particularly in the bone.

5.3.2 Human Studies

The main route of vanadium excretion is through the urine. The elimination half-life of absorbed vanadium has been estimated to be 15–40 hours (Sabbioni and Moroni, 1983). In a healthy young man orally administered sodium metavanadate (12.5 mg/day for 12 days) was completely recovered, largely unabsorbed, in the feces (87.6%) and the remainder (12.4%) in the urine (Proescher *et al*., 1917). Similar results were obtained by Tipton *et al*. (1969). Studies on workers exposed to vanadium through worksite air showed that blood and urinary values of vanadium dropped to half the initial value within a few days after cessation of exposure (Kiviluoto *et al*., 1981a; Thürauf *et al*., 1979). Hauser *et al*. (1998) reported urine vanadium concentrations ranging from 0.19–4.3 mg V/g creatinine in boiler cleaners exposed to air vanadium concentrations of 19.1 ± 10.7 µg V/m^3. In these workers, there was a rapid initial clearance of vanadium followed by a slow clearance that was not completed even 38 hours after the end of exposure.

The pharmacokinetics of vanadium has been studied by Heinemann *et al*. (2003). Five healthy volunteers received an intravenous infusion solution of 20% commercial albumin containing 47.6 µg vanadium as an impurity. Serum concentrations of vanadium were

then followed for 31 days. The data obtained could be fitted to a three-exponential function corresponding formally to a three-compartment model, with an initial rapid decrease in serum concentrations with half-lives of 1.2 and 26 hours, followed by a longer half-life of 10 days. The terminal phase accounted for approximately 80% of the total area under the serum concentration curve (AUC). A total of 24.9 μg (about 52% of the dose) was recovered in the urine after 12 days. The calculated amount of vanadium retained in the body was 16.5 μg. The remaining amount not accounted for (approximately 13% of the administered dose) was possibly excreted in the feces. This was in line with the previously reported fecal excretion of vanadium found in humans and experimental animals after intravenous administration (IPCS, 2001).

6 BIOLOGICAL MONITORING

Biological monitoring of vanadium in serum, blood, and urine has been used to monitor vanadium levels in the general population or occupational exposure to vanadium compounds. Elevated levels above the reference range are often found in workers, although not necessarily associated with clinical symptoms (e.g., eye and respiratory symptoms or gastrointestinal disturbances). The reference range for vanadium used by the Trace Element and Environmental Toxicology Laboratory at the University of Alberta Hospitals in Canada is 0–160 nmol (0–9 μg/L) in 24–hour urine collection (Guidotti *et al*., 1997). The biological exposure index (BEI) Committee of the American Conference of Governmental Hygienists has recommended a BEI of 50 μg/g creatinine for end of shift urine specimens collected at the end of a work week (ACGIH, 1995–1996). The BEI value is exposure related and is based on the calculated urine vanadium concentration arising from exposure at the 8-hour TLV (threshold limit value) of 0.5 mg/m^3 over a 40-hour exposure period (HSE, 2002). According to the UK Health and Safety Executive, a tentative level for a biological monitoring guidance value (BMGV) using the criteria establishing good occupational hygiene practice would be 10 μg/L (HSE, 2002). There is some controversy concerning whether spot samples of urine need to be corrected for creatinine or not. The ideal sample would be urine collected over a 24-hour period. Because this is usually not practical, the ACGIH recommends that samples be collected at a specific time in relation to exposure (preshift and postshift) and corrected for creatinine (ACGIH, 1995–1996). However, the UK Health and Safety Executive does not consider correction to creatinine to be necessary, referring to a study by Barbieri *et al*. from 1988 (HSE, 2002).

Concentrations of vanadium in human serum in Belgium (using RNAA without preseparation) were reported to be 0.024–0.939 and 0.016–0.139 μg/L in nonexposed healthy men and women, respectively (Cornelis *et al*., 1981). Slightly lower values using the same method were reported in whole blood in nonexposed children and adults from Czechoslovakia (e.g., 0.024–0.226 and 0.032–0.095 μg/L, respectively) (Kucera *et al*., 1992). There was no significant difference between children and adults. Children living near a V_2O_5 producing plant had significantly higher blood vanadium concentrations than control children (i.e., median 0.078 vs. 0.042 μg/L). In a study of 65 healthy adult subjects from Northern Italy (using INAA with preseparation), vanadium blood concentrations were between 0.09 and 1.1 μg/L, with a mean of 0.35 μg/L (Minoia *et al*., 1990). Serum concentrations in 415 samples from the same area, (analyzed with GF-AAS) were 0.07–1.80 μg/L (mean, 0.62 μg/L). Suggested references ranges including 95% of the examined population were 0.09–0.75 in blood and 0.07–1.1 μg/L in serum. In a review by Sabbioni *et al*. (1996), it was proposed that normal vanadium values in blood and serum of the general population were approximately 1 nmol/L (0.05 μg/L).

Analyses of vanadium concentrations in urine have been regularly used to monitor exposure to vanadium compounds in different industrial activities. The vanadium concentrations in different studies (between 1987 and 1995) that were reviewed by the UK HSE had a wide range, from 0.1–762 μg/L (HSE, 2002). In recent studies on boiler cleaning operators, the personal exposure to vanadium dusts ranged from <0.04–88.70 μg/m^3, and the vanadium concentrations in urine were between 0.1 and 322 μg/L. The major factors influencing the vanadium uptake were determined to be the cleaning method (wet washing or dry brushing), the level of respiratory protective equipment used, and the vanadium content of the inhaled dust (which ranged from 0.1%–15% vanadium).

Vanadium concentrations in urine were recently analyzed with ICP-MS in Spanish hazardous waste incinerator workers (n=20); these were 3.4–32.8 μg V/g creatinine. The range in a small group of administrative workers (n=3) was 3.2–16.4 μg V/g creatinine (Agramunt *et al*., 2003). Urine vanadium concentrations (using HR-ICP-MS) in 131 children (aged 6–10) in the urban area of Rome were seen to be lower (i.e., 0.02–0.22 μg V/g creatinine) (Alimonti *et al*., 2000).

Concentrations of vanadium in hair, ranging from 20–60 ng/g have been reported by different authors (Byrne and Kosta, 1978; Gordus *et al*., 1974). Ranges

of 4–140 ng V/g in hair and 4–625 ng/g for nails were listed by the U.S. Environmental Protection Agency (EPA, 1979). Kucera *et al*. (1992) did not find a correlation between vanadium concentrations in blood (0.042 ng V/mL) and hair (98 ng/g dry wt) in 17 nonexposed children. On the basis of their results, a level of 30–100 µg V/kg was suggested as the most typical value for vanadium in hair. However, these authors proposed that the vanadium hair level was not the best indicator of environmental exposure to vanadium, because vanadium may be leached out from the hair matrix during washing. For reviews on reference values in serum and tissue levels, the reader is referred to Byrne and Kosta (1978), Nechay (1984), Cornelis *et al*. (1994), and Sabbioni *et al*. (1996).

7 EFFECTS AND DOSE-RESPONSE RELATIONSHIPS

Vanadium is essential for certain bacteria, marine microalgae, lichen, and fungus (IPCS, 2001). In the 1970s, vanadium was reported to be an essential trace element for chicken and rats. These findings have subsequently been questioned, because the diets were unbalanced, and the control diets contained small amounts of vanadium (Sabbioni *et al*., 1996). However, other reports have suggested that vanadium is essential for higher animals (Arnold *et al*., 1993). These studies showed abnormalities in the bone structure in goat kids born by females who had a long-term exposure to vanadium-deficient diets. Anke (2004) recently published a review on studies dealing with the essentiality of vanadium.

Several vanadium-dependent enzymes have been found in lower organisms; in mammals, a specific biochemical function for vanadium has yet to be identified. The possibility has been considered that vanadium might play a role in the regulation of Na^+/K^+ exchanging ATPase, phosphoryl-transfer enzymes, adenylate cyclase, and protein kinases. The possible role of the vanadyl ion in hormone, glucose, lipid, bone and tooth metabolism has also been discussed (see IPCS, 2001; Nechay, 1984; Rehder and Jantzen, 1998; WHO, 1996).

7.1 Local Effects and Dose-Response Relationships

7.1.1 Human Studies

7.1.2.1 Experimental Exposure

Exposure of two healthy volunteers to vanadium pentoxide dust (1 mg/m^3, 8 hours) produced respiratory irritation and sporadic coughing that started 5 hours after the challenge, became persistent after approximately 12 hours, and lasted for 8 days (Zenz and Berg, 1967). Exposure to a lower concentration (0.2 mg V/m^3, 98% particles <5 µm; for 8 hours, 5 subjects) had the same effect, but the induction time for the persistent cough was longer (about 20 hours). The highest concentration of vanadium in the urine (0.13 mg V/L) and feces (0.3 mg/kg) of the subjects was found after 3 days. Vanadium was not detected in their blood. Concentrations of 0.1 mg V/m^3 caused no irritation during 8 hours of exposure (two volunteers not previously exposed), but the formation of mucus was increased 24 hours later, increased further on the second day, and disappeared after 4 days. There were no alterations in pulmonary function tests or chest X-ray; on re-exposure, respiratory irritation occurred at this concentration. It was not possible to identify a no observed adverse effect level (NOAEL) for bronchial effects in this study. The potential for vanadium to produce skin irritation on direct contact is unclear. No skin irritation was noted in 100 human volunteers after skin patch testing with 10% V_2O_5 in petrolatum (HSE, 2002).

7.1.2.2 Occupational Exposure

Acute local effects of occupational exposure to airborne vanadium compounds include irritation of the respiratory tract and the eyes, and occasionally skin manifestations. Mild effects are characterized by sneezing, rhinitis, sore throat, dry or productive persistent cough, chest pain, and conjunctivitis (Roshchin, 1968; Sjöberg, 1950). The signs and symptoms disappear within 2–5 days. No quantitative data on the eye irritation threshold for vanadium pentoxide dust are available. However, exposure to a concentration of 0.018 mg/m^3 of V_2O_5 is known to cause eye irritation. Workers exposed to 1–5 mg/m^3 of V_2O_5 developed dermatitis, as well as conjunctivitis and tracheobronchitis (OSHA, 1999). There was usually a latent period of 1–6 days before the effects; commonly, mucus formation and persistent cough appeared (Roshchin, 1968; Williams, 1952). According to Lewis (1959b), acute effects may occur at concentrations of V_2O_5 as low as 0.1–1.0 mg/m^3. In more severe cases, at concentrations of airborne vanadium up to > 10 mg/m^3, bronchospasm and bronchitis accompanied by wheezing and dyspnea, which also were reversible, were observed (Lewis, 1959b; Zenz *et al*., 1962). Bronchopneumonia may result from very high exposures (Roshchin, 1968; Sjöberg, 1950). In some cases, the severity of respiratory effects increased with repeated exposure of shorter duration and lower intensity, indicating a sensitization reaction

(Roshchin, 1968; Zenz *et al*., 1962). At higher exposure levels, ranging from 2– >100 mg/m^3 of V_2O_5 dust, cases of acute poisoning have been reported. Watering of the eyes, discharge from the nose, green discoloration of the tongue, and sneezing developed immediately on exposure (OSHA, 1999). Green coloration of the tongue, resulting from deposition of vanadium salts, is a common indicator of exposure (Lewis, 1959b; Musk and Tees, 1982; Williams, 1952). This indicator may be absent even with prolonged exposure (Sjöberg, 1950) and is probably evidence of exposure only and not a manifestation of toxicity (Lees, 1980). According to Sjöberg (1950), hypersensitivity to vanadium may result in eczema. Three isolated cases of V_2O_5-induced skin allergy have been reported (Browne, 1955; Proctor *et al*., 1988; Zenz *et al*., 1962). However, the skin-sensitizing potential of vanadium compounds in humans is probably low (HSE, 2002).

Observations on a few vanadium workers suggested that V_2O_5 compounds might produce asthma (Musk and Tees, 1982). One study by Isrigler *et al*. (1999) reviewed by IPCS (2001) claimed a link between exposure to vanadium and hyperresponsiveness or asthma. However, because of an incomplete description of background data, it is not possible to draw any certain conclusions from that study. In regard to chronic respiratory effects of vanadium exposure, the evidence is inconclusive. Roshchin (1968) considered that chronic effects of vanadium exposure included diffuse pneumosclerosis, chronic bronchitis, and chronic rhinitis and pharyngitis. Sjöberg (1950) also recorded some cases of chronic atrophic changes such as atrophic pharyngitis. The NAS (1974) concluded that there was no clinical feature in man to correspond to the emphysema, postpneumonic atelectasis, bronchopneumonic-atelectatic changes, or enteritis seen in animals. An increase in inflammatory cells of the nasal mucosa has been found in exposed workers (Kiviluoto *et al*., 1979; Woodin *et al*., 1998).

Kiviluoto (1980) investigated 63 vanadium workers and matching referents by means of a questionnaire on respiration, chest radiography, and tests of ventilatory functions. No significant differences in the radiographs and tests were found between the reference group and the men with long-term exposure (at least 4 months) to vanadium (at levels of 0.1–3.9 mg/m^3). Breathing zone samples in 58 workers gave a shift length time-weighted mean concentration of 0.028 mg/m^3 vanadium in all but two workers with higher concentrations, 0.13 and 1.7 mg/m^3, respectively. Complaints of wheezing were significantly more common among the workers exposed to vanadium than among their referents, but there were no differences in the prevalence of nasal catarrh, cough, phlegm, or other respiratory symptoms. The effects reported during the 1980s and thereafter confirmed the findings in the earlier studies that exposure to V_2O_5 caused eye and respiratory tract effects and, in some cases, green tongue. There are no studies on the effects on tetravalent vanadium compounds (HSE, 2002).

In summary, the data in the older studies are insufficient to describe a reliable dose-response relationship for the respiratory effects of V_2O_5 dust and fume in humans (HSE, 2002). Some findings of the effects after occupational exposure at different exposure levels are summarized in Table 1. More recent data on exposure levels are reported by IPCS (2001) and HSE (2002).

Since the early 1990s, a research team at the Harvard School of Public Health has been studying the respiratory effects of low-level vanadium exposure in boilermakers. In one study, 26 males had a significant fall in forced expiratory volume during 1 second (FEV_1) related to average PM_{10} after 4 weeks of work on the boiler. The average PM_{10} concentration was 3.22 ± 1.42 mg/m^3, and the vanadium concentration was 2.2–31.3 (mean, 12.2 ± 9.1) μg V/m^3 (Hauser *et al*., 1995). A later study showed significant decreases in peak expiratory flow rate (PEFR) during a day with an average PM_{10} exposure of 2.75 mg/m^3 (Hauser *et al*., 1996). To study biomarkers for acute upper airway inflammation, nasal lavage was performed in 18 boilermakers and 11 controls. The PM_{10} concentrations were approximately four times higher in boilermakers (i.e., 0.47 vs. 0.13 mg/m^3). Compared with the controls, it was found that the levels of cytokine interleukin-8 (IL-8) and the enzyme myeloperoxidase (MPO) increased significantly with exposure and returned to preexposure values after approximately 2 weeks. IL-8 is a protein messenger that is known to play a key role in acute inflammatory responses, and MPO is responsible for the recruitment and activation of polymorphonuclear leucocytes (PMN) to sites of inflammation. Controls showed no significant change in any marker during the study period (Woodin *et al*., 1998). Nasal fluid vanadium levels also increased significantly during the period, but there were no significant correlations between vanadium concentrations and the levels of IL-8 and MPO. This missing association was attributed to the fact that the protein samples had to be collected in the morning approximately 12 hours after the previous working day to allow the recruitment of the biomarkers to the site of inflammation. Because five other metals analyzed simultaneously (using ICP-MS) (e.g., nickel and manganese, as well as ozone) were not elevated, the authors concluded that their contributory role in causing the observed inflammatory reactions were probably small.

TABLE 1 Summary of Effects of Industrial Exposure to Vanadium

Industry	Vanadium compound	Air levels (mg/m³)	Symptoms and signs	Reference
Vanadium refinery	V_2O_5	<12	Mild respiratory irritation	Sjöberg (1950)
Vanadium ore mining and processing	V_2O_5	3–100	Local respiratory effects, no systemic poisoning	Vintinner *et al*. (1955)
Vanadium processing	V_2O_5	0.5–2.2	Eye and bronchial irritation	Gul'ko (1956)
Vanadium refinery	V_2O_5	0.2–0.5	Respiratory irritation	Lewis (1959b)
Vanadium refinery	V_2O_5 and NH_4VO_3	0.25	Green tongue, metallic taste, throat irritation and cough	Faulkner Hudson (1964)
Vanadium factory	V_2O_5	0.2–0.5	Increased number of plasma and round cells in biopsies from nasal mucosa	Kiviluoto *et al*. (1979)
Vanadium factory	V_2O_5	0.01–0.04 (earlier 0.1–3.9)	Complaints of wheezing; no changes in lung ventilation tests	Kiviluoto (1980)
Boiler cleaning	V_2O_5, V_2O_3	2–85	Respiratory irritation	Sjöberg (1955)
Boiler cleaning	V_2O_5	0.5 (15.3% V)	Airway irritation; reversible reduction of lung ventilation (respirators were used)	Lees (1980)
Boiler cleaning	V_2O_5	PM_{10}; mean 0.47. Nasal fluid V; median 0.47 μg/L	Inflammatory reaction as, increased nasal fluid levels of interleukin-8 and polymorphonuclear leukocytes	Woodin *et al*. (1998)

7.2 Systemic Effects and Dose-Response Relationships

7.2.1 Animals

7.2.1.1 Liver and Kidney

Fatty changes with partial cell necrosis in the liver and kidney have been observed in rats and rabbits as a result of long-term exposure by inhalation to vanadium pentoxide, trioxide and chloride (10–70 mg/kg, 2 hours/day, for 9–12 months) (Roshchin, 1968). Fatty changes in the liver of rats after subcutaneous injections of ammonium vanadate (1 mg V/kg/day for 30 days) have also been reported by Kaku *et al*. (1971). Oral exposure was less toxic. No effect on the liver function in groups of normal rats ($n = 10$ in each dose group) was found after exposure to 0, 0.3, 0.6, and 3 mg V/kg body weight/day as sodium metavanadate in drinking water for 3 months (Domingo *et al*., 1985). A deficiency syndrome may increase the susceptibility. In selenium- and vitamin E–deficient rats, liver necrosis developed when the diet contained 50 mg/kg of V_2O_5 (Whanger and Weswig, 1978).

In the study by Domingo *et al*. (1985), histopathological examinations made in three animals from each group revealed dose-dependent changes in the spleen, kidneys, and lungs at the two highest doses (0.6, and 3 mg V/kg body weight/day). However, no histopathological changes in the liver, brain, heart, or blood vessels were detected in rats orally exposed to similar doses of vanadium (0, 0.6, and 2.4 mg/kg body weight in drinking water for 210 days) (Boscolo *et al*., 1994).

Domingo *et al*. (1985) reported that plasma levels of urea and uric acid were significantly greater in the highest dose group compared with controls but were within the normal ranges in the lower dose groups. Further studies have confirmed that vanadate decreases Na^+-K^+-ATPase activity and causes increased urinary excretion of potassium and even hypokalemic renal tubular acidosis (Boscolo *et al*., 1994; Dafnis *et al*., 1992).

Several studies have demonstrated age-dependent differences in the vanadium nephrotoxicity in rats. The kidney concentration of vanadium in adult rats (62 days), receiving intraperitoneal injections of 10 mg/kg/day for 8 days was approximately fourfold higher in young rats, 22 days old (De la Torre *et al*., 1999). Serum creatinine, urea levels, alkaline phosphatase activity (ALP), and lactate dehydrogenase (LDH) were mainly affected in adult rats, with only slight effects in young animals. Vanadium-induced morphologic changes (e.g., proximal tubular necrosis) were also more pronounced with age. A detailed review on the biochemistry and pathophysiology of vanadium in the kidney is available from Dafnis and Sabatini (1994).

There is a species variation in the sensitivity to vanadium. Vanadate given with food for 10 weeks to 6 weaning pigs at a dose of 200 mg V/kg suppressed growth and increased mortality (van Vleet *et al*., 1981).

On the other hand, vanadium was not markedly toxic when 200 mg/kg or less was fed to growing lambs for 84 days (Hansard *et al*., 1978). Vanadate caused increased urinary flow and sodium loss in the rat, but not in the dog or cat (IPCS, 1990).

The effects of oral intake of pentavalent vanadium compounds on weight gain, consumption of water, and urine volume were reviewed by IPCS (2001). Some studies reported no effect on weight gain, and others a decreased weight gain. Decreased water consumption in the exposed animals (possibly related to unpalatability) and concomitant decrease in food intake are confounders, which makes it impossible to draw any valid conclusions regarding the effects of vanadium on growth. For reviews on the toxicology of vanadium compounds see Nechay (1984), Domingo *et al*. (1995), IPCS (2001), and HSE (2002).

7.2.1.2 Nervous System

In acute vanadium poisoning, death is preceded by the paralysis of hind legs, depression of the respiratory center, and convulsions (Browning, 1969; Roshchin, 1968). Llobet and Domingo (1984) also reported paralysis of the hind legs and decreased sensitivity to pain in male mice and rats given sodium metavanadate (31 mg and 41 mg V/kg body weight by gavage). There was a regression of most symptoms 48 hours after treatment in surviving rats. Leg incoordination and paralysis of the hind legs preceded death in 23 heifers (of 98 cattle) that died 14–23 days after being fed fresh hay contaminated with soil and a fertilizer containing 3% vanadium for 7 days (Frank *et al*., 1996). Because of residual neurological disturbances (not described) and decreased milk production, the rest of the herd was slaughtered 5 months after the accidental exposure.

Repeated feeding of vanadium pentoxide (as aqueous solution containing 0.05–0.5 mg V/kg/day, for 80 days) impaired the condition reflex mechanisms in rats (Seljankina, 1961). Doses of 0.005 mg/kg/day did not cause any neurological disturbances. Inhalation exposure to a condensation aerosol of vanadium pentoxide at 0.027 or 0.006 (but not at 0.002) mg/m^3 produced changes in the excitability of the tibial musculature in rats (IPCS, 1990). Vanadium has been reported to have behavioral effects in rats. Oral administration of vanadium at doses between 4.1 and 16.4 mg/kg/day for 8 weeks significantly reduced the general activity and learning of healthy rats (Sanchez *et al*., 1998). However, no differences in external appearance or locomotor behavior were reported in rats administered ammonium metavanadate (1.5 or 5–6 mg V/kg /day for 4 weeks) in drinking water (Zaporowska *et al*., 1993).

7.2.1.3 Hematological and Cardiovascular Effects

Early work (Myers and Beard, 1931) indicated a hemopoietic effect of vanadium chloride (0.6 mg V/kg in diet) in rats previously rendered anemic. More recent studies on the hematological effects of vanadium are not consistent. In the previously described oral study on rats by Zaporowska *et al*. (1993), small decreases in erythrocyte number and hemoglobin concentration in rats given 5–6 mg vanadium/kg body weight/day for 4 weeks were reported. Boscolo *et al*. (1994) found no adverse effect on cardiovascular function or histopathological changes in the heart or blood vessels in rats administered 2.4 mg V/kg body weight/day for 7 months.

Contradictory findings concerning blood pressure, pulse rate, or hematological parameters have been reported (Domingo, 2000; HSE, 2002). Carmignani *et al*. (1996) studied the effects on the cardiovascular regulation in rabbits exposed for 1 year to 1 μg/mL of vanadium (as sodium metavanadate) in drinking water. Blood pressure and heart rate were unchanged in the exposed animals, because the observed decrease of cardiac inotropism and stroke volume was counteracted by an increase of the peripheral vascular resistance. There was also evidence that vanadium reduced the synthesis and/or release of nitric oxide, a vasodilating factor derived from the endothelium.

7.2.1.4 Mutagenicity and Carcinogenicity

The UK Health and Safety Evaluation included a review on 17 *in vitro* studies from the 1990s dealing with vanadium mutagenicity in bacteria and in mammalian cells (HSE, 2002). The overall evaluation was that pentavalent and tetravalent vanadium compounds produced aneuploidy both *in vitro* and *in vivo* (somatic cells). There is evidence that these compounds, as well as trivalent vanadium, could cause DNA/chromosome damage *in vitro*, although both positive and negative results have been reported (IPCS, 2001).

Vanadium pentoxide was not mutagenic in Ames test in concentrations from 0.03–333 μg/plate, with or without induced rat or hamster liver S9 enzymes (NTP, 2002). The combined evidence does not support the claim that vanadium compounds cause gene mutation in standard *in vitro* tests using bacterial or mammalian cells.

In a study by Owasu-Yau *et al*. (1990) reviewed by HSE (2002), the induction of chromosomal aberrations was increased in Chinese hamster ovary cells exposed to vanadium as metavanadate (V^{5+}), vanadyl sulfate (V^{4+}), and vanadium oxide (V^{3+}). In two studies on human lymphocytes reviewed by HSE (2002), no significant chromosomal aberrations were reported after exposure to vanadyl sulfate or four other pentavalent vanadium compounds. However, the number

of polyploid cells and "satellite associations" were increased in one study, indicating that vanadium pentoxide exerts its effect at the level of spindle formation. In another study, the number of hypoploid cells (missing chromosomes) was increased.

Tetravalent vanadium compounds have been found to produce chromosome aberrations *in vivo*. Vanadyl sulfate induced chromosomal aberrations and aneuploidy-related endpoints (hypoploidy and hyperploidy) in mice bone marrow after a single dose of 100 mg/kg (31 mg V/kg) intragastrically administered vanadyl sulfate. Pentavalent compounds (e.g., 75 mg/kg of sodium orthovanadate [21 mg V/kg] or 50 mg/kg of ammonium metavanadate [42 mg V/kg]), caused significant increases in the numbers of cells with hypoploidy and with hyperploidy. Increases of chromosomal structural aberrations were noted, but these were not statistically significant (Cirrani *et al*., 1995).

The toxicity and carcinogenicity of V_2O_5 have been studied in rats (F344/N) and mice (B6C3F1) after inhalation of concentrations of 0, 0.5 (rats only), 1, 2, or 4 (mice only) mg/m^3, 6 hours per day, 5 days per week, for 2 years (NTP, 2002). It is important here to note that the OSHA permissible occupational exposure limit is 0.5 mg/m^3 dust (NIOSH, 1997). In these studies, the survival and body weights were only affected in mice. Female mice of all exposed groups had a lower body weight than controls, and male mice at the highest dose had lower survival and body weights than controls.

Lung alveolar/bronchiolar neoplasms (adenomas or carcinomas) occurred in some of the exposed groups of rats and were significantly increased in all groups of exposed mice. Both exposed rats and mice showed increases in chronic inflammation, interstitial fibrosis, and alveolar and bronchiolar epithelial hyperplasia. The NTP conclusions were "some evidence of carcinogenic activity of vanadium pentoxide" in male rats and "equivocal evidence" in female rats, and "clear evidence of carcinogenic activity" in male and female mice. Vanadium pentoxide has been evaluated by IARC and classified as "possible carcinogenic to humans" (Group 2B) on the basis of sufficient evidence in experimental animals, even in the absence of human data (IARC, 2003).

It has been reported that vanadium can exert preventive effects against chemical carcinogenesis in animals (Evangelou, 2002, review), and anticancer effects of vanadium in experimental animal models have been reported. Suresh Kanna *et al*. (2005) studied the effects of ammonium monovanadate in drinking water on preneoplastic rat colon carcinogenesis induced by 1,2-dimethylhydrazine. They reported significant reductions in DNA-protein cross-links in colon cells, increases in glutathione S-transferase and cytochrome P-450 levels, as well as a marked improvement in colonic architecture, with lower numbers of aberrant crypt foci, compared with control rats not treated with vanadate.

7.2.1.5 Reproductive Toxicity

The findings from studies on developmental and reproductive effects of vanadium are not unanimous. According to Domingo (1996), the reproductive and developmental toxicity of vanadium compounds is well established. However, the UK Health and Safety Executive in their review on reproductive toxicity concluded that the potential for vanadium compounds to affect fertility are poorly investigated (HSE, 2002). Altamirano-Lozano *et al*., (1996) reported an adverse effect on germ cells in mice exposed orally to 60 and 80 mg/kg of metavanadate, causing 50% reduction in successful matings in exposed mice compared with controls. The resorption of embryos was also increased and the number of live fetuses/litter decreased in the exposed animals.

In relation to developmental toxicity, the results indicated that pentavalent vanadium did not cause direct developmental effects. It is true that dose-related skeletal abnormalities were increased in the fetuses of mice administered 0, 37.5, 75, or 150 mg/kg/day of vanadyl sulfate pentahydrate (0, 7.6, 15.1, and 30.2 mg V/kg/day) by gavage on days 6–15 of gestation (Paternain *et al*., 1990, reviewed by HSE, 2002). Fetal body weight and length were significantly reduced at all dose levels. Cleft palate was increased at 75 and 150 mg/kg. Micrognathia and delayed ossification were increased at all dose levels, and hydrocephaly at 75 and 150 mg/kg. However, there was also a significant maternal toxicity as defined by decreased body weight; thus, the fetal effects may be secondary to maternal toxicity. Several other studies have reported increased skeletal abnormalities, increased number of resorbed or dead fetuses, delayed ossification, and decreased fetal body weight. However, in these studies, vanadium was administered through intraperitoneal, subcutaneous, and intravenous routes (HSE, 2002).

7.2.1.6 Metabolic Effects

Mountain *et al*. (1953) reported that rats administered V_2O_5 in the diet (25–1000 mg V/kg feed) had a lower content of cystine in their hair than controls, indicating inhibition of cysteine and cystine synthesis. Such inhibition should affect metabolic processes involving cystine (e.g., biosynthesis of coenzyme A). This was confirmed by Mascitelli-Coriandoli and Citterio (1959), who demonstrated a reduction of coenzyme A in the liver of rats administered sodium vanadate (single intraperitoneal injection of 5–10 mg/kg, or a dietary concentration 500 mg/kg).

Inhibition of the cholesterol synthesis in animals has been reported by vanadyl sulfate both *in vitro* and *in vivo* (Azarnoff and Curran, 1957; Azarnoff *et al*., 1961; Curran, 1954; Korhov, 1965). *In vivo* inhibition of the biosynthesis of cholesterol was reported in young rats because of interference of vanadium with acetoacetyl Co-A decyclase; however, in adult animals, inhibition could be demonstrated only *in vitro* (Curran, 1954; Curran and Burch, 1967). Decreases in plasma cholesterol levels after both acute and long-term administration has also reported (Curran and Costello, 1956; IPCS, 1990). Zychlinski *et al*. (1991) reported that blood glucose was slightly decreased and blood cholesterol markedly reduced in rats IT exposed to 0.56 mg/kg of V_2O_5 once a month for 12 months.

Other effects of vanadium on mammalian metabolism include depression of phospholipid synthesis and lipid peroxidation *in vivo* (Snyder and Cornatzer, 1958; Zaparowska *et al*., 1999), reduction of coenzyme Q levels and succinic dehydrogenase in isolated mitochondria (Aiyar and Sreenivasan, 1961), and *in vitro* stimulation of monoamino oxidase, which oxidizes serotonin (Perry *et al*., 1955). At higher concentrations, vanadium may inhibit the oxidation of serotonin, resulting in a decrease of urinary excretion of 5-hydroxy-indoleacetic acid; this effect has been demonstrated in dogs by Lewis (1959c) and in rabbits and rats by Roshchin (1968).

Wright *et al*. (1960) described how vanadium caused a decrease of adenosine triphosphate (ATP) in mammalian metabolism. In the late 1970s, Cantley *et al*. (1977) isolated a modifier of Na-K-ATPase, previously discovered in commercial ATP extracted from horse skeletal muscle (Cantley, 1977; Charney *et al*., 1975). This modifier was found to be vanadate. It was subsequently shown that vanadate was a potent inhibitor of this enzyme system. Many other enzyme systems are also inhibited or modified by vanadate (e.g., Ca-ATPase, adenylate kinase, ribonuclease, and glucose-6-phosphatase) (for reviews, see Lancet, 1981; Jandhyala and Horn, 1983; and Nechay, 1984). Carmignani *et al*. (1996) reported that the levels of noradrenalin, adrenalin, L-dopa, and dopamine were elevated in rabbits exposed for 1 year to 1 μg/L sodium metavanadate in their drinking water. The catecholamines probably caused the marked increase of the activity of monoamine oxidase observed in renal tubules and liver, and a reduction of glucose-6-phosphatase in the kidney. Vanadium compounds have also been shown to inhibit or stimulate the activity of many DNA or RNA enzymes, giving rise to several genotoxic and mutagenic outcomes (Mukherjee *et al*., 2004).

In 1980, vanadium was reported to mimic the actions of insulin on hexose uptake and glucose metabolism in rat adipocytes (Shechter *et al*., 2003). The effects were not related to the inhibition of Na^+-K^+-ATPase but rather to inhibition of several key metabolic enzyme systems in liver, muscle, and adipose tissues acting toward utilizing or storing glucose, and/or blocking the effects of hormones that oppose insulin action. In 1985, Heyliger *et al*. demonstrated that providing vanadium (in drinking water) to streptozotocin-induced diabetic rats restored elevated blood glucose to normal. Later on it was shown that vanadyl sulfate ($VOSO_4$) could lower elevated blood glucose, cholesterol, and triglycerides in a variety of diabetic rat models. The mechanism of action is not known, but one hypothesis is that vanadium can activate serine/threonine kinases distal to the insulin receptor by preventing dephosphorylation because of inhibition of phosphatases.

7.2.2 Humans

7.2.2.1 Experimental and Occupational Exposure

Evidence of systemic effects in workers exposed to vanadium compounds is scarce. In most cases, no systemic effects have been reported (Eisler *et al*., 1968; Lewis, 1959b; Williams, 1952; Zenz *et al*., 1962). Other reports describe only vague or general signs and symptoms such as weakness, ringing in the ears, nausea, vomiting, and headache (Milby, 1974; Roshchin, 1962). Several observers have reported nervous disturbances described as dizziness or giddiness (Milby, 1974; Sjöberg, 1950), neurasthenic or vegetative symptoms (Minden, 1968; Sjöberg, 1950) and, occasionally, tremor (Sjöberg, 1950). Palpitation of the heart on exercise or at rest was mentioned by Milby (1974). Other cardiac signs include transient coronary insufficiency and an unexpectedly high incidence of extrasystoles (Sjöberg, 1950). Roshchin (1964) and Watanabe *et al*. (1966) related blood changes, such as anemia, leukopenia, and basophilic granulation of leukocytes to vanadium exposure. However, Kiviluoto *et al*. (1981b) found no statistically significant abnormalities in hematological parameters in workers exposed to 0.01–0.04 mg/m^3 of V_2O_5 compared with control employees.

Results from early studies on the effects of vanadium on cholesterol are not unanimous. Reduced levels of cholesterol in the blood were reported by Lewis (1959a), Roshchin (1964), and Watanabe *et al*. (1966). Curran *et al*. (1959) also demonstrated a significant reduction of cholesterol in the serum of five young healthy subjects given diammonium oxytartarovanadate orally for 6 weeks (100–125 mg/day). A similar observation was made by Blankenhorn and Chin (1964), but this could not be confirmed in patients with

hypercholesterolemia (Schroeder *et al*., 1963; Somerville and Davies, 1962). In humans, high doses (300 mg/day) of vanadyl sulfate, which caused gastrointestinal side effects, administered to eight type-2 diabetic patients for 6 weeks caused a decrease of fasting serum cholesterol from 5.28 ± 0.64 after placebo treatment to 4.70 ± 0.51 mmol/L after treatment with vanadyl sulfate (Goldfine *et al*., 2000). This was accompanied by a decrease in high-density lipoprotein (HDL) cholesterol. Lower doses, 75 mg or 150 mg, did not have the same effect.

Mountain *et al*. (1955) and Thürauf *et al*. (1979) reported reduced fingernail cystine in vanadium workers. However, Kiviluoto *et al*. (1980) found no significant correlation between exposure and fingernail cystine in 63 vanadium refinery workers. Similar findings were reported by Kucera *et al*. (1994).

7.2.2.2 Therapeutic and Other Exposures

Early studies on the toxicity to man of sodium metavanadate and related compounds derived from vanadium pentoxide and intended for therapeutic use (such as treatment of syphilis) have been summarized by Faulkner Hudson (1964) and Browning (1969). The main signs and symptoms of intoxication caused by single intravenous doses of the order of 100 mg V_2O_5 included nausea, vomiting, salivation, and lacrimation, disappearance of pulse, and cylindrical casts and albumin in urine. Tolerated and toxic doses did not differ much.

Vanadium compounds have been shown to mimic insulin in *in vitro* and *in vivo* systems and exert antidiabetic effects in rodent models of type I and type II diabetes and in human diabetic subjects. In type I diabetic patients, vanadium lowered the insulin requirement after administration of 125 mg metavanadate/day (equivalent to 0.83 mg vanadium/kg body weight/day) for 2 weeks (Goldfine *et al*., 1995). The same treatment in type II diabetic patients improved insulin sensitivity. Diarrhea was the main side effect observed. For reviews see Sekar and Shechter, 1996; Poucheret *et al*., 1998; and Srivastava, 2000).

Vanadium is claimed to be anabolic and is used as a mineral supplement in bodybuilding. Up to 60 mg/day of vanadyl sulfate, equivalent to 18.6 mg V/day, is reported to be used by weight-training athletes (Barceloux, 1999). Fawcett *et al*. (1996; 1997) studied effects of oral vanadyl sulfate (0.5 mg/kg/day for 12 weeks in 31 weight-training athletes) in a double-blind, placebo-controlled trial. They did not find any vanadyl-related effects on body weight, blood pressure, hematological indices, and blood viscosity or on biochemical parameters relating to liver and kidney function or lipid metabolism.

7.2.2.3 General Population Studies

Studies relating environmental levels of vanadium to morbidity have been reviewed by IPCS (1988). No cause-effect relationships could be established from these studies. One epidemiological study on human exposure to vanadium dust from the vicinity of a vanadium refinery plant in Czechoslovakia is reported by Kucera *et al*. (1992). Children living near the plant were compared with nonexposed children. The most prominent difference between the groups was a decrease in natural cell-mediated immunity. A higher incidence of viral and bacterial infections was registered from the exposed area. However, confounding from exposure to other factors than vanadium could not be controlled. Further studies on reference levels of vanadium in blood, serum, and hair are reviewed in Section 6.

Naylor and Smith (1981) suggested that elevated tissue levels of vanadium were important in the etiology of manic-depressive illness. Improvement after treatment with ascorbic acid or reduced vanadium intake was seen both in manic and depressed patients. Electrolyte changes in these patients have been investigated for many years, and it has been suggested that they might have a defective control of the sodium pump, which makes them vulnerable to vanadate (see the review by Naylor, 1983). Bencherif and Lukas (1992) proposed that vanadate treatment augments inositol triphosphate accumulation *in situ*; because it has been shown *in vitro* that vanadate inhibits hydrolysis of different inositol compounds *in vitro*. This is supported by the fact that lithium, which inhibits hydrolytic degradation of inositol monophosphates, is the most common therapeutic agent used in the treatment of bipolar disorder.

The most important sources of environmental contamination with vanadium are combustion of oil and coal. Therefore, the health risks to the general population are acute and possibly chronic effects on the respiratory system. The lowest observed adverse effect level (LOAEL) for acute exposure is 60 $\mu g/m^3$ (WHO, 2000). Available data from occupational studies suggest that the LOAEL for chronic upper respiratory tract symptoms can be assumed to be 20 $\mu g/m^3$. It is suggested in the Air Quality Guidelines that a 24-hour value of 1 $\mu g/m^3$ environmental exposure is not likely to have adverse effects on health.

8 TREATMENT OF VANADIUM POISONING

Most cases recover after removal from exposure and symptomatic treatment (IPCS, 1990). In case of ingestion, ascorbic acid and local treatment of the mucosa in the

mouth and pharynx should be considered in the acute phase (IPCS, 1990; Jones and Basinger, 1983). BAL (2,3-mercapto-1-propanol) was applied successfully in two human cases of overexposure in the 1950s (Sjöberg, 1955); it is still a useful therapeutic agent and could be used in later stages (IPCS, 1990).

In animal studies, Tiron (sodium 4,5-dihydroxy-bensene-1,3-disulphonate) has been shown to reduce the tissue accumulation and increase the urinary excretion of vanadium (Domingo *et al*., 1995). Experimental studies have shown that the chelating agents, DTPA (diethylenetriamine-pentaacetic acid) and DMSA (dimercaptosuccinic acid), are not effective, and there are no data on the use of DMPS (dimercapto-1-propane sulfonic acid) (IPCS, 1990).

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CHAPTER

47

Zinc*

HAROLD H. SANDSTEAD AND WILLIAM AU

ABSTRACT

Zinc (Zn) is environmentally ubiquitous and essential for life. The curve in Figure 1 represents Zn nutriture. The left and right ascending limbs, respectively, represent risk of deficiency or excess. At the base is the desirable range of intake. The actual shape of the curve is unknown. Human Zn-deficiency is common throughout the life cycle, especially in populations with diets that infrequently include animal flesh, and thus are limited primarily to plant products, many of which are rich in indigestible Zn-binding ligands. One estimate suggests 20% of the world's population is at risk of Zn deficiency. Manifestations include abortion, teratology, prematurity, retarded growth and development, low immunity, poor healing, dermatitis, low physical work capacity, abnormal neuropsychological functions, and other abnormalities.

Human Zn toxicity is considerably less prevalent. Inhalation of Zn oxide fume generated by welding and certain other industrial processes causes metal fume fever, a self-limited flulike illness. In contrast, inhalation of Zn chloride, from smoke bombs, causes severe pulmonary injury and may be fatal. Ingestion of food or drink contaminated by exposure to galvanized surfaces can cause nausea, vomiting, cramps, and diarrhea that is seldom fatal. Uncontrolled consumption of dietary supplements rich in Zn represents a hazard that is not widely appreciated; intestinal absorption of Cu is suppressed, and Cu deficiency can occur. A similar phenomenon can occur when oral intakes of Zn are physiological or near physiological, and intakes of Cu intakes are in the range of the most recent recommended dietary allowance. Present data suggest the risk of Cu deficiency is increased when the oral intake Zn/Cu molar ratio is >18; however, the critical level is unknown.

Preparation of this chapter was facilitated by committee reports and reviews (e.g., the 2001 International Program for Chemical Safety Environmental Health Criteria Report 221, Zinc (Simon-Hettich, *et al*. 2001), the 2005 US Environmental Protection Agency Toxicological Review of Zinc and Compounds (Choudhury *et al.*, 2005), a report sponsored by the International Lead Zinc Research Organization (Walsh *et al.*, 1994), and a review of Zn in soils and crops sponsored by the International Zinc Association (Alloway, 2003). Other sources were identified through PubMed, *http://www.ncbi.nlm.nih.gov/entrez/query.fcgi*, and Google Scholar, *http://scholar.google.com/schhp?hl=en&tab=ws&q=*

1 IDENTITY AND PHYSICAL/CHEMICAL PROPERTIES

Properties of Zn include: atomic weight, 65.38; atomic number, 30; density, 7.13 g/cm^3; Mohs' hardness, 2.5; melting point, 419.5°C; boiling point, 908°C; and electrical conductivity, 28.3% of the international annealed copper standard. Crystalline Zn is a bluish white metal with a distorted hexagonal close-packed structure. There are five stable isotopes: ^{64}Zn (49%), ^{66}Zn (28%), ^{68}Zn (19%), ^{67}Zn (4.1%), and ^{70}Zn (0.62%) and 19 known radioactive isotopes. The half-life of ^{65}Zn is 243.8 days, and of ^{69}Zn is 13.8 hours (Simon-Hettich *et al.*, 2001; Choudhury *et al.*, 2005).

*Dr. Carl-Gustaf Elinder was the author of this chapter in the 2nd edition of the *Handbook on Toxicology of Metals*; his text provided guidance.

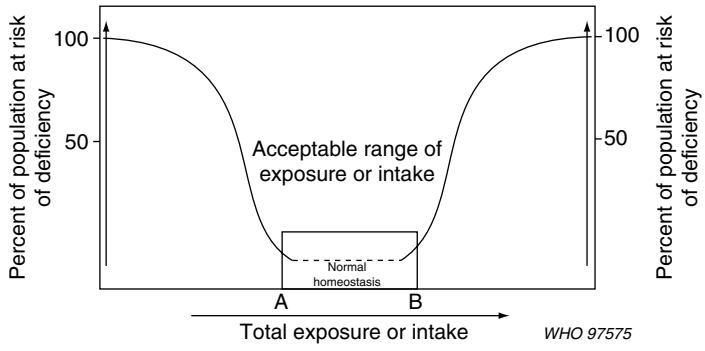

FIGURE 1 Percent of population subjected to deficiency and toxicity effects according to exposure/intake. As intake drops below A, risk for deficiency increases; at extremely low exposures or intakes, all subjects will manifest deficiency. As exposure or intakes increase beyond B, a progressively larger proportion of subjects will exhibit effects of toxicity.

The oxidation state of Zn is +2. It has reducing and several transitional properties and a tendency to react with oxygen, chlorine, and sulfur. It is stable in dry air and is coated by moist air with zinc oxide or basic carbonate. It is amphoteric and forms stable salts that are nonconducting, nonmagnetic, white, or colorless, except for those with a chromophore group such as chromate. At mid-range pH, Zn forms hydroxides that have low solubility in water, whereas at the extremes of pH, solubility is increased, releasing Zn ion at low pH and zincate at high pH.

2 ANALYTICAL METHODS

Because Zn is ubiquitous, special care is required to avoid contamination. Preparation of solid environmental samples typically involves microwave-assisted mineralization with concentrated acids. Water samples may require solvent extraction in the presence of complexing agents and chelating resin separation to preconcentrate Zn (Simon-Hettich *et al.*, 2001).

Element-specific instrumental techniques accurately measure Zn concentrations of 0.006 µg/L in water and 0.01 mg/kg in solid samples (Simon-Hettich, *et al.* 2001). Flame atomic absorption spectrometry, the most common method, has a detection limit of approximately 5 µg/L and an optimal concentration range of 50–2000 µg/L. Other methods include atomic emission spectrometry, X-ray fluorescence, inductively coupled plasma atomic emission spectrometry, inductively coupled plasma mass spectrometry, polarography, anodic stripping voltammetry, and neutron activation analysis. Inductively coupled plasma atomic emission spectrometry and inductively coupled plasma mass spectrometry are more sensitive than flame atomic absorption, and inductively coupled plasma mass spectrometry enables isotope ratio analysis. Specific fluorophores facilitate observation of labile intra- and extracellular Zn (Thompson 2005).

3 SOURCES OF HUMAN AND ENVIRONMENTAL EXPOSURE

3.1 Uses

Fifty-five Zn-containing minerals are known (Choudhury *et al.*, 2005). Commercially, sphalerite (ZnS) is the most important Zn ore, and Zn-oxide is the most common Zn compound in industry. In 1994, world metal production of Zn was approximately 7,089,000 tons, and consumption was approximately 6,895,000 tons. Zinc is used as a protective coating for other metals, in dye casting and the construction industry, and for noncorrosive alloys, and brass. Zinc oxide is insoluble in water and alcohol and soluble in acid and alkali; therefore, Zn is used in the manufacture of rubber and white pigment. Inorganic Zn compounds are used in automotive equipment; storage and dry cell batteries; and dental, medical, and household applications. Organo-Zn compounds are used as fungicides, topical antibiotics, and lubricants (Simon-Hettich *et al.*, 2001).

3.2 General Environment

Zn is the 25th most abundant element, accounting for approximately 0.02% by weight of the earth's crust (Simon-Hettich *et al.* 2001). The average Zn content of rock in the earth's crust is 78 mg/kg^{-1} (Alloway, 2003). Zn occurs only in the divalent state and does not occur as a metal in nature.

3.2.1 Atmosphere

Atmospheric Zn is mostly in aerosols in the oxidized form. Zinc-containing particles up to 5-μm diameter occur in urban areas with industries that release Zn (Nriagu, 1980). Estimates of Zn in the atmosphere from natural sources are 45,000–350,000 tons/year. In 1983–1984, emissions from burning of coal, oil, and refuse were 2570–19,630, 532–3786, and 2950–8850 tons/year, respectively. Zn from industrial emissions decreased from approximately 70,250–193,500 tons in 1983–1984 to approximately 2000 tons in 1996 (Simon-Hettich *et al.*, 2001).

3.2.2 Water

In water, Zn is present in particles of diameter >450 nm as simple hydrated metal ion (e.g., $Zn(H_2O)_6^{2+}$), simple inorganic complexes (e.g., $Zn(H_2O)_5Cl^+$), simple organic complexes (e.g., Zn-citrate), stable inorganic complexes (e.g., ZnS), stable organic complexes (e.g., Zn-humate), adsorbed on inorganic colloids (e.g., $Zn^{+2}Fe_2O_3$), and adsorbed on organic colloids (e.g., Zn^{+2}-humic acid) (Florence, 1980). Emissions into aquatic environments were approximately 77,000–375,000 tons/year in 1983–1984. Since then, there have been substantial decreases (e.g., in the United States discharges into surface water decreased from approximately 386 tons in 1988 to approximately 30 tons in 1993) (Simon-Hettich *et al.*, 2001).

3.2.3 Soil

The zinc content of soils largely depends on weathering of rock, approximately 75% of which is sedimentary (Alloway, 2003). In many places, drift deposits of sand, gravel, and other forms derived from rock cover the underlying rock and are the parent of agricultural soils. Weathering causes release of approximately 915,000 tons of Zn/year in water-soluble forms. Humid hot conditions increase weathering; the resulting acidic soils are low in major and trace plant nutrients. Soils formed on limestone, sandstone, and associated drift tend to have low Zn concentrations in contrast to soils developed on clay, shale, or mafic (basaltic rock, rich in iron and magnesium, that is crystallized from silicate minerals at relatively high temperatures (Lutgens and Tarbuck; 2000)) igneous rock. The low Zn content of soils is an agricultural problem in regions that are sandy or rich in calcium carbonate (Alloway, 2003).

Worldwide, mining and smelting discharge approximately 1–3 million tons of mostly nondispersive Zn onto land. In addition, fertilizer and atmospheric fallout account for approximately $689–2054 \times 10^3$ tons/year, whereas agricultural and animal wastes, sewer sludge, and fly ash contribute $640–1914 \times 10^3$ tons/year (Simon-Hettich *et al.*, 2001). The major impacts of these sources on ecology are local. Worldwide these sources have little impact on Zn-deficient soils, except where Zn amendments are applied (Alloway 2003).

4 ENVIRONMENTAL TRANSPORT, DISTRIBUTION, AND TRANSFORMATION

4.1 Air

Zinc adsorbed on particles of low diameter and density can be transported in air over great distances (Han *et al.*, 2002). Greenland snow records show changes in Zn deposition (e.g., deposition increased fivefold from 1800 to the 1960s, then decreased approximately 40% by the 1990s) (Boutron 1995).

4.2 Water and Sediment

The pH of most freshwaters facilitates adsorption of Zn on particulates. In sediment, Zn is both suspended and in the sediment bed. Zn binds to organic material, especially when the pH is >6.5. Zn also adsorbs to kaolinite and illite clay as hydroxy species in increasing amounts up to pH 10.5 (Farrah and Pickering; 1977). Organic matter is oxidized as it becomes sediment, and because oxygen and nitrate are limited, sulfate is the predominant terminal electron acceptor, thus forming Zn sulfide, which has low solubility (Allen 1993).

Zinc in industrial wastewaters tends to precipitate as Zn hydroxide. At pH 9.5, the lowest soluble Zn concentration is approximately 0.25 mg/L (Patterson *et al.*, 1977). During chemical extraction and acidification of sewage sludge, organic and some insoluble inorganic forms of Zn associated with the tetrasodium pyrophosphate ($Na_4P_2O_7$) fraction compose 18–52% of total Zn. Zinc mobilization begins at pH 6.0, and most of the mobilizable Zn is extracted by pH 2.0. Zinc is more easily extracted from raw sludge than dried forms of activated and digested sludge. Mobilization from liquid sludge begins at pH 4.0 and increases with acidification (Rudd *et al.*, 1988).

4.3 Soil

The Zn content of soils and its bioavailability are critical for plant growth (Adriano, 1986). Soil factors that affect plant nutrition include total Zn, pH, organic matter, calcium carbonate, redox conditions, microbes in the rhizosphere, soil moisture, amounts of other trace elements, and amounts of macronutrients, especially phosphorus. Zinc is present in soil in five pools:

a water-soluble pool; bound to soil particles by electrical charge; bound to organic ligands; nonexchangeable bound to clay and insoluble metal oxides; and weathering minerals (Alloway, 2003). The amphoteric nature of Zn makes it poorly available at intermediate pH but highly available at extremes of pH. Under usual conditions, the proportion of Zn in solution is very small. Below pH 7.7, Zn ion predominates, whereas above 7.7, $ZnOH^+$ is major, and above 9.11, zincates $[Zn(OH)_4]^{2-}$ are soluble (Kiekens, 1995). Low-molecular-weight organic acids that bind Zn are also an important source of soluble Zn.

Adsorption mechanisms determine available Zn ion for plants (Alloway, 2003). Low pH increases cation exchange, and high pH increases chemisorption and complexation to organic ligands. In clay, both reversible Zn ion exchange and irreversible sorption to the mineral lattice occur. The latter mechanism involves fixation of Zn in a hydrolyzed form and precipitation as $Zn(OH)_2$. Over time, Zn fixation decreases Zn availability from Zn fertilizers.

In calcareous soils, Zn chemisorbs on calcium carbonate and forms Zn hydroxycarbonate, which is very stable. Thus, the risk of Zn deficiency in plants is increased. At pH 8 and above, iron oxides precipitate and coat carbonate minerals, which then firmly bind Zn. Zinc coprecipitates with Fe and Mn oxides, and franklinite, $ZnFe_2O_4$. The latter has an important influence on Zn bioavailability apart from adsorption/desorption mechanisms. In addition, reducing conditions that form Zn sulfide impair Zn availability to plants.

Sewage sludge and chemical fertilizers (e.g., Zn nitrate) are effective means of Zn enrichment. Low pH increases Zn mobilization from sludge when it is added to a clay loam or a sandy loam soil but has less effect in the presence of clay (Sanders *et al*., 1987).

4.4 Biotransformation

Biological degradation of Zn complexes and recycling are essential for life. Terrestrial plants assimilate Zn from soil. Factors that affect Zn availability to plants were noted previously. Zinc deficiency in plants is a worldwide problem that is not significantly mitigated by Zn entering the ecosystem from industry (Alloway, 2003). Its impact on humans is primarily through a decrease in the production of edible plants. Zn content of plants effects intake. Zinc enters the terrestrial animal food chain through consumption of plants and soil by herbivores (Thornton and Abrahams, 1983) and by consumption of plants and other animals by omnivores. Under usual conditions, the Zn content of the body cell mass is controlled within the range required for life by importing, exporting, and isolation mechanisms.

5 ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

5.1 Air

Zinc concentrations are usually highest in urban and industrial areas (Simon-Hettich *et al*., 2001). Background concentrations may range from 10–300 ng/m^3. In European rural areas and cities, Zn levels of 0.4–300 ng/m^3 and 10–2400 ng/m^3, respectively, are reported. Indoor urban air Zn concentrations are approximately 0.1–1.0 $\mu g/m^3$. Evidence of Zn effects on human health at air concentrations usually encountered is rare or nonexistent.

5.2 Water

The Zn content of fresh clean water and drinking water worldwide was estimated to be 3.25 µg/L (range, 0.64–16.6) (Zuurdeeg, 1992). However higher Zn concentrations (as much as 2 mg/L) can occur when low pH drinking water passes through Zn-containing pipes (Schroeder *et al*., 1967). Although Zn concentrations in water from active or inactive mines can be substantial (e.g., 900 µg/L in acidic mine tailing ponds in Canada (Simon-Hettich *et al*., 2001), and sometimes enters streams), the effects on human health by contamination on water supplies must be rare. Very high concentrations of Zn are present in the tissues of oysters and similar shellfish that filter seawater. Although the Zn in the flesh of these animals is readily bioavailable, no reports of Zn-related illness associated with frequent consumption of oysters were found.

5.3 Soil

Noted in Section 3.2.3, the Zn content of soils is affected by weathering and varies widely. Average Zn concentrations of noncontaminated soil were reported as 40–90 mg/kg, with a wide range of 1–2000 mg/kg (Adriano, 1986). In addition, discharges from industrial sources contribute to both local and distant Zn concentrations in soil and plants. High concentrations in soil of Zn impair health of susceptible plants (Shuman *et al*., 2001; Palazzo *et al*., 2003) and are reflected in tissues of herbivores that consume soil as they graze (Reif *et al*., 1989; Carpenter *et al*., 2004). Sewage sludge soil amendments also add Zn to soil. Of concern is the accumulation of cadmium in sewage sludge in edible portions of plants (Hooda *et al*., 1997). High concentrations of Zn in sludge inhibit plant uptake of cadmium (Chaney, 1988). Zinc concentrations in composted sewage sludge range from 101–49,000 mg/kg dry weight, with a mean of approximately 1700 mg/kg (Chaney, 1983). Because Zn in soils enters the human food chain primarily through plants and animals whose

TABLE 1 Zinc Content of Foods Commonly Consumed in the United States, per Common Measure (Anon, 2002)

>15 mg	5–10 mg	4–5 mg	3–4 mg	2–3 mg	1–2 mg	<1 mg
Oyster	Beef	Beef liver	Lamb	Lamb	Pork loin	Chicken breast
Peanut Butter Crunch ®	Lamb	Beef	Pork	Pork	Chicken dark meat	Chicken liver
Product 19 ®	Duck	Lamb	Veal	Lobster	Swordfish	Salmon
Total ®	King Crab	Pork	Turkey dark meat	Clams	Shrimp	Tuna
	Wheaties ®	Captain Crunch ®	Blue Crab	Yogurt	Mushroom	Other finfish
		Quaker Oats ®	Rice Chex ®	Skim milk	White wheat flour	Vegetables
			Corn Chex ®	White beans	Navy beans	White rice
			Cheerios ®	Chick peas	Black beans	Eggs
			Whole wheat flour	Lentils	Pinto beans	Tofu
				Corn meal	All Bran ®	Cheddar cheese
					Nuts	Blue cheese
						Cottage cheese
						Nuts

homeostatic mechanisms control the amount of Zn present in tissues, high concentrations of Zn in soil have not been reported to be associated with human disease.

Certain clays consumed during geophagia might adsorb Zn from contents of the small intestine and thus contribute to Zn deficiency (Halsted *et al*., 1972; Çavdar *et al*., 1983).

The Zn content of foods varies widely (Table 1). Reported Zn intake, including children, in several industrialized countries and India varies widely, from 3.1 (Campbell-Brown *et al*., 1985) to 18.6 mg (Simon-Hettich *et al*., 2001). A national survey of the United States found the Zn intakes in Table 2. For comparison, a 2000–2001 survey of British adults, aged 19–64 years,

TABLE 2 Median Zinc Intake of Participants in NHANES-III (Alaimo *et al*., 1994)

	White		Hispanic		Black	
	n	*Median*	*n*	*Median*	*n*	*Median*
Male						
2–11 m	241	5.59	89	6.32	78	6.28
1–2 y	202	6.67	186	5.81	182	6.74
3–5 y	219	7.28	281	7.80	210	8.13
6–11 y	252	9.02	344	9.27	239	9.17
12–15 y	98	11.62	129	10.48	95	8.91
16–19 y	112	13.43	139	12.04	103	12.28
20–29 y	216	13.14	349	13.27	245	12.90
30–39 y	271	13.88	225	13.19	213	10.77
40–49 y	243	12.25	181	12.35	178	10.55
50–59 y	251	12.27	96	9.73	105	8.40
60–69 y	247	11.52	152	8.71	141	8.77
70–79 y	285	10.34	60	8.13	93	7.84
80 y +	250	9.06	19	7.74	21	7.04
Female						
2–11 m	232	5.55	74	5.89	84	5.80
1–2 y	222	5.65	216	5.72	173	5.69
3–5 y	206	6.48	328	6.79	244	7.47
6–11 y	259	7.70	383	8.07	213	8.12
12–15 y	123	8.07	140	8.65	96	8.53
16–19 y	133	8.38	131	8.62	114	9.37
20–29 y	244	8.41	317	8.80	254	8.86
30–39 y	279	8.78	247	8.39	241	7.55
40–49 y	224	8.55	185	7.80	160	7.16
50–59 y	221	7.94	100	8.13	125	7.01
60–69 y	246	7.71	153	6.80	148	6.93
70–79 y	253	7.18	51	6.42	93	6.37
80 y +	251	6.59	23	5.26	35	5.92

found mean Zn intakes of 10.7 ± 5.75 mg, in men and 7.9 ± 3.54 mg in women (Office of National Statistics, 2002), and a survey of boys and girls aged 15–18 years had intakes similar to those of adults, although 11–14-year-olds had intakes of 7.7 mg and 6.7 mg, respectively (Thane *et al*., 2004).

5.4.1 Plants

Pulses and cereals are the major sources of dietary Zn for most people (Gibson 1994). In contrast, in the United States pulses and cereals provide approximately 30% of dietary Zn (Walsh *et al*., 1994). The effect of processing on the Zn content of some plant-derived foods is shown in Table 1. For example, the Zn content of yellow whole grain cornmeal is 2.2 mg/cup (122 g) and of yellow degermed corn meal is 1.0 mg/cup (138 g); the Zn content of cooked long grain brown rice is 1.2 mg/cup (195 g), and of cooked long grain white rice is 0.5 mg/cup (175 g). Pulses are usually richer in Zn than refined cereals. For example, the Zn content of beans ranges from 1.9 mg/cup (172 g) in cooked mature black beans to 2.5 mg/cup (164 g) in boiled chickpeas.

Indigestible plant ligands such as phytate, some dietary fibers and lignin, and products of nonenzymatic Maillard browning formed during cooking inhibit intestinal Zn absorption (Sandstead and Smith, 1996). Removal of Zn-binding ligands by milling and/or fermentation improves Zn bioavailability (Sandstrom and Sandberg, 1992; Larsson *et al*., 1996).

5.4.2 Flesh Foods

Animal flesh is the best source of readily bioavailable Zn. In the United States, meat provides approximately 50% of dietary Zn (Walsh *et al*., 1994). Red meat is the richest common source compared with fowl and fish (Table 1). Preferential consumption of poultry, fish, and dairy products instead of red meat increases the risk of Zn deficiency (Yokoi *et al*., 1994; Yokoi *et al*., 2003).

5.4.3 Dairy Products

Cow milk products provide approximately 20% of the daily Zn intake in the United States (Walsh *et al*., 1994). Adult absorption of ^{65}Zn tracer added to cow's milk was 28% compared with 31% from "humanized" cow's milk infant formula, 41% from human milk, and 14% from soy formula (Sandstrom *et al*., 1983).

5.4.4 Nutritional Supplements

A national survey in the United States found that approximately 40% of the population takes nutritional supplements (Ervin *et al*., 1999). Consumption is common among children, aged 1–5 years, older adults, and individuals with more education and income. By current standards (Trumbo *et al*., 2001), among US adults older than 60 years of age, 35–41% of men and 36–45% of women have "low" dietary intakes of Zn that can be improved by Zn supplementation (Ervin *et al*., 2002). In contrast, a survey of German young persons, aged 2–18 years, found approximately 6% consumed supplemental minerals (Sichert-Hellert and Kersting, 2004).

5.5 Work Environment

5.5.1 Inhalation

Exposure to metallic Zn and Zn compounds occurs during Zn mining, smelting, welding, and other uses of Zn compounds (Simon-Hettich *et al*., 2001). Zinc-oxide fume and dust are regulated in many countries at 5–10 mg/m^3 consistent with guidelines of the International Labor Organization (ILO) to prevent respiratory illness. Data from several European countries and the United States suggest this goal is being achieved.

6 BIOLOGICAL MONITORING

6.1 Direct Indicators of Zn Status

6.1.1 History

Dietary history is a key indicator of risks to Zn nutriture. A focused history of the frequency of consumption of flesh foods, foods rich in nondigestible Zn-binding ligands, and Zn supplements can provide qualitative insights (Yokoi *et al*., 2003). A detailed quantitative history will provide deeper understanding of Zn intake and factors that can affect Zn use (e.g., Ca [Wood and Zheng, 1997], Fe [Solomons, 1986], Cu [Sandstead, 1995], Cd [Brzoska and Moniuszko-Jakoniuk, 2001; Reeves and Chaney, 2002], Pb [Godwin, 2001; Basha *et al*., 2003], phytate [Sandstrom and Sandberg, 1992], dietary fiber [Knudsen *et al*., 1996], Maillard browning products [Lykken *et al*., 1986], and folate [Milne *et al*., 1984; Simmer and Thompson, 1985]). High dietary phytate × Zn and phytate × Ca/Zn molar ratios are indicative of increased risk of low Zn bioavailability (Gibson *et al*., 2003).

6.1.2 Plasma/Serum Zinc

Plasma/serum Zn is the least sensitive indicator of Zn status. Concentrations may (or may not) decrease with Zn deficiency (Pekarek *et al*., 1979) and may (or may not) increase with toxicity (Kumar and Jazieh 2001). Many factors affect plasma Zn concentration:

fasting concentrations are higher in the morning; concentrations decrease after meals; men tend to have higher concentrations than premenopausal women; infants, children, and the aged (Hotz, 2003). The empirical lower limit of normal for fasting plasma Zn is 10.7 μmol/L (700 μg/L) (Pilch and Senti, 1985). However, a study of 33 premenopausal women not taking oral contraceptives found significant correlations between components of the three-compartment kinetics model 24 hours after administration of a stable Zn tracer; the break points in the plasma Zn–Zn kinetics relationship for different kinetic parameters ranged from 9.94 μmol/L (650 μg/L) to 11.5 μmol/L (750 μg/L) (Yokoi *et al*., 2003).

6.1.3 White Blood Cell Zinc

Leukocyte and lymphocyte Zn concentrations are more reflective of Zn status than plasma Zn (Prasad *et al*., 1978; Meadows *et al*., 1981). Unfortunately, isolation and analysis of Zn in white blood cells and platelets is technically demanding (Milne *et al*., 1985).

6.1.4 Hair Zn

The finding of low concentrations of Zn in hair is indicative of Zn deficiency (Strain *et al*., 1966). For example, low hair Zn may be associated with growth failure in children (Ferguson *et al*., 1993; Cavan *et al*., 1993; Gibson *et al*., 1991). The lower limit of "normal" is approximately 1.68 mmol/g. In contrast, hair Zn concentrations were not related to Zn status of 33 premenopausal women who were not taking oral contraceptives (Yokoi *et al*., 2003) or dietary Zn or Zn bioavailability in 330 premenopausal New Zealand women aged 18–40 years (Gibson *et al*., 2001).

Hair Zn is not commonly measured to ascertain increased Zn exposure. However, it increases with increased Zn intake in humans (Pekarek *et al*., 1979) and nonhuman primates (Marriott *et al*., 1996) and, therefore, should be considered a potential marker of excess Zn exposure.

6.1.5 Urine Zinc

Urinary Zn excretion is usually decreased in Zn deficiency (Prasad *et al*., 1963) and increased by Zn excess (Bonner *et al*., 1981; Fuortes and Schenck, 2000; Main *et al*., 1982). Zinc-depleted men displayed urine Zn concentrations <150 μg/day, although at the same time plasma Zn remained >700 μg/L (10.7 μmol/L) (Baer and King, 1984). However, large excretions of Zn can occur in Zn-deficient individuals afflicted by hemolytic anemia (Prasad *et al*., 1975), liver cirrhosis and/or alcoholism (Sullivan and Heaney, 1970; Allan *et al*., 1975), noninsulin-dependent diabetes mellitus (Raz *et al*., 1989; Cunningham *et al*., 1994), or severe catabolism (Cuthbertson *et al*., 1972; Fell *et al*., 1973).

6.2 Indirect Physiological Indicators of Zn Status

6.2.1 Alkaline Phosphatase

Alkaline phosphatase requires Zn for structure and function. If other influences on activity are absent, plasma alkaline phosphatase tends to correlate with plasma Zn concentration. Low activity of plasma alkaline phosphatase is sometimes observed in association with severe Zn deficiency (Arakawa *et al*., 1976).

6.2.2 Ecto 5′-Nucleotidase

Ecto 5′-nucleotidase is a cell membrane enzyme that requires Zn (Zimmermann, 1992). Plasma activity of this enzyme is more sensitive to decreases in Zn status than plasma Zn concentration (Bales *et al*., 1994). Lymphocyte ecto 5′-nucleotidase activity is also a more sensitive index of Zn status than plasma Zn (Meftah *et al*., 1991). The enzyme can be assessed by cell sorter and immunological determination of the percentage of CD73+ cells in the CD8+ T-lymphocyte population (Beck *et al*., 1997).

6.2.3 Immunity

Zinc is essential for immunity (Fraker *et al*., 2000; Fischer Walker and Black, 2004). The high sensitivity of immunity to Zn status is illustrated by findings in mice. Mildly Zn-deprived mice that maintained thymus weight and body weight displayed an increase in the number of apothymulin-positive thymic epithelial cells and the plasma concentration of apothymulin, although plasma thymulin activity decreased (Dardenne *et al*., 1984). Thymulin is a nonapeptide hormone whose active structure requires Zn (Bach and Dardenne; 1989). Thymulin induces differentiation and function of T cells.

The threshold at which limited Zn status impairs immunity in humans is unknown. Human infants recovering from protein-energy malnutrition displayed decreased thymus size and skin reactivity to a common antigen. Zn treatment restored these indices to normal (Golden *et al*., 1977; Golden *et al*., 1978). Consistent with these findings, a Zn-deprived man fed an otherwise adequate oral formula displayed low lymphocyte transformation after *in vitro* stimulation by phytohemagglutinin. Zinc treatment restored function (Pekarek *et al*., 1979). More detailed investigation in men subjected experimentally to mild Zn deprivation revealed suppressed generation

of new CD4+ T lymphocytes and maintenance of cytolytic T lymphocytes and decreases in *in vitro* production of interferon-γ, interleukin-2, and tumor necrosis factor-α by mitogen-stimulated TH1 cells, although TH2 cell functions, such as production of IL-4, IL-6, and IL-10, were unaffected (Beck *et al*., 1997). Practical application of this knowledge by Zn administration to infants in less developed countries prevented diarrhea (18% reduction; 95% confidence interval [CI], 7–28%) and pneumonia (41% reduction; 95% CI, 17–59%) in two studies (Fischer Walker and Black, 2004), and by inclusion with oral rehydration fluids decreased duration of diarrhea by 15% (95% CI, 5–24%) and reduced morbidity from pneumonia (Bhutta *et al*., 2000).

Excess Zn also suppresses some aspects of cell-mediated immunity (Stacey, 1986; Chandra, 1984).

6.2.4 Neuropsychological Functions

Zinc is essential for brain development and function (Sandstead *et al*., 2000). Clinical effects of deficiency range from ataxia, confusion, and dementia (Henkin *et al*., 1975) to relatively mild abnormalities in neuropsychological functions (Penland, 2000). For example, a prospective Zn-deprivation study in 14 men (Johnson *et al*., 1993) induced abnormal neuropsychological functions within 35 days (Penland, 1991). Consistent with these findings, a randomized controlled trial in 740 low-income Chinese school children, aged 6–9 years, found

TABLE 3 Zinc and Copper Intakes, Effects on Copper Nutriture[a]

N	Gender	Time (days)	Cu intake (mg/day)	Zn intake (mg/day)	Zn/Cu molar ratio	Diet	Response	Reference
7	F	165	1.25 ± 0.20	~15	~11.7	Food	Nil	(Johnson *et al., 1988)*
24	M	77	1.03/2850 kcal	~25	23.7	Food: 20% of energy fructose	4/24 Abn ECG, ↓ ESOD with ↑ Fructose, ↓ pl leu- & met-enkephalins, ↑ pl beta-endorphins, ↑cholesterol	(Bhathena *et al*., 1986; Holbrook, Smith, and Reiser, 1989; Reiser *et al*., 1987; Reiser *et al*., 1985)
8	M	105-120	0.89 ± 0.09	19	20.8	Food	1/8 abn ECG, 5/8 ↓ESOD, 2/7 ↓plCu, 4/8 ↓RIDCp, 1/8 ↓ENZCp, 2/8 ↓oral GTT, 1/8 ↑cholesterol	(Klevay *et al*., 1986; Klevay *et al*., 1984; Milne *et al*., 1990)
11	M	42	0.79	~14.7	~18.1	Food and formula	Nil	(Turnlund, Keen, and Smith, 1990)
8	F	84	~0.70	~16	~22.3	Food	8/8 ↓ENZ Cp, ↑stress BP	(Lukaski, Klevay, and Milne, 1988; Milne, Klevay, and Hunt, 1988)
10	M	36	0.6/2500 kcal	11.9/2500 kcal	19.3	Food	↓ ENZ Cp, 4/10 ↓ ESOD, 4/10 ↓MNC CCO, 4/10 ↑glutathione	(Nielsen *et al*., 1990)
6	M	48	0.6/2500 kcal	25.8/2500 kcal	41.9	Food, fructose	3/6 Abn ECG, ↓ESOD, ↓RIDCp	(Milne and Nielsen, 2003)
12	F	105	0.64/2500 kcal	12/2500 kcal	18.3	Food	3/12 Abn ECG; 10/12 ↓Plt CCO, ↓ESOD, ↓Plt Cu, ↓EGPX, ↑Clotting factors V and VIII	(Milne and Nielsen, 1996)
11	M	42	0.38	14.7	37.7	Food and formula	↓plCu, ↓ENZCp	(Turnlund *et al*., 1997)
13	F	90	1.0/2000 kcal	53	51.6		↓ Short term general recall, ↓ number sequence recall. Contact Penland for more formation	(Penland *et al*., 1999)

[a] In part from Milne (Milne, 1998).

* abbreviations: ECG, electrocardiogram; ESOD, erythrocyte superoxide dismutase; plCu, plasma copper; ENZCp, enzymatic activity of ceruloplasmin; MNC (Plt) CCO, monocyte (platelet) cytochrome c oxidase; EGPX, erythrocyte glutathione peroxidase; plZn, plasma zinc; Hgb, hemoglobin

that Zn administration, given other potentially limiting micronutrients, was more efficacious for neuropsychological functions than Zn alone or other micronutrients alone (Sandstead *et al*., 1998). In addition, a study in 221 low-income infants from rural Bangladesh found that Zn given alone, with iron, and/or other micronutrients had a beneficial effect on orientation-engagement behaviors (Black *et al*., 2004).

Zinc is also essential for nerve conduction (Terril-Robb *et al*., 1996; Goto *et al*., 2001). In 12 patients with renal failure undergoing hemodialysis, polyneuropathy and hypogeusia improved after Zn administration (Sprenger *et al*., 1983). In another study, 33 premenopausal women not taking birth control pills displayed an association between plasma Zn <10.7 μmol/L (700 μg/L) and increased sensitivity of gustatory nerves to electrical stimulation, in contrast to subjects with plasma Zn >10.7 μmol/L (Yokoi *et al*., 2003).

In contrast to the preceding, administration of 53 mg Zn daily by mouth adversely affected verbal memory of postmenopausal women in a prospective experiment (Penland *et al*., 1999) (Table 3).

It is unknown how rapidly Zn deficiency or excess can change neuropsychological function or how rapidly recovery occurs.

6.2.5 Dark Adaptation

Zinc and retinol are essential for dark adaptation. Retinol dehydrogenase/retinaldehyde reductase requires Zn for function (Russell *et al*., 1983). The efficacy of Zn for nyctalopia (night blindness) was shown in patients with Zn deficiency and alcohol related or biliary cirrhosis (Morrison *et al*., 1978; Herlong *et al*., 1981) and patients with sickle cell disease (Warth *et al*., 1981). The phenomenon can occur before plasma Zn is decreased and is likely to be associated with low leukocyte Zn.

6.2.6 Taste Acuity

Zinc nutriture is one of many factors that affect the sense of taste (Goto *et al*., 2001; Russell *et al*., 1983). Experimental Zn deprivation (Henkin *et al*., 1975; Ruz *et al*., 1991), low Zn status in Crohn's disease (Solomons *et al*., 1977), and gluten enteropathy (Solomons *et al*., 1976) lowered taste acuity (hypogeusia). A study of 385 older persons from Italy, the United Kingdom, and France associated low Zn status with hypogeusia (Stewart-Knox *et al*., 2005).

6.2.7 Growth and Body Composition

The profound effects of severe Zn deficiency on growth and development prompted the discovery of human Zn deficiency (Halsted *et al*., 1972; Prasad *et al*., 1961; Prasad *et al*., 1963; Sandstead *et al*., 1967; Halsted, 1977). Demonstration of the efficacy of Zn for growth-retarded children is best accomplished by assurance that other potentially limiting micronutrient deficiencies are absent (Ronaghy *et al*., 1974; Sandstead, 1999; Solomons *et al*., 1999). Failure to control treatment experiments for other micronutrient deficiencies can result in no growth (Carter *et al*., 1969; Ronaghy *et al*., 1969) or suboptimal growth (Brown *et al*., 2002).

Because growth is a relatively slow process, observations are usually made over long intervals of time using standardized instruments (Gibson, 1990). Alternately, increases in stature can be detected over considerably shorter intervals by measurement of the lower leg by use of a highly sensitive digitized device (Sandstead *et al*., 1998; Cronk *et al*., 1989; Stallings and Cronk 1993).

Methods that accurately detect small changes in the components of body composition are, for technical reasons, generally not suitable for population surveys or other field studies. Such methods include measurement of ^{40}K by whole-body gamma counter to derive body cell mass, densitometry to derive body fat, and isotope dilution measurement of body water by use of deuterium oxide (Lukaski *et al*., 1981). In addition, dual energy X-ray absorptiometry (DEXA) is used to measure fat mass, lean tissue mass, and total body bone mineral (Haarbo *et al*., 1991).

In contrast to other approaches, body composition (body water, body fat, and fat-free mass) can be derived by use of a portable instrument that measures bioelectrical impedance (Lukaski *et al*., 1985). The method was used to assess patients with HIV (Earthman *et al*., 2000), postsurgical patients (Meguid *et al*., 1992), healthy children (Goran *et al*., 1993), children with acute and subacute hydration disorders (Mazariegos *et al*., 1998), Mexican-American children at risk of type 2 diabetes mellitus (Trevino *et al*., 1999), and changes in fat-free mass of Mexican-American children who participated in a 10-week randomized placebo and micronutrient-controlled trial of Zn or Fe with other micronutrients (Egger *et al*., 1999).

6.2.8 Physical Examination

No physical sign is pathognomonic of Zn deficiency. Short stature and underdevelopment are consistent with long-standing Zn deficiency (Halsted *et al*., 1972; Sandstead *et al*., 1967). Severe Zn deficiency can cause infected dermatitis of all surfaces. The distribution may be perioral-facial, perianal-peroneal-scrotal, and periungual distribution, characteristic of acrodermatitis (Arakawa *et al*., 1976; Kay *et al*., 1976; Neldner *et al*., 1978). Some patients display bilateral angular oral fissures and cheilosis similar to those observed in association with riboflavin (Sebrell, 1979; Sebrell, 1958) and pyridoxine deficiencies (Mueller and Vilter, 1950).

Severe atrophy of lingual papillae may also occur; it commonly occurs in association with severe iron deficiency (Sandstead *et al*., 1969). Other signs include poor healing of wounds (Henzel *et al*., 1970; Husain *et al*., 1970; Larson *et al*., 1970; Myers and Cherry; 1970). Hair may be easily plucked and alopecia evident. In addition, hair texture and color may be abnormal; black hair may be changed to a reddish brown. Effects of Zn deficiency on the nervous system may include decreased nerve conduction (Terril-Robb *et al*., 1996; O'Dell *et al*., 1990), ataxia, and disorientation (Henkin *et al*., 1975).

No physical sign is pathognomonic of Zn toxicity. Myeloneuropathy from Cu deficiency can occur (Kumar *et al*., 2005).

7 EFFECTS ON LABORATORY MAMMALS

7.1 Essentiality

The essentiality of Zn was first shown in plants and laboratory animals (Raulin 1869; Somner and Lipman 1926; Todd, Elvehjem and Hart 1934; Stirn, Elvehjem and Hart 1935). Zinc is the catalytic metal ion in more than 300 enzymes (Vallee and Falchuk 1993). More than 50 different types have been identified, including representatives of all six enzyme classes defined by the International Union of Biochemistry (i.e. oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases). Examples are carbonic anhydrase, alkaline phosphatase, Cu/Zn superoxide dismutase, alcohol dehydrogenase, RNA-polymerase, ecto 5′-nucleotidase, angiotensin-converting enzyme, protein kinase C, aminopeptidase, and δ-aminolevulinic acid dehydratase. In addition, Zn serves a structural function in proteins. Many of these sites are generically referred to as zinc fingers. Their numbers are in the thousands and include single zinc fingers and double zinc fingers such as in LIM, RING, PHD, and FYVE domains that are important for protein–protein, protein–DNA/RNA, or protein–lipid recognition (Berg and Shi 1996; Maret 2004). It is, therefore, not surprising that zinc enzyme.s orchestrate almost all aspects of metabolism. Distribution of Zn to apoenzymes and other proteins involves thionein, a 60–68 amino acid protein with 20 cysteines that bind seven Zn ions in two Zn/thiolate clusters to form metallothionein (MT). Metallothionein is redox active. Redox reactions of its thiolate ligands tightly bind redox-inert Zn released from proteins, thus preventing deleterious effects from free Zn ion, and also make Zn available for binding to apoenzymes and other proteins (Maret 2004). Mammals have at least four different isoforms of MT, 1–4. In addition to binding Zn, and Cu under special circumstances, MTs bind heavy metals (e.g., Cd, which contributed to its discovery in 1957) (Margoshes and Vallee 1957).

7.2 Deficiency

Studies in experimental animals show that Zn is essential throughout the life cycle. Growth failure and abnormal development (Hurley and Swenerton 1966; Hurley and Shrader 1975; Warkany and Petering 1972; Dvergsten *et al*., 1984) are among the most obvious consequences of deficiency. Mechanisms involve decreased transmembrane Ca uptake (O'Dell 2000) and Ca-mediated gene expression. Nucleic acid (Sandstead and Rinaldi 1969; Terhune and Sandstead 1972) and protein (Duerre, Ford and Sandstead 1977; Hicks and Wallwork 1987) synthesis are decreased. Growth hormone–mediated synthesis of insulin-like growth factor-1 (IGF-1) (Roth, and Kirchgessner 1994) and insulin function (Haase and Maret 2005) are also suppressed. Related to effects on growth is Zn deficiency–induced precancerous (Newberne, Broitman and Schrager 1997) hyperplasia of basal cells of the esophagus and tongue in rats (Follis, Day and McCollum, 1941; Barney, Orgebin-Crist and Macapinalac 1968), chicks (O'Dell, Newberne and Savage 1958), and nonhuman primates (Barney *et al*., 1967) (Section 7.5).

The striking effects of Zn deficiency in swine (Tucker and Salmon 1955) led to recognition that acrodermatitis enteropathica of humans (Barnes and Moynahan 1973) and lethal trait-A46 in Friesian cattle (Brummerstedt 1977) are genetic Zn malabsorption diseases and that similar dermatitis are associated with intravenous alimentation without adequate Zn (Kay *et al*., 1976; Arakawa *et al*., 1976). The effects of Zn deficiency on skin also contributed to demonstration of the essentiality of Zn for wound healing (Savlov, Strain and Huegin 1962; Pories *et al*., 1967; Sandstead *et al*., 1970).

Understanding of the essentiality of Zn for immunity of experimental animals (Fraker *et al*., 2000; Shankar and Prasad, 1998) provided part of the basis for the highly efficacious Zn treatment of infants with diarrhea and pneumonia in developing countries (Bhutta *et al*., 2000).

Experiments in animals revealed the critical roles of Zn for brain development and function (Sandstead *et al*., 2000; Frederickson *et al*., 2000). This research provided part of the basis for Zn supplementation studies in humans that confirmed the adverse effects of contemporaneous Zn deficiency on neuropsychological function (Penland 2000) and suggested Zn deficiency might contribute to long-term adverse effects of human gestational malnutrition on the brain (Opler and Susser 2005).

The need for Zn in animal reproduction (O'Dell 1968) led to the demonstration that Zn is necessary for

human reproduction (Jameson 1976). Zinc deficiency impairs spermatogenesis (Barney, Orgebin-Crist and Macapinalac 1968), preimplantation, and survival of embryos (Hurley and Shrader 1975); causes teratology (Hurley and Swenerton 1966); retards parturition; and decreases perinatal survival (Apgar 1985).

Some other Zn-requiring phenomena in experimental animals include platelet aggregation and eicosanoid metabolism (Gordon, Browning and O'Dell 1983; Gordon and O'Dell 1983), protection of liver from carbon tetrachloride (Chvapil *et al*., 1973), transmembrane movement of Ca and K (O'Dell 2000; Bixby *et al*., 1999), various membrane receptors (Frederickson, Koh, and Bush 2005), nuclear receptors for vitamins D and A, and steroid hormones (Bunce 1994).

7.3 Single Toxic Exposure of Animals

The oral and intraperitoneal LD_{50s} for Zn acetate, nitrate, chloride, or sulfate for rats are approximately 237–623 and 28–73 mg/kg, respectively (Domingo *et al*., 1988). In contrast, the inhalation LD_{50} for Zn chloride, a product of zinc hexachloroethane in smoke bombs, is approximately 2000 mg/m^3 in rats (Karlsson *et al*., 1986). Highly water soluble, Zn-chloride causes severe inflammation.

Acute intraperitoneal administration of Zn chloride at concentrations of 7.5, 10, and 15 mg/kg into Swiss albino mice caused chromosomal aberrations in bone marrow cells, although chronic administration at concentrations of 2.0 and 3.0 mg/kg caused similar abnormalities (Gupta, Talukder and Sharma 1991).

7.4 Short-Term Exposure of Animals

Zinc-chloride causes severe injury when placed on the skin at 1% aqueous concentration (Lansdown 1991). In comparison, aqueous Zn acetate at 20% concentration is nearly as harmful, although Zn oxide at 20% concentration suspended in dilute Tween 80, aqueous Zn sulfate at 1% concentration and Zn pyrithione in 20% suspension may induce epidermal hyperplasia and hair growth. In contrast, Zn undecylenate in 20% suspension causes no apparent irritation.

Respiratory exposure of guinea pigs to ultra-fine Zn oxide at 5 mg/m^3 3 hours/day for 6 days caused abnormal pulmonary functions, and bronchial, alveolar duct, and alveolar inflammation, followed by partial recovery (Lam *et al*., 1985). In contrast, inhalation of Zn chloride caused severe pulmonary injury (Karlsson *et al*., 1986).

High dietary exposure to Zn impairs intestinal absorption of Cu and causes Cu deficiency (Fischer, Giroux, and L'Abbe 1983). A dietary Zn/Cu ratio of 40 by weight increased serum cholesterol concentrations of rats (Klevay 1973). The mechanism seems to involve increased hepatic glutathione and increased 3-hydroxy-3-methyl-glutaryl coenzyme A (MHG-CoA) reductase activity (Kim, Chao, and Allen 1992). Associated abnormalities include myocardial hypertrophy, focal hemorrhage, inflammation, subendocardial fibrosis with adherent organizing thrombi, frayed appearing aortic elastic tissue (Allen and Klevay 1978), enlarged myocardial mitochondria, impaired atrial-ventricular conduction, and arrhythmias (Kopp, Klevay and Feliksik 1983). Copper is essential for cross-linking of elastin and collagen (Starcher, Hill and Matrone 1964; Rucker *et al*., 1998). Other experiments showed that Zn/Cu intake of 240:6 mg/kg diet decreased plasma ceruloplasmin (Cp), liver superoxide dismutase (SOD), and heart cytochrome *c* oxidase (CCO) activities (L'Abbe and Fischer 1984; L'Abbe and Fischer 1984).

7.5 Long-Term Exposure and Carcinogenicity

Although excess Zn is not implicated in cancer, Zn deficiency causes single- and double-strand DNA breaks and oxidative modifications of DNA that have implications for cancer risk (Ho 2004). Esophageal epithelium of the Zn-deficient rat develops precancerous basal cell hyperplasia that facilitates tumor induction by *N*-nitrosomethylbenzylamine (Newberne, Broitman and Schrager 1997). The mechanism may involve activation of *N*-nitrosomethylbenzylamine by increased cytochrome P-450–dependent microsomal metabolism and formation of the mutagenic DNA adduct O6-methyl guanine (Barch 1989). Related to this phenomenon, Zn deficiency caused abnormal expression of 33 genes that are involved in cell division, survival, adhesion, and tumor genesis in esophageal epithelium. Zinc repletion caused recovery in 29 genes (Liu *et al*., 2005).

7.6 Reproductive Toxicity

Excess dietary Zn (500 mg/kg diet) disrupts the normal sperm chromatin quaternary structure of rats. For reasons that are unclear, the abnormality is prevented by a high intake of silicon (Si) (Evenson *et al*., 1993).

Fetal development is disrupted by exposure to high concentrations of Zn. For example, 12.5–25 mg Zn/kg as Zn chloride injected intraperitoneally into mouse dams on gestation days 8–11 caused skeletal malformations in pups (Chang *et al*., 1977). In addition, Cu deficiency, induced in pregnant ewes by feeding 750 mg Zn and 2.5 mg Cu/kg of diet for 110 days, increased plasma Zn concentration and caused anorexia, low feed-efficiency, abortions, stillbirths, neonatal deaths, and signs of Cu deficiency (Campbell and Mills 1979).

TABLE 4 Comparison of Factorial Estimates of Adult Zinc Requirements (Sandstead and Smith, 1996)

Source	Group	Absolute need[a] (mg/d)	Need if 20% available[b]	1.3[c] × 20% available	Need if 30% available	1.3 × 30% available
WHO (Committee, 1973)	Adults	2.2	11.0	14.3	7.3	9.5
King[d] (King and Turnlund 1989)	Adults	2.5	12.5	16.3	8.3	10.8
UK (Anon, 1991)	Men	2.2	11.0	14.3	7.3	9.5
	Women	1.7	8.3	10.8	5.5	7.2
Canada (Committee, 1990)	Men	2.1	10.5	13.7	7.0	9.1
	Women	1.8	9.0	11.7	6.0	7.8

[a]Minimal need = factorially calculated "absolute" requirement.
[b]Available = amount of zinc (mg/d) 'bioavailable' (i.e., absorbed and used by the body each day).
[c]1.3 = the amount of zinc bioavailable plus two 15% coefficients of variation (2 × 15 = 30%) of the amount bioavailable.
[d]Based on more recent data than the WHO estimate.
‡The WHO estimate was based on the assumption that the Zn concentration of fat-free tissue is 30 μg/g, equivalent to 2.0 g of total Zn in soft tissue of a man or 1.2 g total Zn in soft tissue of a woman, as determined from 40K derived body cell mass. Bone Zn was not included in the calculation, because bone Zn is relatively sequestered from the exchangeable Zn pool. The Zn content of sweat was based on a surface loss of 1 mg/L. The urinary excretion of Zn was based on reported normal levels.

8 EFFECTS ON HUMANS

8.1 Absorption

Zinc absorption is in part carrier mediated, is concentration dependent, feedback controlled, and occurs throughout the small intestine with the highest rate in the jejunum (Lee *et al*., 1989). Zn is also absorbed by the colon (Sandstrom *et al*., 1986). Fasting subjects may absorb 60–70% of Zn in water (Sandstrom, 1997). Zinc absorption from mixed omnivorous Western diets of common foods is approximately 20–30% (Hunt *et al*., 1995; Gallaher *et al*., 1988) (Table 4). Plant-derived indigestible Zn-binding ligands (e.g., phytate, lignin, certain dietary fibers) and products on nonenzymatic Maillard browning can suppress absorption (Sandstead and Smith, 1996). High intakes of calcium (Ca) can also decrease Zn absorption (Wood and Zheng, 1997), but not in all cases (Dawson-Hughes, Seligson and Hughes 1986). Calcium apparently increases Zn binding of phytate, thus decreasing Zn absorption (Ferguson *et al*., 1989). Limited observations suggest high polyunsaturated fat intakes suppress Zn and Fe absorption compared with saturated fat, a phenomenon that was less apparent for Cu (Lukaski *et al*., 2001).

Findings in rats indicate Zn (in vegetable oil) can be absorbed across the skin (Keen and Hurley, 1977).

8.2 Excretion

The gastrointestinal tract is the primary route for endogenous Zn excretion. Zinc in pancreatic and intestinal secretions varies widely, from 7 μmol (0.5 mg) to >45 μmol (3 mg)/day and thus may equal or exceed dietary Zn (Sandstrom 1997). Some secreted Zn is reabsorbed by the jejunum, ileum, and colon. The remainder is lost in feces. When dietary Zn is restricted, fecal losses decrease, and absorption increases (Milne *et al*., 1983; Krebs and Hambidge, 2001).

Urinary Zn excretion is approximately 7 μmol (0.5 mg)/day and is affected by Zn status (Johnson *et al*., 1993; Milne *et al*., 1983).

Sweat Zn is affected by Zn status (Milne *et al*., 1983). The Zn content of whole-body sweat from men living in an air-conditioned environment and fed 8.3 mg Zn daily in an omnivorous diet was approximately 0.49 mg/day. In contrast, the sweat Zn decreased to approximately 0.24 mg/day when the diet provided 3.6 mg Zn/day, and increased to approximately 0.62 mg/day when the diet provided 33.7 mg/day. Thus, sweat loss can decrease apparent Zn balance by 12–84%.

8.3 Biological Half-Life

The usual biological half-life of Zn is approximately 280 days (Wastney *et al*., 1986). The rapidly exchangeable Zn pool of approximately 1.5–3 mmol (100–200 mg) is related to dietary Zn, plasma Zn concentration (Yokoi *et al*., 2003; Miller *et al*., 1994), endogenous fecal Zn, and absorbed Zn (Sian *et al*., 1996).

8.4 Zinc Content of Tissues and Blood

Zinc abundance in humans is approximately half that of Fe. A 70-kg adult male contains approximately 2–3 g, and women contain less. Approximately 60% is in muscle, 30% in bone, 8% in skin and hair, 5% in liver, and 3% in the gastrointestinal tract and pancreas, and other organs contain <1% (Wastney *et al*., 1986). Soft tissue Zn (μg/g fresh tissue) ranges from approximately 12 in the adrenal glands to 102 in the prostate (Tipton and Cook, 1963). The body has no readily available Zn store simi-

lar to the store of Fe. However, Zn in bone is available to other tissues when bone turns over. When bone turnover is increased, much of the released Zn is excreted (Fell *et al.*, 1973), although some is reused (Masters *et al.*, 1986).

The zinc content of serum/plasma is normally approximately 1 mg/L. The lower limit of "normal" for morning fasting plasma Zn is approximately 700 μg/L (Yokoi *et al.*, 2003). Plasma Zn is higher in the morning and decreases after meals. Approximately 60–80% of plasma Zn is bound to albumin, and most of the remainder is bound to transferrin and an α_2-macroglobulin (Wastney *et al.*, 1986). Plasma Zn concentration is relatively insensitive to changes in Zn status; concentrations may be maintained within the accepted normal range for several weeks–months when subjects are fed 2.6–3.6 mg/day (40–55 5moL/day) (Milne *et al.*, 1983; Milne *et al.*, 1987), amounts of Zn that are inadequate for neuropsychological function (Penland, 1991). In addition, plasma Zn may not be reflective of fetal growth and maternal muscle Zn, although maternal leukocyte Zn is closely related to maternal muscle Zn and fetal growth (Meadows *et al.*, 1981).

8.5 Essentiality and Requirements

The amount of Zn needed daily by adults is approximately 2–3 mg (Sandstead, 1973; King and Turnlund, 1989). Requirements relate to body cell mass, rate of metabolism, growth, tissue turnover, function of homeostatic mechanisms, and effects of catabolism. Zinc released by tissue turnover is partially recycled. Zinc is also lost in secretions, epithelial cells, hair, and nails.

8.5.1 Bioavailability

Bioavailability is the major factor affecting dietary requirements (Sandstrom, 1997). Flesh foods facilitate bioavailability, although indigestible Zn-binding ligands decrease bioavailability (Mills, 1985). Examples of indigestible Zn-binding ligands are phytate, certain dietary fibers (Sandstrom, 1997), lignin (Reinhold *et al.*, 1976), and Maillard browning products (Lykken *et al.*, 1986; Reinhold and Garcia, 1979). Foods rich in Ca and phytate, such as corn tortillas, are potent inhibitors of Zn absorption (Solomons *et al.*, 1979). Dietary phytate/Zn, and phytate × Ca/Zn molar ratios are predictive of bioavailability of dietary Zn (Gibson *et al.*, 1991; Ferguson *et al.*, 1989). By current use, a phytate/Zn molar ratio <5 is consistent with high Zn bioavailability, although a ratio >15 is consistent with poor Zn bioavailability (Committee, 1996). Other factors that affect Zn bioavailability include high concentrations of ferrous iron in Fe supplements (Solomons, 1986; Solomons, 1986), high intakes of Ca (Wood and Zheng; 1997; Spencer *et al.*, 1984), and pharmacological intakes of folic acid (Milne *et al.*, 1984; Simmer *et al.*, 1987).

8.5.2 Methods for Determining Requirements

8.5.2.1 Estimate of Requirement from Metabolic Balance

Metabolic balance concerning a specific substance is the physiological condition in which the intake and loss of the substance from the body are equal. Accurate measurement of balance is tedious and associated with many opportunities for error, especially loss of portions of specimens during collection and processing. Duplicate specimens of all substances consumed (intake) and collections of all materials excreted or otherwise lost are analyzed chemically, and the net retention calculated.

Zinc balance studies conducted in the highly controlled metabolic unit at the USDA, Grand Forks Human Nutrition Center, provide insights concerning Zn requirements (Sandstead and Smith, 1996; Sandstead *et al.*, 1984; Sandstead, 1985; Sandstead, 1986; Sandstead *et al.*, 1990). Forty-five healthy men (subjects) lived in the Center's metabolic unit for intervals of 2–14 months. Diet intervals were 26–30 days. Balance was determined the last 12 days of the diet interval by chemical analysis of all excreta and daily duplicates of the diet. Each subject's specimens were analyzed together. Subjects were fed four precisely prepared constant omnivorous diets of common foods in 3-day rotating menus that provided for individual energy and nutrient needs. Body weight was maintained within 2% of the admission weight by adjusting energy intake with a glucose polymer and controlling exercise. Energy from protein was 8% (~ 60 g) or 15% (~ 110 g) of the total, and fat provided 40%. One hundred eighty grams of leavened bread prepared from 70% extraction wheat flour was fed daily. To determine the effects of nondigestible plant ligands, 26 g of various sources of dietary fiber and phytate were added to the bread by replacement of the same weight of flour. The zinc content of the four diets was (mean ± SD) 7.18 ± 0.72, 9.13 ± 2.03, 12.74 ± 2.56, and 14.74 ± 2.26 mg/day. The dietary fiber and phytate contents of the bran and other fiber sources, diets, and feces were determined by standard methods (Dintzis, Watson, and Sandstead, 1985). Data analysis methods included regression techniques and the analysis of covariance (ANCOVA).

Fecal Zn content was increased by the four different varieties of wheat bran ($P = 0.01$) and carrot powder ($P = 0.01$). Net Zn retention decreased when the diet provided 15% of energy as protein ($P = 0.004$), was rich in phytate ($P = 0.01$), and when the protein and phytate

TABLE 5 Fractional Zn Absorption From Total Diets, Measured by Isotopic Techniques (Commitee, 2001)

Subjects	Isotopes	Diets	Zn content μmol (mg)	Phytate/Zn molar ratio	Zn absorption, % (×±SD)
Young adults (Petterson, Sandstrome and Cederblad, 1994)	Radioactive	High fiber	163 (10.7)	7	27±6
Young women (Knudsen *et al*., 1995)	Stable isotope	Habitual	124 (8.1)	10	34±9
Women (20–42 y)	Radioisotope	Lacto-ovo-	139 (9.1)	14	26±5
(Hunt, Matthys and Johnson, 1998)		vegetarian	169 (11.1)	5	33
		omnivorous			
Women (20–42y) (Hunt, Matthys		vegetarian	169 (11.1)	5	33
and Johnson, 1998)	Radioisotope	"Low meat"	102 (6.7)		30±4.6
Postmenopausal	Radioisotope	"High meat"	198 (13.0)		28±4.6
women (Hunt *et al*., 1995)					

content were both increased ($P = 0.001$) (Sandstead *et al*., 1984; Sandstead *et al*., 1990).

Evaluation of the data by regression analysis identified significant predictors of Zn intake, which is equivalent to the requirement when balance is in equilibrium. The equation that describes these relationships is: Zn intake = −2.02 + 18.21(diet phytate)−3.52(diet phosphorus) + 0.26(diet nitrogen) + 0.31(Zn balance) + 2.84(diet Ca) + 2.81(diet Cu)−9.32(diet Mg) (number of observations = 183, $R^2 = 0.905$, $P < 0.0001$) (Sandstead and Smith, 1996; Sandstead, 1986). This model is consistent with factors that are known to affect Zn bioavailability.

A simplified regression model that included the nitrogen (N) and phosphorus (P) intakes was used to calculate the requirement and compare it with intakes

TABLE 6 Dietary Reference Values for Zinc (mg/d)

	United Kingdom (Department of Health, 1991)			USA RDA[a] (Committee on Micronutrients, 2001)	WHO DRI (Committee, 1996)	European DRI (Commission of the European Communities, 1993)
	LNRI	EAR	RNI			
Infants						
0–3 months	2.6	3.3	4.0	2.0		
4–6 months	2.6	3.3	4.0	3.0		
7–12 months	3.0	3.8	5.0	3.0	5.6	4.0
1–3 y	3.0	3.8	5.0	3.0	5.5	4.0
4–6 y	4.0	5.0	6.5	5.0	6.5	6.0
7–10 y	4.0	5.4	7.0	8.0	7.5	7.0
Males						
11–14 y	5.3	7.0	9.0	8.0	12.1	9.0
15–18 y	5.5	7.3	9.5	11.0	13.1	9.5
19–50+ y	5.5	7.3	9.5	11.0	9.4	9.5
Females						
11–14 y	5.3	7.0	9.0	8.0	10.3	9.0
15–18 y	4.0	5.5	7.0	9.0	10.2	7.0
19–50+ y	4.0	5.5	7.0	8.0	6.5	7.1
Pregnancy				11.0	7.3–13.3	
Lactation						
0–4 months				12.0	12.7	+5.0
4+ months				12	11.7	+5.0

[a]*Age* groups of the RDA do not coincide exactly with the age groups of the other standards.
DRI, Dietary reference intake; EAR, estimated average requirement; LNRI, lower reference nutrient intake; RDA, recommended daily allowance; RNI, recommended nutrient intake.

of persons aged 9–>75 years, who participated in a USDA Food Consumption Survey (Sandstead 1985). Zinc intakes of the survey subjects ranged across age groups from 9.32 mg/day in men >75 years to 13.53 mg in boys, aged 15–18 years, and from 7.04 mg/day in women >75 years to 9.22 mg/day in girls aged 12–14 years. The regression model (Zn intake (requirement) = 1.466 + 0.23(Zn balance) + 5.19(P intake) + 0.40(N intake)−0.30 (P intake−1.389)(N intake−14.646)) was based on data from the 157 subjects whose diet intervals were 28–30 days ($n = 157$, $R^2 = 0.83$, $P < 0.0001$). For all age groups and both genders, the mean percentage (range) difference between the survey Zn intakes and the calculated Zn requirement was 1.5% (−6.5 to −8.1) in males and 11.3% (−1.1–+20.3) in females. Thus, the survey data were generally within the 95% confidence limits of the calculated requirement.

8.5.2.2 Estimate of Requirement by Factorial Calculation

Factorial estimates of requirements are based on estimates of likely losses and anabolic needs (Sandstead, 1973; King and Turnlund, 1989). Estimates of requirements from the past are shown in Table 4, which also shows the amounts of Zn required if the bioavailability is 20% or 30%, and the coefficient of variation (CV) of the absolute requirement is 15%. It is notable that the actual CV is unknown.

In an attempt to improve the estimate of requirement, Zn retention was measured by use of isotopic Zn tracers. The analytical method related to the nature of the isotope (radioactive or stable). In the past, net retention of orally administered radioisotopes (^{65}Zn or ^{69}Zn) was measured by whole-body counting over a period of days and regression of log transformed data to baseline (Lykken *et al*., 1983; Lykken, 1983); Table 5 shows some representative data. More recently, on the basis of evidence that Zn absorption and retention are highly controlled by feedback mechanisms that maintain balance, the fractional retention of orally administered isotope was measured, by use of stable Zn isotopes, by determining the urinary excretion of an orally administered ^{67}Zn relative to urinary excretion of an intravenously administered ^{70}Zn (Krebs and Hambidge, 2001). The data were used to derive the US 2001 Dietary Reference Intakes for Zn, based on a coefficient of variation of 10% for the estimated average dietary requirement (Committee on Micronutrients, 2001) (Table 6). Factorial estimates of Zn requirements by committees of other countries are also shown. With the exception of the recommendation by the World Health Organization (Committee, 1996), the recommendations do not include adjustments for bioavailability.

8.6 Deficiency

Manifestations of Zn deficiency are described in Sections 6 and 7.

8.6.1 Dietary (Primary) Deficiency

The pathogenesis of dietary Zn deficiency is related to the food content of Zn, food selection, and the bioavailability of Zn from the foods consumed. Dietary Zn deficiency was thought highly unlikely before its description by Prasad, Halsted, and colleagues in the 1960s and 1970s in Iranian and Egyptian farmers (Sandstead 2001). Subsequent research found the condition common (Gibson, 1994; Brown *et al*., 2002; Sandstead, 1991; Brown, Wuehler, and Peerson, 2001). On the basis of Food and Agriculture Organization food balance data from 176 countries, a conservative estimate suggests approximately 20% of the world's population is at risk of Zn deficiency (Wuehler, Peerson, and Brown 2005). One suspects the prevalence of Zn deficiency is, in fact, higher given associations of Fe and Zn nutriture (Yokoi *et al*., 1994; Yokoi *et al*., 2003; Gibson, Heath, and Ferguson, 2002) and the high prevalence of Fe deficiency (Stephenson, Latham, and Ottesen, 2000; Hallberg, 2001; Stoltzfus, 2001).

Effects of food selection, the Zn content of foods, and bioavailability on Zn nutriture are noted previously. In affluent societies food selection has a major effect on Zn nutriture. For example, a constant omnivorous diet based on white poultry meat and finfish provided insufficient Zn for adult men (Milne *et al*., 1983). In addition, diets based on a low-income food plan for elderly in the United States provide approximately half the amount of Zn when white chicken meat and finfish were the flesh foods in diets that include red meat or liver (Sandstead *et al*., 1982). Avoidance of red meat by young women was partly responsible for their combined Zn and Fe deficiency (Yokoi *et al*., 1994). Among the poor of all societies, food quality, variety, and availability are limited by available disposable income. As disposable income decreases, diets include more and more inexpensive plant products with limited Zn content and/or bioavailability, and the likelihood of Zn deficiency increases.

8.6.2 Conditioned (Secondary) Deficiency

Many diseases adversely affect Zn status (Sandstead, Vo-Khactu, and Solomons, 1976; McClain, Kasarskis, and Allen, 1985). Intestinal malabsorption and/or increased intestinal losses from Crohn's disease, gluten enteropathy, cystic fibrosis, and other malabsorption syndromes and inflammatory diseases increase the risk of

Zn deficiency (McClain, 1985). The risk of Zn deficiency is increased, by excretion of Zn released from erythrocytes by hemolysis, in patients with sickle cell disease (Prasad, 2002) and thalassemia (Prasad *et al*., 1965). Chronic bleeding, from hookworm and other parasites (Farid, Patwardhan, and Darby, 1969), also causes loss of Zn (Prasad *et al*., 1963), as does menorrhagia (Yokoi *et al*., 1994; Yokoi *et al*., 2003). Increased Zn excretion in urine occurs in some renal diseases (Lindeman, 1989), cirrhosis of the liver and alcoholism (McClain *et al*., 1986), stress (McClain *et al*., 1993), catabolism (Fell *et al*., 1973), and chronic inflammatory diseases that increase interleukin-1 (IL-1) (Goldblum *et al*., 1987). Pharmacological intakes of ferrous Fe (Solomons 1986), Ca (Wood and Zheng, 1997), and folic acid (Milne *et al*., 1984; Simmer *et al*., 1987) can inhibit Zn absorption.

8.7 Toxicity

8.7.1 Dietary and Supplement Intakes

Intakes of Zn and Cu should be proportionate (Sandstead 1995). The copper requirement seems directly related to Zn intake (Table 7). In contrast, when protein intake is increased, the Cu requirement is decreased. The data in Table 7 were derived from balance studies carried out at the USDA Grand Forks Human Nutrition Research Center (Sandstead, 1982). The data were analyzed by multiple stepwise regression analysis of potential predictors, including Cu balance, diet Zn, diet nitrogen (N), diet Ca, and diet phosphorus (P). Copper balance, diet Zn, and diet N had a significant influence ($n = 161$; $R^2 = 0.59$, $P < 0.0001$). It is notable that addition of the ~ 0.34 mg of Cu, lost daily in sweat under moderate conditions (Jacob *et al*., 1981) to the findings in Table 7, increases the total Cu requirement to levels substantially >0.90 mg recommended for adults in the 2001 Dietary Reference Intake (Committee on Micronutrients, 2001).

High oral intakes of Zn typically impair Cu nutriture (Magee and Matrone, 1960). The putative mechanism involves Zn induction of thionein synthesis in enterocytes, which binds Zn and Cu to become metallothionein (MT). Redox conditions affect release of Zn and Cu from MT. Binding of Cu by MT is many times stronger than binding of Zn. It is unknown whether Zn competes with Cu for binding to proteins that mediate the exit of Cu from enterocytes into the body. In any case, Cu retained in the enterocytes is excreted in feces, which in some instances results in Cu deficiency (Sandstead, 1995).

Copper deficiency causes many abnormalities (e.g., decreased iron use, low serum ferritin (Yadrick, Kenney, and Winterfeldt, 1989), and hypochromic microcytic anemia, leukopenia, osteopenia (Graham, 1971), myeloneuropathy (Hedera *et al*., 2003; Rowin and Lewis, 2005), heart arrhythmias (Kopp, Klevay, and Feliksik, 1983; Klevay *et al*., 1984; Reiser *et al*., 1985), increased LDL cholesterol and/or decreased HDL cholesterol (Klevay *et al*., 1984; Reiser *et al*., 1987; Hooper *et al*., 1980), low plasma Cu concentration, low plasma ceruloplasmin activity, low erythrocyte superoxide dismutase (ESOD) activity (Milne, 1998), low erythrocyte glutathione peroxidase (EGPx), low platelet Cu, low cytochrome *c* oxidase (CCO) activity, and increased activity of clotting factors V and VIII (Milne and Nielsen, 1996). Experience suggested to one observer that the most sensitive indicators of Cu status are platelet cytochrome *c* oxidase activity and Cu concentration, erythrocyte SOD and glutathione peroxidase, and activity of clotting factors V and VIII (Milne and Nielsen, 1996). Some of these are noted in Table 3. In addition, findings in Table 3 suggest a Zn/Cu molar ratio >18 over an extended period of time increases the risk of Cu deficiency. It is notable that available data do not identify a threshold for an adverse effect by Zn.

Because chemically similar metals compete for binding ligands (Hill and Matrone, 1970; Allaway *et al*.,

TABLE 7 Copper Requirement (mg/day) of Adult Men Fed Mixed Diets of Common Foods Providing Four Levels of Protein and Zinc (Sandstead, 1982)[a,b]

Protein, g	40	60	80	100
5 mg Zn	1.0 (0.73–1.28) [c] [6.7–3.8] [d]	0.95 (0.67–1.23) [7.3–3.9]	0.89 (0.61–1.17) [7.9–4.2]	0.83 (0.55–1.11) [8.9–4.4]
10 mg Zn	1.26 (0.98–1.54) [9.9–6.3]	1.20 (0.92–1.48) [10.6–6.6]	1.14 (0.86–1.42) [11.3–6.9]	1.08 (0.80–1.36) [12.2–7.2]
15 mg Zn	1.50 (1.22–1.78) [12.0–8.2]	1.45 (1.17–1.67) [12.5–8.8]	1.39 (1.11–1.67) [13.2–8.8]	1.33 (1.05–1.61) [13.9–9.1]
20 mg Zn	1.76 (1.48–2.04) [13.2–9.6]	1.70 (1.42–1.98) [13.7–9.8]	1.64 (1.36–1.92) [14.3–10.2]	1.58 (1.30–1.86) [15.0–10.5]

[a]Cu intake = 0.87 + 0.49 (Cu balance) + 0.05 (Zn intake) – 0.02 (N intake).
Intake = Requirement when balance is in equilibrium.
[b]For total requirement add 0.34 mg to account for daily surface loss of Cu under moderate environmental conditions (Jacob *et al*., 1981).
[c]95% confidence interval.
[d]Zn = Cu molar ratio.

1977), it might be expected that Zn competes with Cu for binding to some Cu-dependent proteins. However, specific examples of such a phenomenon have apparently not been published in recent years.

Other studies not listed in Table 7 also found adverse effects of high oral intakes of Zn. A study of 778 adults, aged 22–80 years, who were administered sufficient Zn to increase serum Zn concentration, found increased serum total and LDL cholesterol, and triglyceride concentrations (Hiller *et al*., 1995). A study of ambulatory men and women, aged >60 years found Zn intakes from over-the-counter supplements adversely affected serum HDL cholesterol concentrations (Goodwin *et al*., 1985). The 180 subjects not taking Zn showed an increase in serum HDL cholesterol when participating in an exercise program, while 90 subjects taking Zn did not. Stopping Zn in 22 subjects taking >15 mg Zn daily was followed by an increase in serum HDL cholesterol. Consistent with these findings, a study of 12 men given 150 mg Zn daily for 5 weeks found a 75% decrease in serum HDL cholesterol, which was restored to baseline when Zn administration stopped (Hooper *et al*., 1980). Similarly, a 12-week double-blind study of 31 men found that 75 mg Zn daily decreased serum HDL cholesterol significantly after 6, 8, and 12 weeks, although 50 mg Zn daily decreased serum HDL cholesterol significantly after 12 weeks (Black *et al*., 1988). Changes in serum total cholesterol, LDL cholesterol, and triglycerides were not significant. In contrast, an 8-week study of 32 young women found little effect of 15, 50, or 100 mg Zn daily on serum HDL cholesterol, except for a decrease in the 100 mg group after 4 weeks (Freeland-Graves *et al*., 1982).

Effects of excess Zn on immunity were measured in 11 men administered 300 mg Zn daily for 6 weeks. Serum Zn increased twofold, although *in vitro* phytohemagglutinin induced lymphocyte transformation, polymorphonuclear leukocyte chemotaxis, and phagocytosis of bacteria, and serum HDL cholesterol concentration decreased (Chandra, 1984). The absolute number of lymphocytes and the proportions of T and B lymphocytes were not significantly different from baseline.

On the other hand, Zn is an important relatively new treatment for patients with the genetic Cu overload disease, known as Wilson's disease (Brewer, 2000). The treatment is highly efficacious (Brewer *et al*., 1998).

8.7.2 Reference Dose (RfD)

The US Environmental Protection Agency (Dourson 1994) used the Zn intake and erythrocyte Cu/Zn SOD data from Yadrick (Yadrick, Kenney, and Winterfeldt, 1989) as the basis for a LOAEL of 60 mg. A reference dose (RfD) of 0.33 mg/kg/day was proposed. The suggested RfD was, in some instances, similar to or less than the 1989 Food and Nutrition Board's Recommended Dietary Allowance for Zn (Committee, 1989) and less than the provisional Zn requirement suggested by a World Health Organization Expert Committee when Zn bioavailability is 15% (Committee, 1973). Because of this unsatisfactory outcome, alternative RfDs of 1.66 and 0.83 mg/kg/day were suggested for bioavailability of 15% and 30%, respectively (Sandstead, 1993). For Zn supplements that might be 95% absorbed, an RfD of 0.25 mg/kg/day was suggested.

The following is speculation. Perhaps estimation of the LOAEL and the upper limit (UL) for Zn should be related to the intake Zn/Cu molar ratio. On the basis of the study of relationships between diet Zn, Cu, and protein (Table 7), a LOAEL for Zn might occur when the Zn intake is 13.7 mg and the intake of Cu is 0.83 mg (Zn/Cu molar ratio = 16.1). Perhaps the UL for Zn should be determined in light of the intake of Cu. For example, at an intake of 1.5 mg Cu and 13.7 mg Zn (Zn/Cu ratio = 8.9), the Zn intake would be safe and adequate. If a Zn supplement of 10 mg were added, thus increasing the Zn intake to 23.7 mg (Zn/Cu ratio = 15.4), the safety of the Zn might be in question. Inclusion of bioavailability data might also improve the estimate of safety.

8.7.3 Poisoning from Ingestion of Zinc-Contaminated Food, Drink, and Other Substances

High concentrations of Zn in drinks, up to 2500 mg/L, caused nausea, abdominal cramping, vomiting, tenesmus, and diarrhea with or without bleeding. The estimated dose was 325–650 mg (Brown *et al*., 1964). Zinc chloride ingestion severely injured the exocrine pancreas, causing insufficiency (McKinney, Brent, and Kulig, 1995). The finding was similar to the effect of Zn poisoning in other species (Hill *et al*., 1983; Smith and Embling, 1993; Gabrielson, Remillard, and Huso, 1996). Ingestion of >1800 g of coins over a period of years caused Cu deficiency with severe sideroblastic anemia and leukopenia and high plasma Zn in a mentally ill patient (Kumar and Jazieh, 2001).

8.7.4 Poisoning from Inhalation

8.7.4.1 Zinc Chloride Fume

Zinc chloride inhalation causes severe chemical pneumonia. Effects on two soldiers are illustrative (Homma *et al*., 1992). After a relative brief exposure, injury seemed minor for 10 days. Then severe respiratory distress and pulmonary hypertension were diagnosed. Death occurred on the 25th and 32nd days, respectively. Autopsy found interstitial and intraalveolar fibrosis, endothelial proliferation, and vascular occlusions.

Another accident briefly exposed 13 soldiers to smoke bombs in the open air for 5–10 minutes or to smoke that entered a house (Kofoed-Enevoldsen *et al*., 1997). Initially there were few complaints, and all subjects were treated acutely with intravenous hydrocortisone; four were treated systemically for 1 month, because exposure was believed longer than 1 minute. During the first 4 weeks, the lung carbon monoxide diffusion capacity decreased 85%. Later, the subjects began to complain of respiratory symptoms 8 or more weeks after exposure. Another study of 20 subjects found that low exposure caused restrictive lung injury that might be somewhat reversible (Hsu *et al*., 2005). High-resolution computerized tomography (CT) 3–21 days after exposure found patchy or diffuse ground-glass opacities with or without consolidation in the lungs. In most subjects, pulmonary function tests (PFT) were consistent with restrictive injury. In 10 subjects who returned for follow-up 27–66 days after exposure, there were improvements in the CT abnormalities and PFT.

The United States Occupational Safety and Health Administration (OSHA) permissible exposure limit (PEL) for Zn chloride fume is 1 mg/m^3 of air as an 8-hour time-weighted average (TWA) concentration and 2 mg/m^3 as a 15-minute TWA short-term exposure limit (STEL) (Anon., 1996). The STEL is the maximum 15-minute concentration to which workers may be exposed during any 15-minute period of the working day. The United States National Institute for Occupational Safety and Health (NIOSH) recommended exposure limit (REL) is 1 mg/m^3 and 2 mg/m^3 STEL. The American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value (TLV) is 1 mg/m^3 as a TWA for a normal 8-hour workday and a 40-hour workweek; the short-term exposure limit (STEL) is 2 mg/m^3 for periods not to exceed 15 minutes.

8.7.4.2 Zinc Oxide Fume

Inhalation of Zn oxide fume causes metal fume fever (MFF), also known as brassfounder's ague, Zn chills, Zn fever, Spelter's shakes, and metal shakes. The illness is an acute, self-limited flulike illness manifested by fever, chills, myalgia, nausea, fatigue, and infrequently shortness of breath that occurs after inhalation of finely dispersed particulates that are formed when Zn oxide is volatilized as particles <1.0 μm aerodynamic diameter (Gordon *et al*., 1992; Gordon and Fine, 1993). Adequacy of guidelines that allow exposures of 5 mg/m^3 8-hour time-weighted average threshold limit value (TLV) for Zn oxide fume was examined by measurement of specific cytokines in blood and bronchoalveolar lavage in 13 naive subjects. On separate days, air, 2.5 or 5 mg/m^3 of furnace-generated Zn oxide fume was administered for 2 hours. Mild fever occurred after exposure to both levels of Zn oxide; plasma IL-6 was increased 3 and 6 hours after exposure to 5 mg/m^3, although tumor necrosis factor did not increase (Fine *et al*., 1997). However, clinical tolerance typically occurs after repeated exposure. Subjects repeatedly exposed to Zn oxide fume displayed a significant decrease in percent neutrophils and concentration of IL-6 in bronchoalveolar lavage fluid compared with baseline, although clinical symptoms were minimal (Fine *et al*., 2000). These findings are consistent with chronically exposed sheet metal workers' being tolerant of Zn oxide fume at the Occupational Safety and Health Administration Permissible Exposure Limit. Although clinical observations of MFF suggest an absence of long-term sequelae, one study prompted conjecture of a relationship to occupational asthma (El-Zein *et al*., 2005).

Safety guidelines for Zn oxide (Anon., 1993) are as follows: OSHA general industry and construction industry PEL for Zn oxide fume is 5 mg/m^3 for the respirable fraction as an 8-hour TWA concentration. The NIOSH REL is 5 mg/m^3 TWA for up to a 10-hour workday and a 40-hour workweek; the STEL is 10 mg/m^3. The ACGIH TLV is 5 mg/m^3 TWA for a normal 8-hour; workday and a 40-hour workweek; and the STEL is 10 mg/m^3.

9 EFFECTS EVALUATION

9.1 Homeostatic Model

Zinc is essential and can be deficient or toxic. The homeostatic model in Figure 1 shows these relationships. These relationships are affected by the physiological controls of Zn metabolism and extrinsic physiochemical factors that affect Zn exposure.

9.2 Risks to Human Health

9.2.1 General Population

Diet and supplements are the major sources of Zn exposure. Populations with ready access to flesh foods have a low risk of deficiency, although populations that subsist on diets based on roots low in Zn and/or cereals and pulses have a high risk of deficiency. Disproportionately high dietary and/or supplemental intakes of readily bioavailable Zn relative to the Cu intake increase the risk of Cu deficiency. The prevalence is unknown. Zn toxicity from consumption of contaminated food or drink is unusual. No scientific reports implicate Zn from anthropogenic sources as a general health hazard.

9.2.2 Occupational Exposure

Exposure to Zn and Zn compounds in dust and fumes occurs when Zn is produced or used. Exposures to Zn oxide fume from smelting, galvanizing, or welding can cause metal fume fever, a short-duration, flulike, self-limited illness. In contrast, Zn chloride fume can cause severe pulmonary injury and disability and/or death.

9.2.3 Risk of Zn Deficiency

A recent estimate suggests that approximately 20% of the world's population is at risk of Zn deficiency. Major underlying determinants are economic status, food availability, and choice. Diet quality (variety of foods and bioavailability of Zn) and quantity (amount of foods providing bioavailable Zn), and conditioning factors that affect Zn retention or loss affect risk.

9.2.4 Risk of Excess Zn

Free access to uncontrolled amounts of Zn in nutritional supplements is the most common cause of Zn excess. It has been suggested that at least 40% of US citizens take some form of nutritional supplement. The proportion taking excess amounts of Zn is unknown.

9.2.5 Environmental Risk Assessment for Zn

Risk of excess Zn in humans from environmental sources is apparently low. Zinc from anthropogenic sources spreads widely in the environment. Although other species can be adversely affected, evidence of adverse effects on humans is rare or nonexistent.

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