ISSUE PAPER ON
THE HUMAN HEALTH EFFECTS OF METALS

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NOTICE

This paper has been developed in support of an ongoing effort within the U.S. Environmental Protection Agency (EPA) to develop an integrated framework for metals risk assessment. In September 2002, the cross-Agency technical panel, organized under the auspices of the Agency's Science Policy Council, discussed plans for the development of the framework and associated guidance with the Agency's Science Advisory Board (SAB). During the advisory, the SAB affirmed the importance of incorporating external input into the Agency's effort. *As part of the effort to engage stakeholders and the scientific community and to build on existing* experience, the Agency commissioned external experts to lead the development of papers on issues and state-of-the-art approaches in metals risk assessment for several key topics. Topics identified include: environmental chemistry; exposure; ecological effects; human health effects; and bioavailability and bioaccumulation. (Some individual EPA experts contributed specific discussions on topic(s) for which he or she has either specific expertise or knowledge of current Agency practice). Although Agency technical staff, as well as representatives from other Federal agencies, reviewed and commented on previous drafts, the comments were addressed at the discretion of each respective author or group of authors. Therefore, the views expressed are those of the authors and should not be construed as implying EPA consent or endorsement.

This draft paper is being made available for public comment consistent with EPA's commitment to provide opportunities for external input. Science-based comments received on this paper will be made available to authors for final disposition. The material contained in this paper may be used in total, or in part, as source material for the Agency's framework for metals risk assessment and EPA's evaluation of this material will therefore include consideration of the Assessment Factors recently published by EPA for use in evaluating the quality of scientific and technical information. The draft framework, as an Agency document, will undergo scientific peer review by the SAB.

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TABLE OF CONTENTS

LIST OF TABLES

1. INTRODUCTION

The objective of this paper is to discuss issues important to consider in developing a framework for performing human health assessments for exposure to metals and metal compounds. The issues involve the unique and specific characteristics of metals and metal compounds that might be applied in metals risk assessments for human health, in contrast to a more general risk assessment approach applied for assessment of organic compounds. Two types of health hazards exist: (1) those with a threshold for the relationship between exposure and the health effect (most target organ effects), and (2) those with non-threshold effects considered to pose some level of risk at any level of exposure (cancer and mutagenic effects). The characteristics of specific metals or groups of metals should be considered in hazard identification or identification of critical effects. Results that include the specific characteristics of metals can contribute to the establishment of guidelines for EPA programs for the assessment of health risks from exposure to metals.

2. CLASSIFICATION OF METALS

All elements in nature can be classified as metals or non-metals based on various sets of criteria. A number of definitions reflect different properties of metals. A general definition based on physical properties is that metals are a large group of substances that are opaque, form alloys, conduct heat and electricity, and are usually malleable. More than 80 of the 125 known elements fit this definition. Also, there are other elements that are intermediate in their physical properties between metals and non-metals and may be referred to as metalloids, such as arsenic, boron, silicon, and tellurium. In terms of metals of importance to human health and the risk assessment process, metalloids are considered as metals, with the possible exception of silica. There are also a number of low molecular weight cations that do not have the physical properties of metals, such as calcium, sodium, potassium, and magnesium. Nevertheless, these cations are important in terms of human health because of their essential role in mammalian metabolism. A characteristic of this group of cations is that they are in themselves, rather than as members of metal-ligand-complexes, responsible for a number of biological responses, including enzymatic reactions in vivo as well as nerve conduction and muscle contraction. They are also important (calcium in particular) in terms of risk assessment because of potential interactions with the principal metals. Like other essential metals, concentrations of cations in the body are controlled by homeostatic mechanisms.

A classification of metals based on characteristics (toxic, beneficial) of health effects, as well as nutritional requirements (essentiality) or benefits, is shown in Table 1. This approach can assist EPA in rank ordering metals and setting priorities in the risk assessment process. The list is not intended to be complete but rather gives examples of how such a classification might be constructed.

Table 1. Classification of Metals Based on Characteristics of Health Effects

Nutritionally Essential Metals	Toxic Metals With Possible Beneficial Effects	Toxic Metals With No Known Beneficial Effects
Cobalt Chromium III Copper Iron Manganese (animals but not humans) Molybdenum Selenium Zinc	Boron Nickel Silicon Vanadium	Aluminum Antimony Barium Beryllium Cadmium Lead Mercury Silver Strontium Thallium Tin

The primary premise for this classification is that assessment of health risks for nutritionally essential metals requires its own approach or process because of the need to ensure that any restrictive standard will also allow sufficient exposure for the general population to prevent deficiencies. On the other hand it must be recognized that metals that are nutritionally essential to human health may cause adverse health effects at some levels below or beyond the level required for optimum nutrition.

2.1 Nutritionally Essential Metals

Metals that are generally regarded as nutritionally essential for humans are cobalt, chromium III, copper, iron, manganese, molybdenum, selenium, and zinc, and must be recognized as such in the regulatory process. While manganese is cited as a nutritionally essential metal (Goyer and Clarkson, 2001), evidence is limited to its role in non-human animal species. Manganese is an activator and constituent of many enzymes, and there have been reports of effects of manganese deficiency in humans, but requirement in humans cannot be established because of an absence of data (WHO, 1996b).

2.2 Toxic Metals With No Known Essential or Beneficial Effects

Arsenic, cadmium, lead and mercury and their inorganic compounds are probably the most toxic metals in the environment. They have no known nutritional or beneficial effects on human health but are ubiquitous in nature and present in air, water, and soil, so that some level of exposure is not readily preventable. Other metals of interest to EPA include aluminum, antimony, barium, beryllium, silver, strontium and thallium. These metals have many industrial uses which increases the probability of human exposure. Industrial activities may also convert the metallic form of the metals to compounds that may be more soluble in various media, with a resultant increase in risk for exposure and toxicity. Because these metals have no known

essential or beneficial effect, guidelines for regulatory activity might limit human exposure to the lowest level known to have a plausible adverse health effect.

2.3 Toxic Metals That May Have Some Beneficial Effect

There is a small group of metals that are not known to be essential to human health but may have some beneficial effects at low levels of exposure. These include silicon, manganese, nickel, boron, and vanadium. These metals are also toxic at higher levels. Arsenic has also been considered by some as possibly having beneficial effects,(WHO,1996; NAS/IOM,2001), but a recent critical review does not support this view for human exposure (NAS/NRC, 1999). However, some organic arsenic compounds have been utilized as growth factors in poultry, and it has been suggested that arsenic deprivation may impair growth of rats, hamsters, goats, miniature pigs, and chicks; the possible beneficial metabolic functions of arsenic for humans have not been established (NAS/NRC, 1999). Furthermore, arsenic has been found to be a human carcinogen at extremely low levels of exposure, which should be the major priority in consideration of regulatory control of human exposure (NAS/NRC, 1999).

Boron, nickel, silicon, and vanadium have been shown to have biological functions in plants and some animals but essentiality for humans has not been demonstrated (NAS/IOM, 2003). However, human studies are limited. Boron is an essential nutrient for plants and some microorganisms and has a function in reproduction and development and possibly carbohydrate and mineral metabolism. Studies of men and post-menopausal women suggest that homeostasis for boron occurs in humans, but this has not been confirmed in other studies (NAS/IOM, 2003).

Nickel has not been shown to be an essential nutrient for humans but it may serve as a cofactor or structural component of specific metalloenzymes with a variety of physiologic functions in lower animals. Nickel has been shown to facilitate ferric iron absorption or metabolism. Rats deprived of nickel exhibited retarded growth, low hemoglobin, and impaired glucose metabolism (NAS/IOM, 2003).

Silicon has been shown to play an essential role in the development of bone in two species of experimental animals, but no data are available to estimate a human requirement (NAS/IOM, 2003).

Vanadium has not been shown to have a functional role in human nutrition, However, it has been found to influence glucose and lipid metabolism in in-vitro studies, NAS/IOM.(2003).

For some of the metals in this group, therefore, it must be concluded that there are no rigorously defined limits or levels that might have a particular beneficial human health effect, but upper safe levels are defined. In terms of a framework for assessment of metals and inorganic metal compounds, potential beneficial human health effects at low levels might be considered, but as yet these metals cannot be regarded as essential for humans. Also, one of the metals in this group, nickel, is regarded as a human carcinogen by inhalation.

2.4 Carcinogenic Metals

Metals are emerging as an important class of human carcinogens. At least five transition metals or metalloids—arsenic, cadmium, chromium VI, beryllium, and nickel—are accepted as human carcinogens in one form or another or in particular routes of exposure (NTP, 2002). Identification of the mechanism(s) responsible for metal carcinogenesis is elusive, partly because of the complex nature of metals interactions in biological systems. Many toxic metals, including carcinogenic metals, follow the metabolic pathways of similar essential metals. This is the likely result of similar binding preferences between carcinogenic metals and nutritionally essential metals (Clarkson, 1986). Metals typically do not require bioactivation, at least not in the sense that an organic molecule undergoes enzymatic modification that produces in a reactive chemical species (Waalkes, 1995). Enzymatic modification is generally not a mechanism available to detoxify metals. However, metals utilize other detoxification mechanisms, such as long-term storage (e.g., cadmium), and biliary and/or urinary excretion. A major problem in recognizing metals as carcinogens in humans is the lack of populations of sufficient size and with definable single metal exposure. The availability of a large Taiwanese population with defined exposure to arsenic in drinking water recently provided sufficient data to provide a statistical link to the development of cancer in this population (NAS/NRC,2001). Target organ sites for metals as carcinogens are summarized by Waalkes (1995). Experimental animal systems have reproduced, to a large extent, the metal-induced tumors found in humans with the exception of arsenic.

It should be noted that essential metals can also be carcinogenic. Chromium is cited as an example of this duality: chromium III is essential and chromium VI carcinogenic. Iron in combination with a carbohydrate produces tumors at the site of injection (Sunderman,1978). Parenteral administration of iron in combination with nitrilotriacetic acid (an iron chelating agent) is a potent hepatocarcinogen, whereas similar exposure to inorganic iron compounds is not carcinogenic (Cia et al.,1998). While these observations may be dismissed as not relevant for health risk assessment for humans, they do demonstrate the complexity of the carcinogenic process for metals. Persons with hemochromatosis (iron storage disease) develop hepatic cirrhosis and have a possible risk for hepatocarcinoma (NAS/IOM, 2003). Several epidemiological studies have reported a possible correlation between measures of iron status and cancer among people in the general population (NAS/IOM, 2003). One study found higher serum iron concentrations in individuals with colorectal cancer than control subjects (NAS/IOM, 2003). It concluded that “there is no doubt that iron accumulated in the liver is a risk factor for hepatocellular carcinoma in patients with hemochromatosis” (NAS/IOM, 2003). However, the evidence for a relationship between dietary iron intake and cancer, particularly colorectal cancer in the general population, is inconclusive (NAS/IOM, 2003). Updated EPA (2003a, 2003b) guidelines for carcinogenic risk are presently in draft form or under review.

3. ROLE OF SPECIATION OF METALS AND METAL COMPOUNDS

Issues discussed in this paper relevant to a framework for assessing the toxicity of metals and metal compounds are focused on the inorganic species of metals and metal compounds. Chemical speciation has an impact on solubility, bioavailability, and persistence of metals and metal compounds in the environment. The impact of speciation on health effects of metals may be more limited. For inorganic species it is generally assumed that the potential toxicity is related to the interaction of the metal ion with the cellular target. Differences in potential levels of toxicity between various inorganic compounds of a metal may be related to solubility and toxicokinetics, which is discussed later in this paper. The toxicity of organic species of metals have different biological behavior than inorganic metal compounds and do not fit the suggestions for metals risk assessment discussed in this document. Organic species of metals may be more or less toxic than the inorganic forms. For example, inorganic arsenic compounds such as oxides of As(III) and As(V) are very toxic. Acute exposures produce multiple organ toxicity and may be fatal, and these compounds are carcinogenic with long-term exposures. These compounds occur naturally at low levels in drinking water, so they must be carefully regulated. However, organic forms of arsenic present in seafood have no significant toxicity to humans compared to the very toxic inorganic compounds. On the other hand, the organic species of mercury, methyl mercury occurring in seafood, is very toxic to neurological development in utero at very low levels of exposure. Lead occurs in nature in various minerals and as multiple inorganic salts, ranging from the slightly soluble lead chloride to less soluble lead oxides and lead sulfate. While the toxic moiety of inorganic lead salts is ionic lead, the varying degrees of solubility influence absorption and level of exposure. Cadmium also exists in nature in the form of minerals and inorganic salts. There is presently little known about differences in solubility and absorption in the gastrointestinal tract for different inorganic species. However, studies do suggest that protein-bound cadmium (cadmium metallothionein), as present in food, may be less well absorbed by the gastrointestinal tract than inorganic salts (WHO/IPCS, 1992).

4. DIFFERENCES IN BIOLOGICAL BEHAVIOR (KINETICS) BETWEEN METALS AND ORGANIC COMPOUNDS

An objective of the draft Framework for Metals Assessment (USEPA 2002) is to identify issues for "hazard and risk assessments of metals and metal compounds not generally encountered with organic chemicals." Recognition of these differences will assist in refining the health assessment process. A number of these differences, summarized in Table 2, result in differences in biological behavior that affect the kinetics of these substances; that is, differences in rate of absorption in the gastrointestinal tract, lungs, and skin; deposition and retention in tissues; and excretion from the body. General pathways for biotransformation of organic compounds are generally extensive and often species-specific, involving enzymatic pathways concerned with degradation of the compound. On the other hand, metabolism of metals is usually limited to oxidation-reduction reactions or alkylation/de-alkylation reactions. In these reactions, new inorganic species or metal organic complex may be formed but the metal ion persists.

There are major differences between the persistence of metals or inorganic metal compounds in the body and the persistence of organic compounds. Metals are neither created nor destroyed by biological and chemical processes, but may be biotransformed from one chemical species to another. That is, the metal ion thought to be responsible for the toxicity of a metal may persist in the body regardless of how the metal is metabolized.

Table 2. Summary of Major Differences in Kinetic Behavior of Organic Compounds Compared to Metals and Inorganic Metal Compounds

Organics	**Metals**
Metabolism is generally extensive and often species specific.	*Metabolism* is usually limited to oxidation state transitions and alkylation/dealkylation reactions.
Persistence in body fat is common because of lipid solubility (not capacity-limited).	Often *sequestered*, bound to specific plasma or tissue proteins (intrinsically capacity-limited) or bone.
Predominantly *eliminated* by excretion in urine and exhaled air after biotransformation from lipophilic forms to hydrophilic.	Predominantly *eliminated* in urine and bile. Metal compounds are hydrophilic.
Tissue uptake is most commonly a blood flow–limited process, with linear portioning into tissues.	Metals and their complexes are often ionized, with *tissue uptake* (membrane transport) having greater potential to be diffusion-limited or use specialized transport processes.
Interactions with other structurally similar compounds may occur, especially during metabolism.	*Interactions* among metals and between metals and organics are numerous and occur commonly during the processes of absorption, excretion, and sequestration.

Lipid-soluble organic compounds readily diffuse into richly lipophilic tissues such as the brain, liver, and neutral fat stores where they are difficult to excrete. Biotransformation of lipophilic organic compounds usually results in conversion of the original compound to a more hydrophilic form to enhance excretion in urine and feces. Entrance of metals or inorganic metal compounds into lipid-rich tissues like the brain depend on hydrophilic pathways. Metals or metal compounds do undergo some metabolic alterations that involve processes that influence behavior in the body, such as absorption, transport. deposition in tissues, and excretion, but they retain their hydrophilic nature. Retention in tissues of metals or metal compounds is generally related to formation of inorganic complexes or metal protein complexes, e.g., lead in bone and cadmium in tissues bound to the low molecular weight protein, metallothionein.

Absorption of organic xenobiotics in the gastrointestinal tract is favored by the lipid nature of intestinal cell membranes, but this is complicated by the lack of solubility of lipophilic compounds in the hydrophilic contents of the gastrointestinal tract (preabsorption). In the lungs, the absorption of aerosols of particulate forms of metals and metal compounds and of lipophilic organic compounds may not be as dependent on the lipophilic or hydrophilic nature of the substance, but are more dependent on particle size and on whether the substance is presented as a vapor or gas (not a likely property of metals). Human skin is not very permeable and provides a good barrier against absorption of metals and metals compounds as well as highly lipophilic organic compounds, but the mechanism for absorption may differ. Polar substances, like metal compounds, appear to diffuse through the outer surface of protein filaments of the stratum corneum, which is hydrated, whereas lipophilic nonpolar organic molecules diffuse through the lipid matrix between the protein filaments (Rozman and Klaassen, 2001).

Additional issues concerning interaction differences between organic chemicals and metals and inorganic metal compounds are discussed in later sections.

5. EXPOSURE TO METALS

The goal of metals risk assessment is to estimate the consequences of exposure to a metal. To attain this goal, a quantitative measurement of the chemical (metal), as well as the length of the exposure to it, must be determined (WHO, 1993). In cases where the response of exposure is a local effect, such as skin irritation—which can occur directly with beryllium salts—knowledge of the environmental concentration is sufficient for the assessment. For a systemic effect, the uncertainty of the risk assessment is decreased considerably when the internal concentration of a metal, and more preferably the biologically effective dose at the target organ (as opposed to environmental concentration), is quantified. For a number of reasons, however, it is not always feasible to determine the internal or biologically effective dose of the metal at the target tissue. The use of biological indicators or markers of exposure, also termed "biomarkers of exposure" is a way to link external exposure of a metal to internal dose (e.g., lead in blood and bone, arsenic and cadmium in urine, and mercury in maternal hair and umbilical cord blood).

5.1 Biomarkers of Exposure

The World Health Organization (WHO/IPCS, 1993) defines a biomarker of exposure as "an exogenous substance or its metabolite or the product of an interaction between a xenobiotic agent and some target molecule or cell that is measured in a compartment within an organism." In the case of metals, urinary cadmium and blood lead are examples of exogenous substances of biomarkers of exposure. The measurement of metals in biological fluids is the primary means of quantifying biomarkers of exposure for metals by occupational health organizations such as the American Congress of Governmental Industrial Hygienists. An interaction between a metal and a target molecule, such as the adduction of chromium VI with DNA and protein, is used to a more limited extent. The chromium VI adducts could also be classified as biomarkers of effect.

The “ideal” biomarker of exposure has several characteristics (Grandjean et al., 1994). These include that the sample collection and analysis are simple, sensitive, and reliable; the biomarker is specific for a particular type of exposure; the exposure results in a reversible change; intervention or prevention of exposure is considered if exposure is confirmed by the biomarker. There should also be a well-established relationship between biomarker of exposure and outcome, in that the biomarker provides information not only about exposure levels, but is also predictive of an effect. For example, urinary cadmium is directly correlated to the concentration of cadmium in the renal cortex, which is one site for toxicant action of this metal.

Biomarkers of exposure are a measure of aggregate exposure to a metal or chemical, and the exposure may occur simultaneously. Sources of metal exposure include environmental (water, air, soil, dust), occupational, medicinal, and dietary. For this reason, use of biomarkers increases the need for comprehensive, multi-pathway assessments of exposure. Reference or background levels of biomarkers of exposure are essential for any assessment. Several metals, such as arsenic and selenium, are found naturally in the diet. A lack of consideration of dietary sources of metals may result in a misinterpretation of the exposure. Arsenobetaine is an organic and non-toxic form of arsenic found naturally in shrimp and other seafood. The analysis of total unspeciated urinary arsenic of individuals who consume seafood, without recognition of their diet history, will lead to an overestimation of exposure to toxic (inorganic) arsenic species. As a result, some assessments of arsenic exposure have assumed that 10% of total elemental arsenic in seafood and 100% of arsenic in all other foods is a toxic, inorganic form (NAS/NRC 1999). The use of biomarkers of exposure in risk assessment requires that the biomarker be well-grounded or valid. The validity of a biomarker is supported by three aspects: analytical, toxicokinetic, and biological relevance (Grandjean et al., 1994; Schulte and Talaska, 1995; WHO/IPCS, 1993).

5.2 Analysis of Metals

Key analytical issues include specificity, sensitivity, standardization of methodologies (to reduce intra- and interlaboratory variability), speciation, quality assurance, and the availability of reference samples. Technology has advanced significantly in the past decade such that analytical methods for the detection of metals, such as inductively coupled plasma mass spectrometry, hydride generation atomic absorption, or fluorescence spectrometry and others have increased the sensitivity of detection. When coupled with HPLC, these methods are enhanced because of the ability to detect speciated parent metal and metabolites. While these methods can be very reliable for the analysis of metals in biological fluids, using them for tissue analysis is more difficult. In many cases tissues must be digested or the metals extracted out of it before analysis, and these procedures may hinder the ability to fully speciate the metal, or there may be interfering matrix factors. Another drawback to these types of methods is the lack of reference standards in the appropriate matrix. X-ray fluorescence spectrometry, used to detect lead in bone (Ambrose et al., 2000), and neutron activation analysis, used for manganese in liver (Arnold et al., 1999), are highly powerful non-invasive in vivo techniques. The accumulation of metals in organs that results from chronic exposure to metals can be monitored and quantified using these techniques. Also, these techniques are able to detect more than one metal at a time, which may occur after exposure to a mixture of metals. A disadvantage to the in vivo methods is that they

are not able to speciate the metal of interest, so the exposure to the toxic metal species may be estimated incorrectly.

The frequency and timing of sampling of biological fluids and tissues and correctly interpreting the results are dependent on knowing the elimination half-life of the metal. The half-life of lead in plasma, blood, soft tissues and bone ranges from hours to months to years (Sakai, 2000). The detection of lead in plasma above background levels would be indicative of an acute exposure, whereas its detection in bone would be indicative of chronic exposure. Thus the sampling of plasma every other day or week, or the analysis of bone, would not be the best way to determine if an acute exposure to lead occurred.

5.3 Biological Relevance

Biomarkers of exposure that have a biological relevance are one part of the overall process, starting from exposure to a metal and ending with a defined outcome. For example, the presence of a known toxic species of a metal (cadmium) in a target organ (kidney), a specific biomarker of exposure, most certainly would be biologically relevant because cadmium is nephrotoxic. Thus the validity of a biomarker of exposure for a metal is dependent on the link between exposure to it and biological effect. However, for many of the metals of interest, and particularly in humans, the relevance of biomarkers of exposure are not well characterized.

6. INTERACTIONS BETWEEN METALS

There are generally three classes of interactions between metals: interactions between essential metals, between toxic metals, and between essential and toxic metals.

6.1 Interactions Between Essential Metals

An objective of the interactions between essential metals is related to maintaining optimal nutritional levels by synergisms and antagonisms at both physiological and extrinsic (dietary) sites. These interactions are often complex and have been summarized in a WHO publication (WHO, 1996c). One physiological variable that influences essential metal bioavailability and utilization involves changes in the gastrointestinal absorptive process due to developmental stage (i.e., infancy or senility, adaptation due to low trace-element status or high demand such as during pregnancy). Other extrinsic or dietary variables include the solubility or molecular dimensions of the essential metal species within food, digestive media, and factors within the gut mucosa that may influence uptake. Examples of metals or metal compounds that reduce availability include iron oxalates, copper sulfides, trace element silicates, and phytates associated with calcium.

During the past three decades, there has been considerable focus on the bioavailability as well as the nutritionally essential role of trace elements, such as zinc, copper, molybdenum, manganese, iron, selenium, chromium, boron, and cobalt. The Food and Nutrition Board has provided recommended dietary allowances (RDA) for these trace metals and guidance for

assessing risk from dietary exposures to these elements (NAS/IOM, 2003). While there is concern for adequate dietary availability of these elements, there has also been a growing awareness that excess exposure to nutritionally essential metals may result in toxicity. The World Health Organization (WHO/IPCS, 2002) has provided guidance on methods of assessing risks from excessive exposures to nutritionally essential metals. This concern regarding bioavailability is timely given the increase in use growth of dietary supplements and other consumer products or remedies which may contain high levels of metals (examples include colloidal silver "cure-alls" and Mexican folk remedies containing lead tetroxide) (Bose et al., 1983; CDC 1981, 1982, 1983; Geffner and Sandler, 1980; McKinney, 1999; Pontifex and Garg, 1985; Trotter, 1985; Yanez et al., 1994).

RDAs are defined as "levels of intake of essential nutrients considered on the basis of available scientific knowledge to be adequate to meet the known nutritional needs of practically all healthy persons" (NRC/IOM, 2003). This is a public health concept based on the premise that if the requirement of each individual in a population is not known, the allowance must be high enough to meet the needs of those with the highest requirements. RDAs for essential nutrients cannot, therefore, be equated with average requirements; they must exceed the requirements of most of the members of the population group for whom the recommendation is made (NAS/IOM, 2003).

The factors that are considered in setting RDAs for trace elements are:

1) Scientific evidence about requirements of man. For iron, estimates are based on iron stores in tissues formed during growth in children, iron loss in menstruating women, and losses in tissues sloughed off in adult men. For zinc, copper, and iodine, balance studies in humans have been considered in setting RDAs.

2) Approximate estimate of nutrient consumption by population that shows no evidence of nutritional deficiency.

3) Age, sex, body weight, physiological state, inter-individual variability and activity. These are important for estimating RDAs for different population groups.

4) Estimates of biological availability, which may depend on the form in which the element occurs in food, the presence of phytates and other substances that bind the element, the presence of substances that facilitate absorption (e.g., ascorbic acid facilitating absorption of iron), the occurrence of antagonistic compounds, such as goitrogens which reduce the effectiveness of iodine, and the presence of toxic heavy metals as contaminants which may act as antagonists to essential elements. Arsenic, cadmium, lead, and mercury, which act as toxic elements either alone or in combination, may antagonize the availability of zinc, copper, and selenium when these essential elements are present in marginal amounts in diets.

6.1.1 Homeostatic Mechanisms for Maintaining Optimum Levels of Essential Metals

Nutritionally essential metals have homeostatic mechanisms that maintain optimum tissue levels over a range of exposures and may involve metal interactions. This function is required to reduce excessive exposure or deficiency and to regulate essential functions over a wide range of intakes. Homeostasis (e.g., chemical adaptation) is an inherent biological property. These mechanisms involve regulation of absorption and excretion as well as retention or storage of metals. It is these mechanisms that provide for the flexibility in nutritional supplies while maintaining levels that provide optimum nutrition but are not excessive enough to result in toxicity. The efficiency of the homeostatic mechanism may be related to factors that influence absorption (bioavailability), age-related factors, and dietary and nutritional interactions. The homeostatic mechanism may also involve an interaction with another essential metal. The efficiency of the homeostatic mechanism varies within populations and individuals. However, identification of the prevalence of a variation would require the study of large populations. Defects in homeostasis that might occur secondary to certain disease states may result in exceptionally high nutritional requirements (e.g., disorders with a decrease in gastrointestinal absorption). On the other hand, specific genetic abnormalities in the metabolism of an essential metal might result in enhanced sensitivity to toxicity (e.g., iron in hemochromatosis or copper in Wilson disease) (WHO/IPCS, 2003).

6.2 Interactions Between Toxic Metals Within Mixtures

Arsenic, cadmium, lead, and zinc are most predominantly found at Superfund sites, specifically at sites with mining and smelting operations (Brown et al., 1999). Studies of populations around these sites are available (ATSDR, 1995), but this report did not explore the issue of exposure to this quaternary mixture (Sheldrake and Stifelman, 2003; von Lindern et al., 2003). In general, human health studies have addressed blood lead levels in children and urinary cadmium excretion in adults (Idaho Department of Health and Welfare Division of Health, 2000). Blood lead and urinary cadmium levels were elevated relative to those in reference populations. Similarly, a survey of wildlife in the vicinity of a zinc smelter site reported higher concentrations of cadmium in kidney and lead in bone than seen in animals from a relatively uncontaminated area,but did not address potential interactions among the studied components (Cd, Pb, Zn, and Cu). Arsenic was not specifically discussed, but was present at the site (Storm et al., 1994).

Results from a ternary mixture of Cd, Pb, and Zn study in rats indicated slightly more marked adverse hematological effects in ternary mixture exposure than binary mixtures (Thawley et al., 1977). However, inconsistencies in dietary levels of calcium and vitamin D in this study made comparisons problematic. A well-controlled rat study has reported protective effects of high dietary levels of zinc against some of the testicular effects of a mixture of cadmium and lead ***(REF?).*** Significance of these data in human exposure scenarios are unavailable in the current literature. In another study, a relatively wide range of endpoints were investigated in studies (Fowler and Mahaffey, 1978), which included each metal singly and all possible binary and ternary mixtures. Body weight gain was depressed equally by the ternary

mixture and by the Cd-Pb mixture, and to a lesser extent by the As-Pb and Cd-Pb mixtures, whereas food utilization was depressed to a greater extent by the ternary and As-Cd mixtures than by the other binary mixtures. In general, the biological parameters studied in this report indicated changes of smaller magnitude and inconsistency in direction when binary mixtures were compared with ternary mixtures.

The data regarding interactions of As, Cd, Pb, and Zn, summarized above, are not adequate for predicting the magnitude of interactions. Experimental efforts to identify interactions between these toxic metals are needed. For some endpoints, the data are not robust regarding whether the joint action will be additive or greater or less than additive. In this case, the default approach—assumption of dose additivity for individual components—is often used. This approach, which involves calculation of a hazard index, is most appropriate for chemicals that produce the same effects by similar modes of action. Superfund guidance (U.S. EPA, 1989) states that a strong case is required to indicate that two chemicals that produce adverse effects on the same organ system, although by different mechanism, should not be treated as dose additive. In the case of chemicals with different critical effects, separate effect-specific hazard indexes are estimated for the critical effects and the other major effects of the chemicals in the mixture, using the RfD as the toxicity value for each effect. The animal studies discussed in brief in this report used commercial diets or semi-purified diets which may be restricted or enhanced in terms of level of essential metals as compared to human diets. Much higher doses of the metals appear to be required to elicit effects when commercial diets are used than when semi-purified diets are used. At the other extreme, effects seen at very low doses when deficient diets are used. Comparisons among studies are therefore problematic, particularly when the diets are not specified.

6.3 Interactions Between Essential and Toxic Metals Within Mixtures

Toxic elements normally found in the environment, unless the exposure is overwhelming, are antagonized by essential nutrients found in foods we eat. Diet, therefore, can be a major factor in determining the appearance of adverse health effects following exposure to toxic elements. For example, humans can be exposed to mercury by consuming fish that have absorbed mercury from contaminated bay water, whereas selenium present in the same water body can act as a natural antagonist for mercury toxicity; cadmium in contaminated soil can enter our food chain by eating fruits and vegetables grown in contaminated soil, while zinc found in nuts can antagonize cadmium toxicity. Appearance of toxicity also depends to a great extent on absorption and retention of both toxic and essential elements. In the case of copper, a particular level of intake can lead to signs of either copper deficiency or of copper toxicity in humans. Relative intakes of zinc, sulfur, or iron play a significant role in modulating copper deficiency or toxicity. Suttle and Mills (1966) showed that dietary levels of copper at 425 mg/kg caused severe toxicosis in pigs. However, all signs of toxicity were prevented by simultaneously supplementing the diet with 150 mg/kg zinc and 150 mg/kg iron.

In different geographical situations, contamination of air, water supply, and food with trace elements, arising from agricultural practices and from increasing motorization and

urbanization, may have deleterious effects on the long-term health and welfare of human populations. These types of human exposure scenarios have stimulated increasing concerns about the concentrations and movement of trace elements in the environment and about the maximum permissible intakes by humans. Such contamination primarily involve mercury, lead, cadmium, and arsenic. Additionally, it has become evident that the prevalence of processed foods in developed countries can lead to deficient or marginally deficient intakes of other trace elements, for example zinc and chromium.

6.3.1 Role of Molecular or Ionic Mimicry in Essential-Toxic Metal Interactions

The term molecular or ionic mimicry has been applied to those situations in which a toxic metal forms a complex with an endogenous ligand, and the resulting compound mimics the behavior of a normal substrate, disrupting normal function. Awareness of such interactions could be considered in health assessments for exposure to specific metals. A number of reviews discuss this phenomenon along with examples that explain the mechanism of toxicity for specific metals (Clarkson, 1993; Ballatori, 2002). One well studied example is the replacement of zinc by lead in heme synthesis by inhibiting the function of heme synthesizing enzymes (Goyer and Clarkson, 2001). In another study, the substitution of calcium by lead resulted in toxicity of several vital enzyme systems in the central nervous system. This toxicity was responsible for impairment of the development and function of enzymes involved in the production and transport of neurotransmitters (NAS/NRC, 1993). Divalent inorganic mercury forms linear bonds that form a complex that structurally mimics oxidized glutathione. Arsenate complexes with phosphate in the sodium dependent transport system in renal cells and the arsenate replaces the phosphate in mitochondria, impairing syntheses of ATP and energy metabolism. Wetterhahn-Jenerette (1981) explain why chromium VI in the form of chromate can readily enter cells, whereas chromium III cannot enter cells. This may have implications as to why chromium VI is carcinogenic, but the essential metal chromium III is not a carcinogen. There is a large body of literature providing examples of molecular or ionic mimicry that involve most toxic metals. Most of these examples involve replacement of an essential metal with a non-essential or toxic metal, and molecular or ionic mimicry may be viewed as a form of metal-metal interaction; most such examples involve essential and toxic metal interactions, rather than toxic-toxic metal interactions.

6.4 Health Assessment for Exposure to Mixtures

The preferred approach for risk assessment of a mixture is to use exposure data and a toxicity value, such as a reference dose (RfD), for the specific mixture of concern to characterize risk or hazard; however, relevant data are rarely available (U.S. EPA, 1989). Exposure to some of the elements, such as cadmium, lead, arsenic, and zinc, may vary from site to site. The toxicity data for a mixture containing these components in a fixed proportion might not be fully applicable to site assessments involving different proportions. Some judgment as to whether the mixtures are sufficiently similar would need to be made. When adequate health effects data on the same or a similar mixture are lacking, health effects data for the components of the mixture, along with data regarding interactions, are to be used for risk assessment (U.S. EPA, 1989). If adequate quantitative data on interactions of the components are available, the data would be

used to predict the pattern of the interactions for various proportions of the mixture components or to modify the risk assessment. Firmly established biomarkers of exposure can be used to assess exposure models by comparing the predicted model results to those observed in the population studied. A recent study by Choudhury et al. (2001) used urinary cadmium as a biomarker of exposure to evaluate a cadmium dietary exposure model linked to a biokinetic model. The predicted urinary cadmium and kidney cadmium burden levels of the model were in general agreement with those observed from human population mixtures. More accurate model predictions of metal levels in tissue or fluids (i.e., biomarkers of exposure) may be obtained by linking exposure models with physiologically-based pharmacokinetic (PBPK) models as described in Section 7.1 (Andersen, 1995; Clewell, 1995; O'Flaherty, 1998).

7. HUMAN HEALTH RISKS

Assessment of health risks for toxicity from metals involves determinating the probability of an adverse event occurring at a particular level of exposure. Risks are usually assessed for chronic exposures from either environmental or workplace exposure, but may also be expressed for acute or short-term exposures. Acute exposures are characteristically the concern of emergency room physicians or poison control centers, whereas lifetime risks are the concern of regulatory or public health agencies such as the Food and Drug Administration, Environmental Protection Agency, the Agency for Toxic Substances and Disease Registry, and the National Institute of Occupational Safety. International agencies, such as the World Health Organization's International Programme for Chemical Safety and the International Labor Organization Agency, provide guidelines for member nations. The Joint FOA/WHO Expert Committee on Food Additives serves as the scientific advisory body to member states of the WHO regarding the safety of food additives, residues of Veterinary Drugs in Foods, naturally occurring toxicants, and contaminants in foods including metals. The methodologies followed by these agencies result in general agreement regarding health risks, but actual regulatory decisions become the purview of political and social policies.

Risk assessment typically involves four steps. The first step is to determine what the potential health effects are of toxic endpoints that may result from excess exposure to a metal. This is followed by dose-response studies, conducted either through large-scale human epidemiologic studies on human populations with a broad range of human exposures or based on animal studies. Appropriate human populations are seldom available (notable exceptions exist for lead, methylmercury, and arsenic), so for most metals the initial steps in the risk assessment process are done based on laboratory animals. From these studies the no-observed and lowest observed adverse effect level (NOAEL or LOAEL) is determined. The NOAEL may vary between studies depending on experimental design, species of animals, dose of metal, and time and route of exposure. For these reasons the NOAEL approach has become controversial in recent years among risk assessors and regulators, and alternative approaches have been proposed. The actual derivation of a tolerable intake (TI), or RfD incorporates a margin of safety (uncertainty factor) because of uncertainties related to extrapolation of results from animal studies to humans. Even data obtained from studies on humans contains uncertainties due to variations in biology or lifestyle. For these reasons there has been increasing emphasis on

toxicokinetic risk assessment models that incorporate a number of physiological or biological variables. There is additional need to account for differences in mechanisms for different metals and metal compounds and variables in human susceptibility to specific metals.

7.1 Toxicokinetics-PBPK Models

Toxicokinetics of metals entails the mathematical description and modeling of their absorption, distribution, metabolism, and excretion. A typical physiologically based pharmacokinetic (PBPK) model consists of multiple compartments representing tissues or tissue groups that are linked by blood flow. These models are valuable risk assessment tools for purposes of interspecies, high-dose/low-dose, route to route, and exposure scenario extrapolation (Krishnan and Andersen, 1994). A PBPK model for any given metal or metalloid provides an integrated framework for addressing issues related to risk assessment, as well as being a tool for hypothesis testing and experimental design. This is because a PBPK model allows one to define the relationship between external exposure and an internal measure of biologically effective dose in both experimental animals and humans. Use of PBPK models can account for nonlinear uptake, metabolism and clearance; toxicity associated with products of metabolism rather than parent chemical only; and tissue interactions. The underlying assumption is tissue dose equivalence, i.e., that health effects are caused by the toxic form(s) of the chemical measured at the biological target (Krishnan and Andersen, 1994).

PBPK models are often capable of predicting aggregate exposures. For many metals, they can be scaled across species, and the kinetic parameters (tissue blood flow, metabolic constants, chemical binding constants) within the PBPK model are generally reflective of what occurs in vivo. PBPK models have historically been developed and used for risk assessment mainly with volatile organic compounds (e.g. methylene chloride) (Andersen et al., 1987), but have also been applied to many metals (Clarke, 1995; White et al., 1998). Metals differ in their kinetic behavior compared to volatile organic chemicals in a number of ways as discussed by O'Flaherty (1998). Whether using PBPK models or other dosimetric adjustments in the risk assessment process for metals, the following kinetic factors need to be explicitly considered: (1) oral bioavailability, (2) inhalation bioavailability, (3) cellular uptake, (4) toxic metal-essential metal interactions, (5) protein binding behavior and function, (6) incorporation into bone, (7) metabolism, and (8) excretion. The issues (specific determinants) surrounding these factors are outlined in Table 3. To facilitate model evaluation, predicted model compartments should be linked to biomarkers or other measures of exposures, for example, urinary cadmium levels (Choudhury et al, 2001).

Many of the processes controlling the disposition of metals are intrinsically capacity-limited and highly metal-specific. This implies the need to have sufficient understanding of physiology to model these processes and methods to estimate binding constants. Another overarching theme is that metal-metal interactions of multiple types (e.g. competition, antagonism, synergism, as well as essential-nonessential metal interactions) commonly occur at multiple points during the processes of absorption, distribution, metabolism, and excretion. Another distinctive characteristic of metals is that common sequestration mechanisms, such as incorporation into bone and binding to storage proteins, can result in extended residence times.

Constructive use of PBPK models in the risk assessment process also requires some consensus concerning mode(s) of action and the form of the chemical responsible for the effect of greatest toxicological concern in order to select an appropriate dose metric. The issue of which endpoints are matched with what form or species of the metal or metalloid will influence both the functional form of the model and hence dose metric selection. The major challenge here is to balance the complexity of the biology with the data available to parameterize the model. Estimation of many parameters from the same data or insufficient data (over-parameterization) leads to greater uncertainty in model predictions and limits the utility of the model for regulatory purposes.

7.2 Susceptibility Factors

7.2.1 Solubility of Metals and Metal Compounds

Solubility is one of the major factors influencing bioavailability and absorption of metals and metal compounds. The solubility of metal compounds is dependent on its chemical species, on pH of media (H+ ions), and on the presence of other chemical species in the media. Nitrates, acetates, and all chlorides of most metals except silver, mercury, and lead are soluble. Also sulfates of most metals are soluble except for barium and lead. On the other hand, most hydroxides, carbonates, oxalates, phosphates, and sulfides are poorly soluble. Another factor influencing absorption of poorly soluble compounds is particle size; fine particles are usually more soluble. Metallic lead is absorbed from body tissues, probably after being oxidized to soluble salt. Metallic mercury is corrosive and embedded in body tissues, but metallic mercury swallowed into the gastrointestinal tract is not soluble (Goyer and Clarkson, 2001).

Table 3. Kinetic Factors To Consider When Evaluating the Use of PBPK Models or Other Dosimetric Adjustments in the Risk Assessment Process

Kinetic Factor	Physiologic Impact
Cellular Uptake	Carrier-mediated uptake (e.g., phosphate or sulfate transporters) Facilitated transport in the form of organic complexes
Toxic metal-essential metal	Competition for binding sites on membrane transport proteins Interactions at enzyme active sites? Systemic level interactions altering absorption
Protein binding	Capacity limited to binding to specific proteins Inducibility of binding proteins (Zn,Cu, Cd to metallothionein) Protein binding as sequestration mechanism Pb-binding protein in inclusion bodies
Sequestration in bone	Lead sequestered in bone
Metabolism	Relative contribution to overall elimination compared to excretory mechanisms
Excretion	Relative contribution of urinary and biliary excretion Capacity limitation (saturation kinetics)

Bioavailability of several metals has been shown to be influenced by an association with phytates in the gastrointestinal tract. Dietary components such as cereals and grains are rich in phytates and form phytate/metal particularly with nutritionally essential metals. There is more concern regarding zinc availability in milk substitutes (soy protein) in diets for infants than bioavailability in the more complex conventional diets of adults. The calcium-phytate complex has a strong affinity for both lead and calcium. High dietary calcium and iron restrict intestinal absorption of lead (WHO, 1996c).

7.2.2 Metal Protein Interactions

Metals react with many different proteins in the body that may modify their toxicity and kinetics. Examples are the interaction of lead with heme synthesizing enzymes. Arsenic, cadmium, mercury, and lead interfere with enzymes involved with energy metabolism by substituting with essential metals (see Section 6). Many metals bind with albumin for purposes of transport in the circulatory system and across cell membranes and within cells. In addition there are a number of specific metal-binding proteins (Goyer and Clarkson, 2001).

Metallothioneins. The metallothioneins are a group of low molecular weight proteins (MW about 6,000 Daltons), rich in sulfhydryl groups which serve as ligands for several essential and nonessential metals). In vitro studies have found that the highest affinity is for silver, then in descending order, mercury, copper, bismuth, cadmium, lead, and zinc (Kagi and Kogima, 1987). However, studies of in vivo metallothioneins from various sources contain zinc, copper, and cadmium. The type of metals bound to metallothioneins differ depending on the species, the organ, and previous exposures to metals, but most of them contain at least two different types of metals. For example, metallothioneins isolated from adult or fetal human livers contain mainly zinc and copper, while those from human kidney contain cadmium, copper, and zinc (Cherian and Goyer, 1995). In most cases the metallothioneins are inducible and perform a number of functions, including serving as a storage protein for zinc and copper in the liver, kidney, brain, and possibly skin, and having an important protective role in cadmium toxicity (Goyer and Clarkson, 2001).

There has been recent interest in the role of metallothionein as a modulator of immune response, and it is suggested that assessment of metallothionein status in peripheral blood monocytes may provide a non-invasive approach to assessing the risk of metal exposure to immunotoxicicty (Pillet et al., 2002). While metallothioneins have an affinity for lead in vitro, in vivo binding to lead has not been demonstrated. Also, mercury may induce synthesis of metallothionein in vivo, but binding is only temporary regardless of the demonstrated in vitro affinity.

Transferrin. Transferrin is a glycoprotein that binds most of ferric ion in plasma and has a role in transporting iron across cell membranes. This protein also transports aluminum and manganese.

Ferritin. Ferritin is primarily a storage protein for iron in reticuloendothelial cells of the liver, spleen, and bone. It plays an important role in turnover of iron. It has also been suggested that ferritin may serve as a general metal agonist since it binds a number of toxic metals including cadmium, zinc, beryllium, and aluminum.

Ceruloplasmin. Ceruloplasmin is a copper-containing glycoprotein oxidase in plasma that converts ferrous to ferric iron, which then binds to transferrin.

Lead-Binding Protein(s). Lead binds with a number of lead-binding proteins but the identity or function of these proteins is not as well defined as other metal-specific proteins. The most studied lead-binding protein is the denatured lead-protein complex identified as the intracellular inclusion body occurring cells, particularly in the liver and kidney in persons with high-level lead exposure. It has been suggested that lead-binding proteins may have a protective effect for lead (Goyer and Clarkson, 2001).

Membrane Carrier Proteins. There are a number of recently discovered carrier proteins that transport metals across cell membranes. Many metals are transported as complexes with endogenous ligands; no transport systems are intended for the ligand itself. Many of these carrier proteins are multi-specific, accepting substrates that vary considerably, but are recognized by the attached metal ion (Dawson and Ballatori, 1995)

7.3 Variability in Susceptibility

7.3.1 Age

It is well documented that infants and children have a greater intake per unit of body weight of soil, air, certain types of food, and water (U.S. EPA, 1997). Consequently, for a given concentration of a pollutant or contaminant in soil, air, food or water, a child will receive a different exposure (in terms of mg/kg/bw) than will an adult exposed to the same medium (Plunkett et al., 1992). Usually a child's intake or unit of body weight is higher than an adult's.

There are also differences in pharmacokinetic behavior of metals at difference stages in the life cycle, particularly for the nutritionally essential metals (WHO, 1996). During the immediate post-natal period, absorption of essential metals is poorly regulated (e.g., chromium, iron, zinc) until homeostatic regulatory mechanisms become established with increasing gut maturity. Much of what is known about gastrointestinal absorption during infancy is derived from animal studies. Few studies have been conducted on humans. On the other hand, there are numerous studies on the effects of lead and on the developing nervous system in humans (WHO/IPCS, 1995; NAS/NRC, 1993). It is suspected that the human placenta is resistant to transport of cadmium (Goyer, 1995). It has also been shown that neonate experimental animals have a higher absorption of both lead and cadmium (Kostial et al., 1978). The efficiency of intestinal uptake of some trace metals, particularly zinc, declines in the elderly. But differences between mature adults for other trace elements and toxic metals has not been demonstrated (WHO, 1996c).

7.3.2 Gender

Pregnancy and lactation increase demand for some essential metals, particularly copper, zinc, and iron (Picciano, 1996; NAS/IOM, 2003). References to women as being a highly susceptible group for metal toxicity usually refers to effects on the fetus during pregnancy, e.g., lead and mercury, but there may also be basic gender differences independent of pregnancy that would account for differences in toxicokinetics between women and men. Women have only

about two-thirds the fat-free body mass of men, while having a larger percentage of body fat. The male/female ratio for urinary creatinine excretion (an index of body muscle mass) is 1.5. Men are generally larger than women. Skeletal size as well as body calcium are a function of height. Because adult females have only two-thirds as much fat-free body mass as men, protein and energy requirements are correspondingly less. These differences have an impact on body content of minerals (WHO/IPCS, 2002).Women also have significant loss of iron during menstruation, and it has been shown that absorption and toxicity of cadmium is greater in women, related to decrease in iron stores (Berglund et al., 1994).

7.3.3 Genetically Determined Human Variability (Polymorphisms)

There is considerable variability between individuals in the nature and severity of response from exposure to metals and metal compounds. Some of these differences in responses to metals may be due to subtle genetic differences or genetic polymorphisms that may alter the metabolism of a metal. The most apparent genetic polymorphisms affecting metabolism and toxicity of metals are disorders in homeostatic mechanisms for nutritionally essential metals. There are two disorders affecting copper metabolism. Wilson disease is an autosomal recessive abnormality (prevalence of 1 in 30,000), believed to be due to impaired biliary excretion of copper resulting in copper accumulation in most organs of the body, particularly the liver, brain, and kidney, which provide the most apparent clinical manifestations. The second disorder of copper metabolism is Menkes disease, an X-linked recessive disorder of copper metabolism (prevalence of 1 in 200,000) that resembles copper deficiency regardless of level of copper intake (WHO/IPCS, 2002).

Hemochromatosis is a common inherited disorder of iron homeostasis. This disorder is characterized by excessive iron absorption, elevated plasma iron concentration, and altered distribution of iron stores (altered iron kinetics). One long-term effect is liver cirrhosis, with increased risk of liver cancer (WHO/IPCS, 2003).

A genetic polymorphism for a heme metabolizing enzyme affecting lead metabolism was identified in 1973 (Granick, 1973), but the molecular characteristics and potential clinical implications have only recently received attention (Fleming et al., 1998; Smith et al., 1998). It is suspected that genetic polymorphisms also exist for arsenic metabolism (NAS/NRC, 2001), but these have not yet been defined. Other genetic polymorphisms that may affect the metabolism of toxic chemicals are being described but their role in the toxicity of metals and metal compounds is yet to be defined (Parkinson, 2001).

8. TARGET ORGAN EFFECTS

Metals and metal compounds may produce health effects in any organ system. These include the neurological, cardiovascular, hematological, gastrointestinal, musculoskeletal, and immunological systems. Many factors act as determinants of a target organ effect following exposure to a toxic metal. Some of these factors are exposure issues, e.g., dose rate factors: high level, short-term versus low dose, long-term exposure. Other factors are related to issues

identified in PBPK models and susceptibility factors as described below. Short-term exposures may produce target organ effects very different from those produced by a similar exposure in terms of dose but over a longer period of time. Short-term, high-level exposure by ingestion may give rise to well recognized acute toxicity syndromes, usually involving the gastrointestinal tract initially and possibly involving secondarily renal, cardiovascular, nervous, and hematopoetic systems. Survivors of acute high-dose arsenic ingestion usually experience multiple organ effects, sometimes with long-term sequelae. Long-term, low-dose exposure by ingestion is the route of exposure in food and water of metals that accumulate in target organs over time. Such exposures may involve any organ system over time but does not usually produce overt gastrointestinal symptoms. For example, low-level, long-term exposure to cadmium in food sometimes combined with inhalation exposure from cigarette smoking will accumulate in target organs, but not produce any obvious clinical effects until "excess" capacity is diminished to a point where the normal function is lost (e.g. onset of renal disease and/or osteoporosis later in life).

Several factors are important to selective target organ toxicity, including: distribution to the target organ and metabolic activation in the target organ (as discussed above). In addition, specific functions of the differentiated cell types in the target organ (electrical excitability, hormone production) may be differentially vulnerable.

Metals can be contrasted to an extent with organics in terms of lipid solubility and the need for metabolic activation, but this provides limited information on selective target organ toxicity. Although low lipid solubility of metal ions could be a factor in limiting their accessibility to tissues and cells, recent rapid progress in identifying metal transporters (Foulkes, 2000) suggests that generalizations are not appropriate, and each metal must be assessed in terms of its ability to access transporters and the presence of transporters in potential target organs. Further, complex lipids can offer high affinity binding sites for metal ions, such as phosphates and sulhydryl groups, and some metals, such as thallium, have a demonstrated affinity for adipose compartments. In terms of metabolic activation, a parallel process for metals that are active as ions is binding and displacement from metal-binding proteins. Thus many of the same considerations apply to metal and nonmetal toxicants.

Target organ function does not appear to create a differential vulnerability for metals and organics. A thorough review of all organ systems is required to characterize target organ toxicity. ATSDR monographs, or *Toxicological Profiles,* part of the EPA Superfund program, review all toxicological data by organ system effects (cancer, immune, reproductive, developmental, renal, respiratory, etc.). Toxicological profiles for 24 metals generally reveal that across organ systems, metals show a spectrum of toxic action similar to organic compounds. It is possible that subgroups of metals (heavy metals, Group III metals, transition metals, divalent metals) can be constructed that have common patterns of target organ toxicity, as has been done for subgroups of organics (halogenated hydrocarbons, organic acids, chlorinated solvents, aromatic solvents, PM_{10}, etc.). These groups should be formed based on an empirical basis after thorough literature reviews.

9. INPUTS TO THE REGULATORY FRAMEWORK

The background information provided in this "issues" paper has a number of specific implications when considered in the context of a Framework for Metals Assessment. Several basic differences have been elucidated that distinguish metals and metal compounds from organic compounds that impact on the risk assessment process. These have been enumerated in Section 4. An obvious consequence of these differences is that an independent risk assessment process should be developed for metals and metal compounds that embodies these differences while recognizing generic features common to all toxicants. These issues are discussed in the following sections.

9.1 Pre-Exposure Issues

Some of the issues discussed in this report might be considered as pre-exposure issues. These include classification of metals, role of essentiality, and exposure issues including route of exposure (e.g., inhalation, oral, or dermal) and mixtures. After absorption, these factors may influence toxic kinetics.

The classification of metals presented in this paper emphasizes the differences in significance in terms of health between nutritionally essential metals, primarily toxic metals, and metals with carcinogenic potential. Separation of metals into these groups impacts all three of the EPA risk assessment scenarios. Nutritionally essential metals are of less significance at cleanup sites not only because of their importance in terms of moderating bioavailability of toxic metals but also their potential interaction with highly toxic metals following exposures to complex mixtures. Examples include the protection afforded by zinc from the toxic effects of cadmium; protection provided by calcium and iron from toxic effects of lead and cadmium; and the protection selenium provides against mercury toxicity.

Recognition of differences in potential toxicity between nutritionally essential metals and purely toxic metals should impact EPA risk assessments associated with National Hazard/Risk Ranking Characterization. The implication is that purely toxic metals should be given higher hazard ranking than essential metals or those thought not to be as hazardous at lowest levels of exposures. These decisions must be further refined with dose/response data for specific metals. This approach does not exclude essential metals from hazard assessment, but only relates to characterizing level of risk. The challenge for EPA programs and assessment scenarios is to avoid excessive exposure to nutritionally essential metals to prevent toxicity while ensuring adequate exposure to prevent deficiency (WHO/IPCS, 2002). The optimum dietary intake or exposure is a range between the minimum level required to prevent deficiency and the maximum safe level of exposure to prevent toxicity. This range has recently been referred to as the "Acceptable Range of Intake," and is represented by a trough in the U-shaped dose response curve (WHO/IPCS, 2002). The application of a risk assessment model based primarily on health considerations should not exclude risk assessment based on a classification for hard and soft acids and bases for ecological/environmental considerations.

For metals with no known nutritional requirement, concern must be focused on excess exposure, recognizing that the no adverse effect level is a function of analytical sensitivity and sensitivity of the methodology used to determine the health endpoint. Carcinogenic metals might have their own guidelines within the EPA regulatory framework for carcinogens. There may be future questions arising with methodology for the risk assessment process for the potential carcinogenicity of the nutritionally essential metals. Speciation and oxidation state may be included in the process as discussed regarding iron and chromium.

Assessment of human exposure to a metal or metal compound is critical in health risk evaluations for Site-Specific Assessments, National Regulatory Assessments, and National Hazard/Risk Ranking and Characterization. While there is no specific guidance for exposure assessment to metals, EPA has a published guidelines for exposure assessment (U.S. EPA, 2003b) and guidelines for assessment of susceptible populations (U.S. EPA, 2003b). For site-specific assessments, mixtures of metals and mixtures of metals with organic chemicals may be of great concern. While there is limited information or guidance on issues of exposure to mixtures of metals, there is published guidance for health risk assessment of chemical mixtures (U.S. EPA, 1986,1989, 1992a, 1992b, 2000).

In terms of Hazard/Risk Ranking, consideration must be given to likely routes of exposure. Historically, lead has been a major concern for the general population via inhalation in addition to food and water. Presently, the primary concern might be lead in soil for toddlers. Air levels of mercury are not of major concern in terms of direct health effects from inhalation, but from the indirect effect of deposition in sediments in aquatic sites and ultimate human exposure to methyl mercury through eating fish exposed to methyl mercury in the aquatic food chain. On the other hand, inhalation of cadmium can have direct adverse health effects. These are primarily exposure issues and should be considered by the appropriate EPA risk assessment scenario.

9.2 Human Health Issues

Human health issues considered in this paper include biomarkers of exposure and effect, and factors that influence human health outcomes. Human health risk assessment largely concerns the relationship between exposure and various host factors that are considered in toxicokinetic or physiologically based pharmacological kinetic (PBPK) models. These risk assessment models include a number of variables that permit consideration of factors specific to the metal of concern and the host. Presently the EPA National Regulatory Assessment scenario involving the setting of media standards (e.g., soil, air, and water) establishes Reference Doses (RfDs) as an expression of risk for non-cancer health endpoints from exposure to toxic substances including metals. PBPK models can be used to predict health effects from a particular level of exposure. Differences between PBPK models for metals and organic toxicants have been discussed in this paper. PBPK models for lead and cadmium are available, and animal models are being developed for other metals, e.g., chromium and uranium. The models are necessarily complex but may be useful for converting environmental data into human health risk assessment data.

The area in which toxicokinetic issues specific to metals can have the most influence on the regulatory framework is at the level of national regulatory assessments for specific metals (e.g., ambient water quality criteria, MCLGs, RfDs, and RfCs). The extent to which such health-based criteria are used as inputs to site-specific assessments (e.g. Superfund assessments) and national hazard/risk ranking and characterization will determine the impact of toxicokinetic issues in these areas. Metals in general require special consideration of the processes controlling their disposition that may be intrinsically capacity-limited and highly metal-specific (e.g., specific protein binding, specialized transport processes). This implies the need to have sufficient understanding of the underlying physiology to model these processes and methods to estimate binding constants.

Another theme is that metal-metal interactions of multiple types commonly occur at multiple points during the processes of absorption, distribution, metabolism and excretion. The implication of multi-level, metal-metal interactions is that addressing issues related to groups of metals is critical, i.e., risk assessment for metals has to consider the issue of exposure to multiple metals simultaneously.

Another distinctive characteristic of metals is that common sequestration mechanisms, such incorporation into bone and binding to storage proteins, can result in extended residence times. O'Flaherty (1998) has pointed out that this requires that models describing metal kinetics over an extended time frame incorporate age dependence into the model, i.e., anatomic measures and physiological processes that are critical determinants of metal disposition can be expressed as mathematical functions of age or body weight (e.g. O'Flaherty, 1995). It is also necessary to evaluate whether metal binding to specific proteins is a sequestration mechanism or part of the pharmacodynamic process leading to toxicity.

EPA risk assessment scenarios are concerned with effects on the most sensitive populations. Susceptibility factors such as age/gender may be included in the risk assessment process, and remedial efforts may be directed toward correcting nutritional deficiencies, However, variability in the general population now recognized with the emerging discoveries in human polymorphisms presents new challenges.

9.3 Issues Related to Regulatory Applications

9.3.1 Grouping Chemical Forms of Metals for Risk Assessment

The scientific literature amply demonstrates that the pattern, effective doses, and species-specific toxicity of a metal will vary widely depending on its form. This potentially has implications concerning the separation of metals from organics, and the separation of different forms of a metal (for example inorganic and organic), for hazard assessment.

Precedent in EPA national regulatory programs varies widely in grouping of metal forms for health risk assessment. For example, the IRIS program provides 42 metal-associated RfDs, including independent RfDs for seven different thallium salts and a single RfD for “beryllium

and compounds." Some RfDs identify a general subcategory of the metal (inorganic, soluble, elemental). This issue is not unique to metals; a similar variability is found in the designation of RfDs for organics. For example, the RfD for xylenes includes all three structural isomers, di-methyl substituted xylenes, as well as mixtures, while there is an oral RfD for trans-1,2 dichloroethylene that excludes the cis-isomer.

One possible approach to specifying metal forms for health risk assessment is to divide organic from inorganic forms. However, consideration of toxicity data suggests that this division is often inadequate from the viewpoint of health risk. For example, valence is an important factor in subdividing inorganic forms of transition metals like chromium and arsenic in terms of their toxicity. Also, distinctions between various organic forms can be important. Inorganic tin (stannous chloride) has a much higher toxic effect threshold than organotins. However, among organotins, both the pattern of toxicity and threshold toxic doses vary for aryl (triphenyltin, fenbutatin) and alkyl tins, as well as for alkyl tins of various chain lengths (triethyltin, trimethyltin) (ATSDR, 1992). Further, the mechanism of action of dibutyltin, a reproductive toxicant in marine snails, may be species-specific, requiring separate consideration for human and aquatic risk assessments (Gooding and LeBlanc, 2001). This issue is not unique to metals; toxicity of organics can vary depending on optical or structural isomers, substitutions, and target species. These considerations suggest that toxicity information on all forms of the metal must initially be reviewed and that wide discretion is needed in deciding what groupings are appropriate for the hazard identification and dose-response assessments that are provided for specific regulatory purposes. These groupings are most appropriately based on the empirical data concerning toxicity. Further, these groupings may need to be revised as new data are published. For instance, concern about thimerosal, an ethyl mercury-containing preservative, has led to new studies of ethyl mercury toxicity that will help clarify the appropriateness of grouping organic mercury compounds, or alkyl mercury compounds, together for health risk assessment in national regulatory programs.

Many times, a risk assessment is available from a national regulatory assessment for a specific form or subgroup of metal compounds, but the risk manager conducting a site assessment must deal with a different form of the metal, or unspecified forms of the metal as represented in an elemental analysis. A further review of adjunct scientific information on physical chemistry, bioavailability, structure activity, etc., is needed to decide the applicability of the assessment from the national regulatory program. With this in mind, a detailed discussion of the factors that led to the original grouping in the national regulatory assessment would be valuable. In addition, a full presentation of adjunct data on toxicokinetics in national regulatory programs is valuable.

Similarly, if toxicity data are being used in ranking/prioritization, the grouping that was used in the national regulatory risk assessment is most appropriately used in the ranking/prioritization based on toxicity, with generalizations applied with a defined level of uncertainty based on review of adjunct data.

9.3.2 Generalizing From Forms of Metals Administered in Animal Toxicology Studies to Forms of Metals Found in Environmental Media

In order to achieve an adequate internal dose for the study of toxicity, animal toxicologists often use bioavailable form of metals. For the initial characterization of a toxicity syndrome, it is not practical to simultaneously test all forms of a metal that may be involved in human exposures. For example, aluminum researchers commonly use aluminum lactate which is known to reliably provide elevated tissue concentrations in laboratory animals. Aluminum maltolate is also used because it provides a stable ion pool in water solution, as opposed to other salts which are progressively hydrated as the solution stands. However, a site assessor is very unlikely to encounter aluminum in the lactate or maltolate form. Thus the situation arises where toxicity data have been generated for a bioavailable form of a metal, but the site assessor must deal with another form. Several approaches are possible: (1) use a default assumption that the metal in the environmental samples is in its most toxic form; (2) use adjunct scientific data to derive an adjustment to the effective dose identified in the animal study; (3) conduct new animal toxicology studies using the metal form encountered in the site assessment. The first approach is the most health conservative and the second is more scientifically sound. The third option might be available in some circumstances but is usually precluded by time and financial resource limitations.

A fourth alternative that is less often used is to estimate bioavailability through solubility studies or limited bioavailability studies of samples from the site. For example, arsenic bioavailability has been estimated for soils from various contaminated sites (Freeman et al., 1995; Freeman et al., 1993; Ng et al., 1998) and also through a series of solubility studies of soil from a site contaminated with mine tailings (Ng et al., 1998; Salocks et al., 1996).

An example of adjunct data useful for generalization from the administered to encountered form can be provided for aluminum. Pharmacokinetic information for several aluminum forms has been provided in review articles (Yokel and McNamara, 2001; DeVoto and Yokel 1994). Other studies provide data on tissue concentration after dosing with equivalent molar amounts of different aluminum salts (Dlugaszek et al., 2000). An empirical comparison of the LD_{50} of a number of administered salts is also available (Llobet et al., 1987) and another series of studies looked at developmental toxicity of several salts (Domingo, 1995).

9.3.3 Evaluation of Research Reports of Metal Toxicity

Research reports need to be evaluated for adequacy of design, confounding factors, accurate identification of administered dose, and quality of the study. The same principles apply to organics and metals. Some specific applications of these principles for animal studies of metals follow.

Adequacy of Counter-ion Controls. When a salt of a metal is administered it is important to consider whether the counter-ion could possess toxicity and whether this needs to be

controlled. For example, if lead acetate is studied, is it necessary to use sodium acetate as a control?

Dosing Solubility, Ionization, Hydration, and Speciation of Metals Administered in Water. Metal compounds may be in suspension or in solution and may be differentially hydrated depending on the concentration in which they are prepared and the length of time the preparation stands. Water pH and mineral content are also relevant. These different species may in turn have different pharmacokinetic and toxic properties.

Trace Element Content of Food and Drinking Water. Because of the well-known interaction of metals with essential trace elements, the trace element content of the animal feed and drinking water should be reported or controlled. Inconsistent results across experiments could be due to this factor. Trace element content of vehicles for gavage or injection should also be considered.

Acute Stress in the Experiment. A component of acute stress in the experiment can induce hepatic metal-binding proteins (acute phase proteins) and alter the toxic efficacy of a given administered dose.

Selection of Short Term versus Chronic Safe Exposure Levels for Metals That Accumulate in End Organs. Separate safe exposure levels are often derived for short-term and long-term exposure. The duration of an exposure that is appropriately classified as short term may need to vary with dose for metals that accumulate in end organs.

9.3.4 Use of Biomarkers of Dose, or Pharmacokinetic Estimates of Systemic Exposure, To Identify Safe Exposure Levels

Because metals can persist in biological systems, target organ accumulation rather than administered dose (mg/kg/day) may be a more accurate metric for identifying effective dose levels (NOAELs and LOAELs) across target organs. Most of these same principles apply to human studies.

More recently, the definition of biomarkers has been expanded to include measures of gene expression and protein regulation (i.e., genomics and proteomics). It is anticipated that emerging tools will benefit risk assessments by identifying more sensitive health endpoints and measures of exposure proximal to adverse health effect, and elucidating modes of action and quantitative measures of homology as indices of intra- and interspecies variability.

9.3.5 Changes in Essential Trace Element Status as an Adverse Effect in Metal Risk Assessment

Metals can have a secondary impact by interacting with essential trace elements (Section 6.1). In this case the organ systems affected would be anticipated to coincide with those affected in trace element deficiency. Following this line of thought, an alteration of trace element status

(for example changes in circulating concentrations or storage depots [ferritin, bone] or reduced activity of a marker enzyme (Cu/Mn SOD) could be identified as an adverse effect without further target organ studies. For example, the oral RfD for "zinc and zinc compounds" is based on a reduction in erythrocyte superoxide dismutase, a copper-dependent enzyme, as the adverse endpoint. However, the presence of a metal toxicant in a biological system may alter the relationship between a marker of trace element status and a state of deficiency. Further, group differences in markers may represent a range within a normal and physiologically tolerable nutrient status profile.

In reviewing health effects, EPA should discuss essentiality as it contributes to the understanding of target organ and mechanism. Although the EPA does not regulate the commercial food supply, risks from consumption of contaminated foods have been assessed for Native American subsistence populations. As applied to the general population, an RfD or parallel exposure guideline can only be applied to the portion of daily exposure that comes from pesticides, chemicals, waste streams, and other sources of toxicants regulated by EPA. It is important to consider the possibility that the intake of one nutrient may alter in detrimental ways the health benefits conferred by an other nutrient. Any such alteration (referred to as an adverse nutrient-nutrient interaction) is considered an adverse health effect. Further, an alteration that has been clinically defined "abnormal," such as a TIBC or hemoglobin value below an age cut-off, could be considered as the basis for risk assessment without further target organ information.

9.3.6 Biological Plausibility and Cellular Actions of Metals

A final step in characterizing target organ toxicity is establishing a link between known biological actions of a toxicant and the functions of a target organ. For example, sensitive target organs for toxicants that interfere with cell proliferation might be expected to be organs that rely heavily on ongoing cell proliferation for their function, such as skin, immune system, and the embryo. While it is rare that the mechanism of action of a toxicant will be completely defined by basic research, establishing biological plausibility for target organ effects is often possible and is a well-recognized component of risk assessment, particularly at the weight-of-evidence step.

Because of common physical chemistry properties, metals are sometimes investigated as a group for mechanism of action. For example, transition metals have the potential for promoting ROS generation through the Fenton reaction and other pathways (Ercal et al., 2001). Trivalent metals can modify the structure of lipid membranes to promote generation of lipid peroxidation (Verstraeten et al., 1997). The metal-binding capacity of metallothionein is principally limited to divalent cations, and of transferrin to trivalent cations.

However, metals can also be active at most cellular sites where organic toxicants have their effects. Metals can directly interfere with receptor activation (Stoica et al., 2000), ion channel regulation (Kiss and Osipenko, 1994), cell signaling (DeMoor and Koropatnick, 2000), cell adhesion (Prozialeck, Grunwald et al., 2002) and gene transcription (Meplan et al., 2000). Recent data suggests that metals can directly activate apoptotic cell death programs independent of cell damage (Chen and Shi, 2002). Thus metals are not readily distinguished from organics in

the range of their potential mechanisms of action at the cellular and molecular level. In general, the fact that a toxicant is a metal rather than an organic neither simplifies or complicates consideration of biological plausibility in a risk assessment.

10. RESEARCH NEEDS

- Research should be conducted to determine potential essential or beneficial effects of metals and inorganic metal compounds (especially as these effects impact low-dose extrapolation).

- There should be further research concerning the potential interactions between essential metals and toxic metals and between toxic metals per se.

- Research should be conducted concerning the applicability of toxicokinetic models for risk assessment for metals and inorganic metal compounds. Consideration should be given to differences in models for essential metals and toxic metals with no known beneficial effects.

- There should be further research and development regarding the use of gene and protein biomarkers as endpoints in the risk assessment process for regulatory issues

- Speciation of metals in tissues of target organs should be determined. Research should be conducted on mechanisms of toxicity including carcinogenicity, and whether carcinogenicity of specific metals is a threshold or non-threshold event.

- Research is needed to meet the needs of sensitive individuals on the basis of genetic and developmental factors.

11. REFERENCES

Ambrose, T.M., M. Al-Lozi, and M.G. Scott. 2000. Bone lead concentrations assessed by *in vivo* x-ray fluorescence. Clin. Chem. 46:1171-1178.

Andersen, M.E. 1995. Development of physiologically based pharmacokinetic and physiologically based pharmacodynamic model for applications in toxicology and risk assessment. Toxicol. Lett. 79:35-44.

Andersen, M.E, H.J. Clewell, M.L. Gargas, F.A. Smith, and R.H. Reitz. 1987. Physiologically based pharmacokinetics and the risk assessment process for methylene chloride. Toxicol. Appl. Pharmacol. 87:185-205.

Arnold, M.L., F.E. McNeill, and D.R. Chettle. 1999. The feasibility of measuring manganese concentrations in human liver using neutron activation analysis. Neurotoxicology 20:407-412.

Agency for Toxic Substance and Disease Registry (ATSDR). 1995. Multiple lead and cadmium exposure study with biological markers incorporated. Atlanta, GA.

Ballatori, N. 2002. Transport of toxic metals by molecular mimicry. Environ. Health Perspect. 110 Suppl. 5):689-694.

Berglund, M., A. Askesson, B. Nermell, and M. Vahter. 1994. Intestinal absorption of dietary cadmium in women depends on body stores and fiber intake. Environ. Health Perspect. 102:1058-1065.

Bose, A., K. Vashistha, and B.J. O'Loughlin. 1983. Azarcon por empacho–another cause of lead toxicity. Pediatrics 72:106-8.

Brown, G.E., Jr., A.L. Foster, and J.D. Ostergren. 1999. Mineral surfaces and bioavailability of heavy metals: A molecular-scale perspective. Proc. Natl. Acad. Sci. USA 96:3388-95.

Centers for Disease Control and Prevention (CDC). 1981. Use of lead tetroxide as a folk remedy for gastrointestinal illness. Morb. Mortal. Weekly Rep. 30:546-7.

Centers for Disease Control and Prevention (CDC). 1982. Lead poisoning from lead tetroxide used as a folk remedy—Colorado. Morb. Mortal. Weekly Rep. 30:647-8.

Centers for Disease Control and Prevention (CDC). 1983. Leads from the MMWR. Folk remedy-associated lead poisoning in Hmong children. J. Am. Med. Assoc. 250:3149-50.

Chen, F and X. Shi. 2000. Signaling form toxic metals to NF-kappaB and beyond: not just a matter of reactive oxygen species. Environ. Health Perspect. 110Suppl5:807-11.

Cherian, M.D. and R.A. Goyer. 1995. Part Three, Chapter 9, Section A, In: Berthon, G. ed. Handbook of metal-ligand interactions in biological fluids, Vol. 1. New York: Marcel Dekker, Inc. pp. 648-654.

Choudhury, H., T. Harvey, W.C. Thayer, T.F. Lockwod, W.M. Stiteler, P.E. Goodrum, J. Hassett, and G.L. Diamond. 2001. Urinary cadmium elimination as a biomarker for evaluating a cadmium dietary exposure-biokinetic model. J. Toxicol. Environ. Health, Pt. A 63:321-350.

Cia, L., G. Tsiapalis, and M.G. Cherian. 1998. Protective role of zinc metallothionein on DNA damage *in vitro* by ferric nitriloacaetate (Fe-NTA) and ferris salts. Chem-Biol. Interact. 115:141-151.

Clarke, R.H. 1995. ICRP recommendations applicable to the mining and minerals processing industries and to natural sources. International Commission on Radiological Protection. Health Phys. 69:454-60.

Clarkson, T.W. 1993. Molecular and ionic mimicry of toxic metals. Annu. Rev. Pharmacol. Toxicol. 32:545-571.

Clarkson, T.W. 1986. Effects-general principles underlying the toxic action of metals. In: Friberg, L., G.F Nordberg, and V. Vouk, eds. Handbook on the toxicology of metals, 2nd ed., Vol. 1. Amsterdam: Elsevier, pp. 85-127.

Clewell, H.J. 1995. The application of physiologically based pharmacokinetic modeling in human health risk assessment of hazardous substances. Toxicol. Lett. 79:207-217.

Dawson, D.C. and N. Ballatori. 1995. Membrane transporters as site of action and routes of entry for toxic metals. In: Goyer, R.A. and M.G. Cherian, eds. Toxicology of metals: Biochemical aspects. New York: Springer-Verlag, pp. 53-76.

DeMoor, J.M. and D.J. Koropatnick. 2000. Metals and cellular signaling in mammalian cells. Cell. Mol. Biol. 46:367-81.

DeVoto, E. and R.A. Yokel. 1994. The biological speciation and toxicokinetics of aluminum. Environ. Health Perspect. 102:940-51.

Dlugaszek, M., M.A. Fiejka, A. Graczy, J.S. Aleksandrowicz, and M. Slowikowska. 2000. Effects of various aluminum compounds given orally to mice on Al tissue distribution and tissue concentrations of essential elements. Annu. Rev. Pharmacol. Toxicol. 86:135-9.

Domingo, J.L. 1995. Reproductive and developmental toxicity of aluminum: A review. Neurotoxicol. Teratol. 17:515-21.

Ercal, N., H. Gurer-Orhan and N. Aykin-Burns. 2001. Toxic metals and oxidative stress part 1:mechanisms involved in metal-induces oxidative damage. Cur. Top. Med. Chem. 1:529-39.

Fleming, D.E.B., D.R. Chettle, J.G. Wetmur, R.G. Desnick, J. Robin, D. Boulay, N.S. Richard, C.L. Gordon, and C.E. Webber. 1998. Effect of the d-aminolevulinic dehydratase polymorphism on the accumulation of lead in bone and blood in lead smelter workers. Environ. Res. 77:49-61.

Foulkes, E.C. 2000. Transport of toxic heavy metals across cell membranes. Proc. Soc. Exp. Biol. Med. 223:234-40.

Fowler, B.A. and K.R. Mahaffey. 1978. Interactions among lead, cadmium and arsenic in relation to porphyrin excretion patterns. Environ. Health Perspect. 25:87-90.

Freeman, G.B., J.D. Johnson, J.M. Killinger, S.C. Liao, A.O. Davis, M.V. Ruby, R.L. Chaney, S.C. Lovre, and P.D. Bergstrom. 1993. Bioavailability of arsenic in soil impacted by smelter activities following oral administration in rabbits. Fundam. Appl. Toxicol. 21:83-8.

Freeman, G.B., R.A. Schoof, M.V. Ruby, A.O. Davis, J.A. Dill, S.C. Liao, C.A. Lapin, and P.D. Bergstrom. 1995. Bioavailability of arsenic in soil and house dust impacted by smelter activities following oral administration in cynomolgus monkeys. Fundam. Appl. Toxicol. 28:215-22.

Geffner, M.E. and A. Sandler. 1980. Oral metallic mercury: A folk medicine remedy for gastroenteritis. Clin. Pediatr. 19:435-7.

Goyer, R.A. 1995. Transplacental transfer of lead and cadmium. In: Goyer, R.A. and M.G. Cherian, eds. Toxicology of metals. New York: Springer-Verlag, pp. 1-13.

Goyer, R.A. and T.M. Clarkson. 2001. Toxic effects of metals. In: Klaassen, C.D., ed. Casarett & Doull's toxicology. New York: McGraw-Hill, pp. 811-868.

Grandjean, P., S.S. Brown, P. Reavey, and D.S. Young. 1994. Biomarkers of chemical exposure: State of the art. Clin. Chem. 40:1360-1362.

Granick, J.L., S. Sassa, R.D. Granick, R.D. Levere, and A. Kappas. 1973. Studies in lead poisoning. II: Correlation between the ration of activated to inactivated d-aminolevulinic acid dehydrates of whole blood and the blood lead level. Biochem. Med. 8:149-159.

Idaho Department of Health and Welfare, Division of Health. 2000. Coeur d'Alene River Basin environmental health assessment. Agency for Toxic Substances and Disease Registry, Atlanta, GA. pp. 67.

Kagi, J.H.R. and Y. Kogima, eds. 1987. Chemistry and biochemistry of metallothionein. Boston: Birkhäuser Boston, pp. 25-61.

Kiss, T. and O.N. Osipenko. 1994. Toxic effects of heavy metals on ionic channels. Pharmacol. Rev. 46:245-67.

Kostial, K., D. Kello, S. Jugo, I. Rabar, and T. Maljkovic. 1978. Influence of age on metal metabolism and toxicity. Environ. Health Perspect. 25:81-86.

Krishnan, K. and M.E. Andersen. 1994. Physiologically based pharmacokinetic modeling in toxicology. In: Hayes, A.W., ed., Principles and methods in toxicology, 3rd ed. New York: Raven Press, Ltd., pp. 149-188.

Llobet, J.M., J.L. Domingo, M. Gomez, J.M. Tomas, and J. Corbella. 1987. Acute toxicity studies of aluminum compounds: Antidotal efficacy of several chelating agents. Annu. Rev. Pharmacol. Toxicol. 60:80-3.

McKinney, P.E. 1999. Elemental mercury in the appendix: An unusual complication of Mexican-American folk remedy. J. Toxicol.: Clin. Toxicol. 37:103-7.

Meplan, C., M.J. Richard and P. Hainaut. 2000. Redox signaling and transition metals in the control of the p53 pathway. Biochem. Pharmacol. 59:25-33.

National Academy of Sciences (NAS). 2003. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. Food and Nutrition Board, Institute of Medicine, Washington, DC. ISBN 0-309-7279-4 <http://www.nap.edu/catalog/10026.html>.

National Academy of Sciences (NAS) and National Research Council (NRC). 1999. Arsenic in drinking water. Washington, DC, pp. 251-257.

National Academy of Sciences (NAS) and National Research Council (NRC). 2001. Arsenic in drinking water. 2001 update. Washington, DC.

National Academy of Sciences (NAS) and National Research Council (NRC). 1993. Measuring lead exposure in infants children and susceptible populations. Washington, DC.

Ng, J.C., S.M. Kratzmann, L. Qi, H. Crawley, B. Chiswell, and M.R. Moore. 1998. Speciation and absolute bioavailability: Risk assessment of arsenic contaminated sites in a residential suburb in Canberra. Analyst 123:889-92.

O'Flaherty, E.J. 1998. Physiologically based models of metal kinetics. Crit. Rev. Toxicol. 28:271-317.

O'Flaherty, E.J. 1995. Physiologically based models for bone-seeking elements. V: Lead absorption and disposition in childhood. Toxicol. Appl. Pharmacol. 131:297-308.

Parkinson, A. 2001. Biotransformation of zenobiotics. In: Klaasen, C.D., ed. Casarett & Doull's toxicology. New York: McGraw-Hill, pp. 133-224.

Picciano, M.F. 1996. Pregnancy and lactation. In: Ziegler, E.E. and L.J. Filer, Jr., eds. Present knowledge in nutrition, 7th ed. Washington, DC: ILSI Press, pp. 384-395.

Pillet, S., M. Fournier, L.N. Measures, J. Bousqsuegneau, and D.G. Cyr. 2002. Presence and regulation of metallothioneins in peripheral blood leukocytes of grey seals. Toxicol. Appl. Pharmacol. 185:207-217.

Plunkett, L.M., D. Turnbull, and J.W. Rodricks. 1992. Differences between adults and children affecting exposure assessment. In: Guzelian, P.S., C.J. Henry, and S.S. Olin, eds. Similarities & differences between children and adults: Implications for risk assessment. Washington, DC: ILSI Press, pp. 79-94.

Pontifex, A.H. and A.K. Garg, 1985. Lead poisoning from an Asian Indian folk remedy. Can. Med. Assoc. J. 133:1227-8.

Prozialeck, W.C., G.B. Grunwald, P.M. Dey, K.R. Reuhl, A.R. Parrish, and Cadherins.2002. NCAM as potential targets in metal toxicity. Toxicol. Appl. Pharmacol. 59: 25-33.

Rozman, K.K. and C.D. Klaassen. 2001. Biotransformation of zenobiotics. In: Klaassen, C.D., ed. Casserett and Doull's toxicology. New York: McGraw-Hill, pp.107-132.

Sakai, T. 2000. Biomarkers of lead exposure. Ind. Health 37:127-142.

Salocks, C., T. Hathaway, C. Ziarkowski, and W. Walker. 1996. Physical characterization, solubility and potential bioavailability of arsenic in tailings from a former gold mine. Toxicologist 16:48.

Schulte, P.A. and G. Talaska. 1995. Validity criteria for the use of biological markers of exposure to chemical agents in environmental epidemiology. Toxicology 101:73-88.

Sheldrake, S. and M. Stifelman. 2003. A case study of lead contamination cleanup effectiveness at Bunker Hill. Sci. Total Environ. 303:105-23.

Smith, C.M., X. Wang, H. Hu, and K.T. Kelsey. 1998. A polymorphism in the d-aminolevulinic acid dehydratase gene can modify the pharmacodynamics and toxicity of lead. Environ. Health Perspect. 103:248-253.

Stoica, A. B.S. Katzenellenbogen and M.B. Martin. 2000. Activation of estrogen receptor-alpha by the heavy metal cadmium. Mol. Endocrinol. 14: 545-53.

Storm, G.L., G.J. Fosmire, and E.D. Bellis. 1994. Heavy metals in the environment: Persistence of metals in soil and selected vertebrates in the vicinity of Palmerton zinc smelters. J. Environ. Qual. 23:508-515.

Sunderman, F.W., Jr. 1978. Carcinogenic effects of metals. Fed. Proc. 37:40-46.

Suttle, N.F. and C.F. Mills. 1966. Studies of the toxicity of copper to pigs. 1: Effects of oral supplements zinc and iron salts on the development of copper toxicosis. Br. J. Nutr. 20:135-149.

Thawley, D.G., S.E. Pratt, and L.A. Selby. 1977. Antagonistic effect of zinc on increased urinary delta-aminolevulinic acid excretion in lead intoxicated rats. Environ. Res.14:463-475.

Trotter, R.T., 2nd. 1985. Greta and azarcon: A survey of episodic lead poisoning from a folk remedy. Hum. Organ. 44:64-72.

U.S. Department of Health and Human Services (U.S. DHHS). 2002. 10th report on carcinogens. National Toxicology Program. Washington, DC. <http://ehp.niechs.nih.gov/roc/toc10.html>.

U.S. EPA. 2003a. Draft final guidelines for carcinogen risk assessment. (External review draft, February 2003). EPA/630/P-03/001A, NCEA-F-0644A. Risk Assessment Forum, U.S. EPA, Washington, DC. pp. 120. <http://www.epa.gov/ncea/raf/cancer2003.htm>.

U.S. EPA. 2003b. Supplemental guidance for assessing cancer susceptibility from early-life exposure to carcinogens. External review draft. EPA/630/R-03/003. Risk Assessment Forum, U.S. EPA, Washington, DC. pp. 80. <http://www.epa.gov/ncea/raf/cancer2003.htm>.

U.S. EPA. 2002. Draft action plan: Development of a framework for metals assessment and guidance for characterizing metals. EPA/630/P-02/003A. Washington, DC.

U.S. EPA. 2000. Supplementary guidance for conducting health risk assessment of chemical mixtures. EPA/630/R-00/002.

U.S. EPA. 1997. Exposure factors handbook. EPA/600/P-95/002Fc. Office of Research and Development, U.S. EPA, Washington, DC.<http://www.epa.gov/ncea/exposfac.htm>.

U.S. EPA. 1992a. Dermal exposure assessment: Principles and application. EPA/600/8-91/011B.

U.S. EPA. 1992b. Guidelines for exposure assessment. Fed. Reg. 57(104):22888-22938.

U.S. EPA. 1989. Risk assessment guidance for Superfund, volume I. Human health evaluation manual, Part A. Office of Emergency and Remedial Response, U.S. EPA, Washington, DC.

U.S. EPA. 1986. Guidelines for the health risk assessment of chemical mixtures. Fed. Reg. 51(185):34014-14025.

Verstraeten, S.V., I.V. Nogueira, S. Schreier, and P.I. Oteiza. 1997. Effect of trivalent metal ions on phase separation and membrane lipid packing: role in lipid peroxidation. Arch. Biochem. Biophys. 338:121-7.

von Lindern, I., S. Spalinger, V. Petroysan, and M. von Braun. 2003. Assessing remedial effectiveness through the blood lead: Soil/dust lead relationship at the Bunker Hill Superfund site in the Silver Valley of Idaho. Sci. Total Environ. 303:39-170.

Waalkes, M. 1995. Metal carcinogenesis. In: Goyer, R.A. and C.D. Klaassen, eds. Metal toxicology. New York: Academic Press, pp. 47-67.

Wetterhahn-Jenerette, K. 1981. The role of metals in carcinogenesis: Biochemistry and metabolism. Environ. Health Perspect. 40:233-252.

White, P.D., P. Van Leeuwen, B.D. Davis, M. Maddaloni, K.A. Hogan, A.H. Marcus, and R.W. Elias. 1998. The conceptual structure of the integrated exposure uptake biokinetic model for lead in children. Environ. Health Perspect. 106(Suppl. 6):1513-30.

World Health Organization (WHO). 1996a. Trace elements in human health and nutrition. Chapter 3: Trace element bioavailability and interactions. Geneva. pp. 23-41.

World Health Organization (WHO). 1996b. Trace elements in human health and nutrition. Chapter 10: Manganese. Geneva. pp. 163-167.

World Health Organization (WHO). 1996c. Trace elements in human health and nutrition. Chapter 17: Arsenic. Geneva. pp. 217-220.

World Health Organization (WHO). 1995. Guiding principles for the use of biological markers in the assessment of human exposure to environmental factors: An integrative approach of epidemiology and toxicology. Toxicology 101:1-10.

World Health Organization (WHO) and International Programme on Chemical Safety (IPCS). 2002. Principles and methods for the assessment of risk from essential trace elements. Environmental Health Criteria Document No. 228. Geneva.

World Health Organization (WHO) and International Programme on Chemical Safety (IPCS). 1995. Inorganic lead. Environmental Health Criteria Document No. 165. Geneva. pp. 152-192.

World Health Organization (WHO) and International Programme on Chemical Safety (IPCS). 1993. Biomarkers and risk assessment: Concepts and principles. Environmental Health Criteria Document No. 155: Geneva. pp. 25.

World Health Organization (WHO) and International Programme on Chemical Safety (IPCS). 1992. Cadmium. Environmental Health Criteria Document No. 134. Geneva. p. 69.

Yanez, L., L. Batres, L. Carrizales, M. Santoyo, V. Escalante, and F. Diaz-Barriga. 1994. Toxicological assessment of azarcon, a lead salt used as a folk remedy in Mexico. I: Oral toxicity in rats. J. Ethnopharmacol. 41:91-7.

Yokel, R.A. and P.J. McNamara. 2001. Aluminum toxicokinetics: An updated minireview. Annu. Rev. Pharmacol. Toxicol. 88:159-67.