ISSUE PAPER ON THE HUMAN HEALTH EFFECTS OF METALS

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1. INTRODUCTION

This paper discusses issues important to consider in developing a framework for performing human health assessments for exposure to metals and metal compounds (U.S. EPA, 2002). The Framework is intended to provide guidance to risk assessors within EPA (the "Agency") as they develop program-specific risk assessment methods. It will complement other general Agency guidance on the risk assessment process, and contain metal-specific information that should be considered. This involves 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. This issue paper provides some of the scientific basis that underlies metal-specific characteristics of human health effects assessment. It is not intended to be comprehensive, but does provide appropriate and sufficient reference material for the interested reader to find additional detail on any of the topics.

There are two types of health hazard: (1) hazards with a threshold for the relationship between exposure and the health effect (most target organ effects) and (2) hazards 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 help EPA establish guidelines for programs assessing the 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. 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. As with other essential metals, concentrations of cations in the body are controlled by homeostatic mechanisms.

The paper on environmental chemistry issues discusses various approaches to classification of metals in detail. The concept of hard and soft acid and base relationships and the applicability of hard and soft properties to the formation of metal complexes, as summarized in the environmental chemistry paper, has relevance to solubility and mobility of metals in the environment. The term "heavy metals" is sometimes used to suggest pollution and toxic effects;

it implies metals of high density, but has also been used for other metals. A recent IUPAC Technical Report (Duffus, 2002) discusses the inappropriateness of this term and the term is avoided. In the context of this paper it is most appropriate to classify metals of interest by their impact on health effects—nutritionally essential, nonessential with a possible beneficial effect, or nonessential with no beneficial effects. Table 1, below, lists the metals identified in the environmental chemistry paper as metals of concern; it also lists iron and magnesium, which are nutritionally essential.

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

Nutritionally Essential Metals	Metals with Possible Beneficial Effects	Metals with No Known Beneficial Effects
Cobalt	Boron	Aluminum
Chromium III	Nickel	Antimony
Copper	Silicon	Arsenic
Iron	Vanadium	Barium
Manganese		Beryllium
Molybdenum		Cadmium
Selenium		Lead
Zinc		Mercury
		Silver
		Strontium
		Thallium

The primary premise for this classification is that assessment of health risks for nutritionally essential metals requires its own approach or process: restrictive standards must allow sufficient exposure for the general population to prevent deficiencies, but nutritionally essential metals 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. Nevertheless, manganese is regarded as essential for human nutrition because it is an activator and constituent of many enzymes present in humans (NAS/IOM, 2003).

2.2 Metals with No Known Essential or Beneficial Effects

Arsenic, cadmium, lead, and mercury, and their inorganic compounds, are probably the most potentially 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 concern 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 forms 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 Metals That May Have Some Beneficial Effect

A few metals are not known to be essential to human health but may have some beneficial effects at low levels of exposure. These include silicon, nickel, boron, and vanadium. (These metals are toxic at higher levels.) Some have said arsenic may have beneficial effects (WHO, 1996b; NAS/IOM, 2003), but a recent critical review does not support this view for human exposure (NAS/NRC, 1999). However, some organic arsenic compounds have been used as growth factors in poultry, and it has been suggested that arsenic deprivation may impair the 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). 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—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). The mechanism(s) responsible for metal carcinogenesis is elusive, partly because of the complex nature of metals' interactions in biological systems. Many metals, including carcinogenic metals, follow the metabolic pathways of similar essential metals. This is probably the 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 a reactive chemical species (Waalkes, 1995). Enzymatic modification is generally not a mechanism available to detoxify metals. However, metals use 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 the metal-induced tumors found in humans to a large extent, except for arsenic.

It should be noted that essential metals can also be carcinogenic. For example, chromium III is essential and chromium VI is 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 does not produce cancer (Cia et al., 1998). While these observations may be dismissed as not relevant to 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 guidelines for carcinogenic risk (U.S. EPA, 2003a, 2003b) are presently in draft form or under review.

3. ROLE OF SPECIATION AND SOLUBILITY OF METALS AND METAL COMPOUNDS

This paper focuses 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; for some metals, speciation may influence the pattern of toxicity (e.g., inorganic arsenic versus organic compounds, inorganic and organic mercury compounds). The role of speciation in bioavailability and bioaccumulation within the environment and bioaccessibility to human receptors is discussed in the papers on exposure issues and bioavailability and bioaccumulation. For inorganic species it is generally assumed that the potential toxicity is related to the presence of the cation in body tissues (in most cases, bound to a tissue ligand). The intracellular context and nature of ligand or protein binding may influence the potential or availability of the metal for interacting at a specific cellular target, such as an enzyme or transport protein, to produce a toxic effect.

Solubility is one of the major factors influencing bioavailability and absorption of metals and metal compounds. The solubility of a metal compound depends on its chemical species, on the pH of its medium (H+ ions), and on the presence of other chemical species in the medium (see the environmental chemistry paper). Nitrates, acetates, and all chlorides of most metals except silver, mercury, and lead are soluble. Sulfates of most metals are also 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 in body tissues (as may occur following gunshot wounds) is probably absorbed 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).

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

An objective of the draft Framework for Metals Assessment (U.S. EPA, 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 the 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/dealkylation reactions. In these reactions, new inorganic species or metal organic complexes may be formed but the metal ion persists.

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

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.

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 can be fatal, and long-term exposures can cause cancer. These compounds occur naturally at low levels in drinking water, so they must be carefully regulated. Organic forms of arsenic present in seafood, on the other hand, have no significant toxicity to humans compared to the potentially toxic inorganic compounds. However, recent experimental studies have shown that dimethylarsinic acid may be carcinogenic (NAS/NRC, 2001). Meanwhile, 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 potentially 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 (IPCS, 1992).

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.

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 depends 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 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, depending more on particle size and on whether the substance is presented as a vapor or a gas (e.g., elemental mercury). Human skin is not very permeable and provides a good barrier against absorption of metals and metal compounds as well as highly lipophilic organic compounds, but the mechanism for absorption may differ. Dimethylmercury is a notable exception (Siegler et al., 1999). 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).

Although metal ions' low lipid solubility could limit 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, 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 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, 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.

5. MEASURES OF EXPOSURE TO METALS

In terms of health assessment, the extent of exposure to a metal is best determined by measuring its internal concentration, and more preferably the biologically effective dose at the target organ (as opposed to environmental concentration). 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. For example, activity of the heme-synthesizing enzyme aminolivulinic acid dehydrate (ALAD) in red blood cells is directly related to the concentration of lead in blood and therefore may be used as a surrogate for the measurement of lead in blood. 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 or umbilical cord blood).

5.1 Biomarkers of Exposure

The World Health Organization (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 or biomarkers of exposure.

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; that the biomarker is specific for a particular type of exposure; that the exposure results in a reversible change; and that 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 not only provides information about exposure levels but can also be 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.

A biomarker of exposure is a measure of cumulative exposure to a metal—and also of metal actually existent in tissue or chemical, as occurs with chronic exposure for metals. However, such an approach may not be appropriate for metals that are not extensively

accumulated in tissues, and it does not differentiate between metal present in a tissue in a sequestered or inactive form and metal engaged in toxic or pathological processes.

There are environmental (water, air, soil, dust), occupational, medicinal, and dietary sources of metal exposure. 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, as discussed in the exposures issue paper. Several metals, such as arsenic and selenium, are found naturally in the diet. Therefore, failure to consider dietary sources of metals may result in a misinterpretation of the exposure. For example, arsenobetaine is a non-toxic organic 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 potentially toxic (inorganic) arsenic species—some assessments of arsenic exposure have assumed that 10% of total elemental arsenic in seafood and 100% of arsenic in all other foods is in 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 kinds of relevance: analytical, toxicokinetic, and biological (Grandjean et al., 1994; Schulte and Talaska, 1995; IPCS, 1993).

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. Some biomarkers of exposure such as the DNA adducts of chromium VI might also be classified as biomarkers of effect.

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: analytical methods for the detection of metals, such as inductively coupled plasma mass spectrometry, hydride generation atomic absorption, 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 before analysis; these procedures may make it difficult to fully speciate the metal, or there may be interfering matrix factors. Another drawback to these 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. However, the sensitivity of techniques such as X-ray fluorescence is extremely limited with respect to general

population monitoring. The accumulation of metals in organs that results from chronic exposure to metals can be monitored and quantified using these techniques. “Accumulation” in this context refers to the capacity-limited sequestration of metals in a specific organ or tissue, not to the bioaccumulation or biomagnifications discussed in the bioavailability and bioaccumulation issue paper. Some of these techniques can detect more than one metal at a time (multiple metals may be present after exposure to a mixture of metals). A disadvantage of the in vivo methods is that they cannot speciate the metal of interest, so the exposure to the potentially toxic metal species may be estimated incorrectly.

The correct frequency and timing of sampling of biological fluids and tissues, as well as the correct interpretation of the results, depends 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). A detection of lead in plasma above background levels would be indicative of an acute exposure, whereas a detection in bone would be indicative of chronic exposure. Thus sampling plasma every other day or week, or analyzing 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 that starts with exposure to a metal and ends with a defined outcome. For example, the presence of a known potentially 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 depends on the link between exposure to it and biological effect. However, for many of the metals of interest, and particularly in humans, the role or relevance of biomarkers of exposure may not be well characterized. Nevertheless, biomarkers of exposure and effect are basic tools for population or molecular epidemiology studies of effects of exposure to humans of various metals.

6. INTERACTIONS BETWEEN METALS

There are generally three classes of interactions between metals: between essential metals, between nonessential metals, and between essential and nonessential metals. Antagonisms between metals, and indeed much of the uptake and/or sequestration behavior of metals, occurs as a result of commonalities in uptake mechanisms. For example, it might be specified that the protective effects of zinc against copper toxicity are most likely due to diminished gastrointestinal uptake of copper. Such interactions are also at play in the consideration of essential and nonessential metals. The uptake of lead from the gastrointestinal tract likely occurs via both passive diffusion processes and via active transport mechanisms used in the uptake of essential minerals such as calcium. Calcium deficiency will increase the uptake of lead into the body, presumably as a result of lead uptake via calcium active transport processes. Calcium supplementation will then diminish lead uptake via both competitive binding to uptake proteins and down-modulation of active transport activity. There is a large body of literature providing examples of molecular or ionic mimicry that involve most 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, which are often complex, 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 (e.g., 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. There may be competitive interactions for absorption between essential metals, e.g., zinc and copper. Examples of metals or metal compounds that reduce availability are iron oxalates, copper sulfides, and trace element silicates. Phytates reduce gastrointestinal absorption of lead by binding in association 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 (RDAs) for these trace metals and guidance for assessing risk from dietary exposures to these elements (NAS/IOM, 2003). 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" (NAS/IOM, 2003). This public health concept is 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 following factors are considered when RDAs are set for trace elements:

1) Scientific evidence about human requirements. 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.

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 (e.g., goitrogens that reduce the effectiveness of iodine), and the presence of metals as contaminants that 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 high enough to result in toxicity. The efficiency of the homeostatic mechanism may be related to factors that influence absorption, age-related factors, and dietary and nutritional interactions. The homeostatic mechanism may also involve an interaction with another essential metal. Its efficiency varies within populations and individuals, but one would have to study large populations to find the prevalence of a variation. 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) (NAS/IOM, 2003).

6.1.2 Deficiency Versus Excess (Toxicity) of Essential Metals

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 can be toxic. This concern is timely given the increase in use of dietary supplements and other consumer products or remedies that 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). The World Health Organization (IPCS, 2002) has provided guidance on methods of assessing risks from excessive exposures to nutritionally essential metals, including the use of an "Acceptable Range of Oral Intake" (AROI). To accommodate an AROI, there must an estimate of the minimal requirement to prevent deficiency and an upper limit that will produce toxicity. A basic principle for establishing the AROI is that one must balance toxicity against the potential health effects of deficiency. In finding such a balance, one might find it helpful to consider effects in terms of four levels: (1) lethal effects; (2) clinical effects (e.g., anemia, neurodevelopmental impairment); (3) subclinical biomarkers of effect with functional impairment, such as change in enzyme activity (hepatic transaminase); and (4)

biochemical markers without functional impairment (erythrocyte superoxide dismutase, E-SOD) (Nordberg et al., 2000).

As a case study, the AROI for zinc may be established by determining the RDA for a selected population (women of childbearing age during pregnancy/nursing) and a Tolerable Upper Intake Level (UL). A Tolerable Upper Intake Level is defined as the highest average daily nutrient intake level that is likely to pose no risk of adverse health effects to almost all individuals in the general population. As intake increases above the UL, the potential risk of adverse effects may increase (IOM, 2001). Based on guidelines contained in the IOM Dietary Reference Intakes (NAS/IOM, 2003), the RDA for lactation is 12 mg/day (ages 19 to 50 years) and UL for lactation is 40 mg/day. Accordingly, the AROI for lactating women aged 19 to 50 might be an oral intake between 12 and 40 mg/day.

The effects of mild zinc deficiency are diverse, but the requirements during lactation are increased because of contributions to milk. A UL may be based on decreased E-SOD, an indicator of copper deficiency. There may be debate on the point at which E-SOD changes are functionally significant. Determining an AROI for zinc is somewhat more complex than presented in this case study. Zinc, like all essential metals, has homeostatic mechanisms as discussed above. Also, the AROI for the general population may differ depending on health endpoints selected, variability in susceptibility of the population under consideration, and other uncertainty considerations.

6.2 Interactions Between Nonessential Metals Within Mixtures

Exposure to mixtures may be reflective of concomitant release of substances. For example, individuals in the vicinity of a zinc smelter will have higher concentrations of cadmium in their kidneys, more likely as a result of increased cadmium emissions than because of the emission of zinc. This is probably also true for increased levels of lead in bone. These materials are co-generation products and can be released together.

Although arsenic, cadmium, lead, and zinc are ubiquitous in soil and sediment samples worldwide, many Superfund sites include these metals as chemicals of potential concern (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).

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).

A study of a ternary mixture of cadmium, lead, and zinc study in rats found slightly more marked adverse hematological effects with ternary mixture exposure than with 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 (Saxena et al., 1989). The current literature do not explain the significance of these data in human exposure scenarios. In another study (Fowler and Mahaffey, 1978), a relatively wide range of endpoints were investigated in studies that covered each metal singly and all possible binary and ternary mixtures. Body weight gain was depressed equally by the ternary mixture and the cadmium-lead mixture, and to a lesser extent by the arsenic-lead and cadmium-lead mixtures, whereas food utilization was depressed more by the ternary and arsenic-cadmium 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 arsenic, cadmium, lead, and zinc, summarized above, are not adequate for predicting the magnitudes of interactions. Experimental efforts to identify interactions between these metals are needed. For some endpoints, the data are not robust in showing 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, even by different mechanisms, 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 reference dose (RfD) as the toxicity value for each effect. The animal studies discussed in brief in this report used commercial diets or semi-purified diets that may have higher or lower levels of essential metals than 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 are 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 Nonessential Metals Within Mixtures

Nutritionally nonessential elements normally found in the environment, unless the exposure is overwhelming, can be antagonized by essential nutrients found in foods we eat. Diet, therefore, can be a major factor in the appearance of adverse health effects following exposure to 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 a food chain whose members eat 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 nutritionally essential and nonessential elements. In the case of copper, a particular level of intake can lead to signs of either copper deficiency or 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 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 involves 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-Nonessential Metal Interactions

The term “molecular” or “ionic mimicry” has been applied to those situations in which a metal forms a complex with an endogenous ligand and the resulting compound mimics the behavior of a normal substrate, disrupting normal function. Such interactions could be considered in health assessments for exposure to specific metals. A number of reviews discuss this phenomenon, giving examples of the mechanism of toxicity for specific metals (Clarkson, 1993; Ballatori, 2002). One well-studied example way lead replaces zinc 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 impaired 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 synthesis of ATP and energy metabolism. Wetterhahn-Jenerette (1981) explains why chromium VI in the form of chromate can readily enter cells, whereas chromium III cannot. This may have implications as to why chromium VI is carcinogenic, but the essential metal chromium III is not a carcinogen.

Most of these examples involve replacement of an essential metal with a nonessential metal, and molecular or ionic mimicry may be viewed as a form of metal-metal interaction; most such examples involve interactions between nutritionally essential and nonessential metals, rather than nonessential-nonessential metal interactions.

Molecular mimicry is central to aspects of uptake and biokinetics for toxic metals within the body. For example, lead will be actively taken up into the body and sequestered into the bone

because of ionic mimicry for calcium. Similarly, cadmium uptake may in large part be related to ionic mimicry of zinc.

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 an RfD, for the specific mixture of concern to characterize risk or hazard; however, relevant data are rarely available (U.S. EPA, 1989). The traditional alternative has been to combine exposure data and route-specific toxicity values for each component metal in the mixture. A hazard index is then generated for the target organ/system by aggregating exposure amounts of metals with the same mode of action (MOA) and comparing the aggregates to a toxicity threshold based on the most toxic metal. (This process is based on the assumption of additivity of effects of metals with like MOAs.) This topic is further discussed in an EPA document (U.S. EPA, 2000) and an ATSDR report (ATSDR, 2004). Exposure to some of the elements, such as cadmium, lead, and arsenic, 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, but such data may be difficult to obtain. For example, in vitro studies showed that chromosome mutagenicity resulting from coexposure to arsenic and antimony was subadditive, causing less cell damage than would an additive effect from the two metals (Gerbel, 1998). However, it may be difficult to validate lab data in the absence of comparable field (epidemiological) data (McCarty et al., 2004).

One can use firmly established biomarkers of exposure 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. One can obtain more accurate model predictions of metal levels in tissue or fluids (i.e., biomarkers of exposure) 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 determining the probability of an adverse event 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, the Environmental Protection Agency, the Agency for Toxic Substances and Disease Registry, and the National Institute of Occupational Safety and Health. 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 depend on political and social policies. The information in the EPA Integrated Risk Information System (IRIS) program is intended for use in protecting public health through risk assessment and risk management. These two processes are briefly explained below.

Risk assessment has been defined as “the characterization of the potential adverse health effects of human exposures to environmental hazards” (NAS/NRC, 1983). In a risk assessment, the extent to which a group of people has been or may be exposed to a certain chemical is determined, and the extent of exposure is then considered in relation to the kind and degree of hazard posed by the chemical, thereby permitting an estimate of the present or potential health risk to the group of people involved. Risk assessment typically involves four steps: exposure assessment, toxicity assessment, risk characterization, and uncertainty analysis. The first step is to determine the potential health effects of toxic endpoints that may result from excess exposure to a metal. This is followed by dose-response studies, either conducted through large-scale human epidemiologic studies on human populations with a broad range of human exposures or based on animal studies. These studies are used to develop RfDs. Appropriate human populations are seldom available (notable exceptions exist for lead, methyl mercury, and arsenic), so for most metals the initial steps in the risk assessment process involve laboratory animals. From these studies the no-observed- and lowest-observed-adverse-effect levels (NOAEL and LOAEL) are 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 empirical studies on humans contain uncertainties due to variations in biology or lifestyle. For these reasons there has been increasing emphasis on predictive assessments or use of toxicokinetic/pharmacodynamic risk assessment models that may be conducted at contaminated sites. The predictive risk assessment models 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 Pharmacokinetic/Pharmacodynamic Modeling of Behavior of Metals in Humans

Pharmacokinetic/pharmacodynamic (PBPK/PBPD) modeling of behavior of metals entails the mathematical description and modeling of their absorption, distribution, metabolism, and excretion. The biokinetics of absorption is described in the exposure issue paper and the related terms and concepts of bioavailability and bioaccumulation are defined and discussed in the bioavailability and bioaccumulation issue paper, largely in terms of transfer of metals in the environment and exposure to human receptors. However, the concepts of bioaccumulation and persistence are questionable when it comes to metals risk assessment for humans. Generally, for most metals, all the body compartments are in dynamic equilibrium with other body compartments and turnover rates differ between compartments. Differential turnover, while it may lead to accumulation in some body parts, does not equate with bioaccumulation because dynamic equilibria are maintained and accumulation is capacity-limited and generally reversible. The concept of PBT (persistence, bioavailability, and chronic toxicity, discussed in the bioavailability paper) regarding metals in environmental media may not be a valid way to predict chronic toxicity in humans because of the complexity of distribution between various target organs and differences in retention time between different metals.

A typical physiologically based PBPK model for the behavior of metals in humans consists of multiple compartments representing tissues or tissue groups that are linked by blood flow. PBPD models describe the relationship between target tissue dose and health endpoints or target tissue effects. Combined use of PBPK and PBPD models provides understanding of the complex relationships between exposure and target organ effects. 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 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 the 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 generally reflect 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 from volatile organic compounds 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, one must explicitly consider the following kinetic factors: (1) oral bioavailability, (2) inhalation bioavailability, (3) cellular uptake, (4) nutritionally essential and nonessential 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).

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

Kinetic Factor	Physiologic Impact
Cellular uptake	Carrier-mediated uptake (e.g., phosphate or sulfate transporters) Facilitated transport in the form of organic complexes
Nutritionally essential and nonessential 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, As, Ni, Hg 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)

Many of the processes controlling the disposition of metals are intrinsically capacity-limited and highly metal-specific. This makes it necessary to understand physiology well enough 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, and 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. But in using biokinetic models it is important to have the most reliable and current data. Modern PBPK models for lead predict that bone lead levels are constant under steady-state exposure conditions. However, misconceptions have arisen as a consequence of changing metal sequestration within specific body compartments and changing patterns of human exposure. For example, lead levels in human bone have been proposed to increase as a function of age; more recent studies have observed that this increase is likely an "exposure cohort" effect reflective of both the slow turnover kinetics of lead in bone and higher historical levels of lead exposure (i.e., the concentration of lead in bone increases with age because older individuals had higher levels of lead exposure in previous years).

Constructive use of PBPK and PBPD 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 will influence 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.

There are three pharmacokinetic models currently being considered for lead risk assessment. The O'Flaherty Model is a PBPK model for children and adults. It includes the movement of lead from exposure media (i.e., intake via ingestion or inhalation) to the lungs and gastrointestinal tract; and subsequent exchanges between blood plasma, liver, kidney, and richly and poorly per fused tissues; and excretion from liver and/or kidney (O'Flaherty, 1995). The Integrated Exposure Uptake (IEUBK) Model was developed by EPA for predicting lead levels in children (U.S. EPA, 1994). The Leggett Model allows simulation of lifetime exposures and can be used to predict blood lead concentrations in both children and adults (Leggett, 1993).

To develop and apply biokinetic models, one must understand not only the relationships between exposure and body burden of metals but also the pharmacodynamics of metals within body compartments and relationships between internal doses and at target organ sites and toxic effects. EPA has a research program for the development of an All Ages Lead (biokinetic) Model and a cadmium biokinetic model based at least initially on the Kjellstrom and Nordberg Model (Kjellstrom and Nordberg, 1978).

7.2 Uncertainty Factors in Evaluating Health Effects of Metals

Uncertainty issues in toxicology are generally expressed in calculation of a tolerable level of intake or reference (RfD). An *uncertainty factor* is usually expressed as the value of the product of several single factors or issues that include variation in susceptibility as discussed below. Factors that impact measures of exposure include data regarding dietary intake, nutritional confounders (as mentioned in the discussion of mixtures), co-exposure to other toxins

with similar or identical critical endpoints (as in the evaluation of the toxicological effects of methyl mercury); there was also concern regarding the co-exposure to PCPs, another neurotoxin (NAS/NRC, 2001). Other issues of concern to evaluating health effects from exposure to metals are whether the available measures of exposure actually measure peak exposures (e.g., methyl mercury or arsenic) or cumulative exposure when health effects are the product of long-term exposure (e.g., cadmium and lead).

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 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 per unit of body weight is higher than an adult's.

There are also differences in pharmacokinetic behavior of metals at different stages in the life cycle, particularly for the nutritionally essential metals (WHO, 1996a). 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 (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 metals of interest to EPA 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 highly susceptible to metal toxicity usually refer to effects on the fetus during pregnancy (e.g., of 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—so that their protein and energy requirements are lower—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. These differences have an impact on body content of minerals (IPCS, 2002). Women also have significant loss of iron during menstruation, and it has been shown that absorption and toxicity of cadmium are greater in women, related to decrease in iron stores (Berglund et al., 1994).

7.3.3 Genetically Determined Human Variability (Polymorphisms)

Individuals vary considerably in the nature and severity of their response to exposure to metals and metal compounds. Some of these differences may be due to subtle genetic differences or genetic polymorphisms that may alter the metabolism of a metal. The most apparent of these genetic polymorphisms affecting metabolism and toxicity of metals are disorders in homeostatic mechanisms for nutritionally essential metals. Two disorders affect copper metabolism: Wilson disease and Menkes disease. 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. Menkes disease is an X-linked recessive disorder of copper metabolism (prevalence of 1 in 200,000) that resembles copper deficiency regardless of level of copper intake (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 (NAS/IOM, 2003).

A genetic polymorphism for a heme-metabolizing enzyme affecting lead metabolism was identified in 1973 (Granick et al., 1973), but the molecular characteristics and potential clinical implications have only recently received attention (Smith et al., 1995). Fleming et al. (1998) found that the relationship of bone lead to the cumulative blood index for workers with occupational exposure to lead was greater in those workers with the ALAD1 allele, suggesting that the ALAD2 genotype decreased transfer of lead from blood to bone. This effect was only demonstrated in workers with higher blood lead levels than the general population with only environmental exposures.

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 chemicals are being described, but their role in the toxicity of metals and metal compounds has yet to be defined (Parkinson, 2001).

7.3.4 Metal-Protein Interactions

Metals react with many different proteins in the body that may modify their toxicity and kinetics. An example is 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. There are also several proteins that bind to specific metals (Goyer and Clarkson, 2001).

Metallothioneins. The metallothioneins are a group of low-molecular-weight proteins (MW about 6,000 daltons), rich in sulfhydryl groups that 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 included zinc, copper, and cadmium. Metallothioneins have multiple binding sites that have different affinities for metals. Also, the types of metal 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 kidneys 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 the 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 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 their identity or function is not as well defined as that of other metal-specific proteins. The most studied lead-binding protein is the denatured lead-protein complex identified as the intracellular inclusion body occurring in 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).

8. TARGET ORGAN EFFECTS

Metals and metal compounds can produce health effects in any organ or physiological system extending from those arising through a limited exposure to those assumed over a lifetime of exposure to a metal. These effects may be identified through target organs, or end organs, that reflect the clinically relevant effects. For the EPA IRIS program, the target organ effect may be the *Critical Effect,* or the first adverse effect, or its known precursor, that occurs to the most sensitive species as the dose rate of an agent increases. Affected target organs can include the neurological, cardiovascular, hematological, gastrointestinal, musculoskeletal, immunological, and epidermal organ systems.

8.1 Determinants of Target Organ Effects

Many factors act as determinants of a target organ effect following exposure to a metal. Some of these factors are exposure issues, e.g., dose rate factors: high-level, short-term versus low-dose, long-term exposure. Retention time and binding or sequestration of the metal in a non-toxic form allows the metal to reside in the body without producing a toxic or pathological effect. Arsenic and mercury have relatively short biological half-lives that can be measured in days, whereas cadmium and lead can be bound or sequestered in inactive forms for years. Cadmium is retained in soft tissues (e.g., liver and kidney) for 10 to 20 years by intracellular binding with metallothionein. This is capacity-limited, and toxicity to liver and kidney occurs when the capacity is exceeded. The limits of cadmium retention by metallothionein are influenced by synthesis of metallothionein and competitive binding by other metals, particularly zinc and copper.

Lead is bound within different body compartments and may be judged to “accumulate” in one or more of them, but the most toxicologically relevant systemic lead is that within the relatively labile plasma fraction. “Free lead” in blood plasma is amenable to either rapid excretion for transfer to soft tissues. Observations of a non-linear relationship between blood lead concentration and lead intake in humans suggest the existence of a saturable absorption mechanism or some other capacity-limited process in the distribution of lead to various tissue sites. Lead is considered to have at least three different tissue pools. Blood lead is the most labile, with a half-life of 36 days; bone lead is the most stable, with a half-life of several decades. Lead in soft tissues has a half-life of approximately 40 days. These factors are considered in PBPK models. Lead uptake may increase as a non-linear function of dose as lead intake rises and the ratio of lead to calcium decreases. Absorption of lead, at least in children, is inversely affected by iron status. These relationships demonstrate the previously discussed interactions between nutritionally essential and nonessential metals.

Other factors are related to issues identified in PBPK models and susceptibility factors, as described above. 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 secondarily involving 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 can involve any organ system over time, but do 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 cause cadmium to 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).

8.2 Target Organ Effects of Arsenic, Cadmium, Mercury, and Lead

Excess exposure to metals, particularly the nutritionally nonessential metals, can produce toxicity or pathological effects on most organ systems. Arsenic, cadmium, lead, and mercury have been the most studied for target organ effects because of their prevalence in the environment and documented human health effects. Their potential health effects are evaluated in detail in reports from EPA (IRIS reports), the ATSDR Toxicological Profiles, and reports from the World Health Organization's International Programme for Chemical Safety, as well toxicology textbooks. The following brief summaries are intended to illustrate differences between acute and chronic exposures (arsenic and lead), the diversity of target organ effects that can result from differences in dosage and susceptible populations, and differences in effects between inorganic forms and organic forms (mercury and arsenic).

Arsenic (inorganic). Target organ effects depend on dose, as well as mode and duration of exposure. Oral ingestion of a single high dose (300 mg) can be fatal to an adult. Single or repeated oral high doses (0.04 mg/kg/day) for weeks or months can produce overt non-specific effects, including gastrointestinal effects such as diarrhea and cramping, hematological effects including anemia, and leucopenia, peripheral neuropathy, and cardiovascular effects. These effects are usually reversible, but can permanently damage affected organ systems. Chronic exposure (inhalation or oral) to small doses (0.01 mg/kg/day or higher) for 3 to 5 years can result in diffuse or spotted hyper-pigmentation of the skin, and if continued for years can produce benign skin lesions (hyperkeratosis) and cancer of the skin. Chronic exposure can produce liver disease reflected by abnormal porphyry metabolism. Chronic inhalation can cause lung cancer. Chronic exposure to levels in drinking water as low as 10 µg/L can cause cancer of internal organs, particularly the urinary bladder, lung, liver, and kidney. While these effects have been described as the result of exposure to inorganic forms of arsenic, there is experimental evidence that one organic species of arsenic—dimethyl arsinic acid, a normal metabolite of exposure to inorganic arsenic—may be carcinogenic to rodents (NAS/NRC, 2000).

Cadmium (inorganic). Acute effects from oral cadmium exposure are uncommon, but high exposure to cadmium fumes (which can occur in some occupational settings) can cause acute bronchitis or even chronic disease, such as emphysema or pulmonary fibrosis and lung cancer (Davison et al., 1988). Chronic exposure over several years to low doses of cadmium—which might, for example, occur through cigarette smoking or daily ingestion of cadmium-contaminated rice—can cause kidney tubular dysfunction and osteoporosis in susceptible populations (elderly women with iron deficiency) (Jarup et al., 1998). Chronic inhalation of cadmium may cause lung cancer, but cancer has not been observed from oral ingestion only (Goyer and Clarkson, 2001). Health effects from cadmium in humans are the result of exposure to inorganic compounds of cadmium. Organic forms of cadmium do not exist in nature, and health effects have not been noted in humans.

Lead. Exposure to inorganic compounds of lead may affect multiple organ systems. Infants and young children in the neonatal period and early childhood are particularly susceptible to health effects from exposure to lead, including impairment of motor function and cognitive development. Anemia may also occur. Chronic high-level exposure to lead in older children will also produce anemia and central nervous system effects, including impaired motor function and cognitive function and even seizures, coma, and death with markedly elevated blood lead levels (i.e., greater than 80 µg/dL). Adults with high blood lead levels (greater than 40 µg/dL) may have impaired heme synthesis and chronic kidney disease (blood lead levels above 60 µg/dL), and sustained blood lead levels above 80 µg/dL can cause lethargy and impairment of cognitive function. Epidemiological studies suggest a small dose-effect on blood pressure for blood levels up to 30 to 40 µg/dL. Lead produces tumors in experimental animals, but there is not enough evidence to regard lead as a human carcinogen (IPCS, 1995; ATSDR, 1999).

***Mercury*.** Three species of mercury are of toxicological concern: elemental mercury, inorganic mercury, and methyl mercury. The target organ for mercury exposure should be viewed in terms of the species of interest. Exposure to elemental mercury occurs mainly in an occupational setting, taking the form of mercury vapor inhalation. There are two target organs, the central nervous system and the kidney. The toxicity of elemental mercury is believed to be due to mercuric mercury. Inhaled elemental mercury vapor readily crosses the blood-brain barrier and is oxidized to mercuric mercury, which becomes bound to macromolecules in the brain. Effects include tremor, psychiatric disturbances, and altered behavior; they are generally not reversible and there is no apparent mechanism for rapid removal of mercury from the brain. The renal toxicity of mercury vapor may involve an immunological mechanism resulting in glomerulnephritis, which may progress to renal failure. Exposure to methyl mercury follows the consumption of fish that have accumulated methyl mercury from the aquatic food chain. The target organ is the brain. The most susceptible population is the unborn fetus. Methyl mercury readily crosses the placenta resulting in exposure and toxicity to the developing brain. Low levels of exposure result in impaired development of motor and language skills during neonatal life and early childhood, but larger exposures can produce severe cognitive effects, including paresthesia, blindness, deafness, and—with more severe exposures—fetal death and abortion. Methyl mercury in the brain is slowly transformed into inorganic mercury; it is questioned

whether the actual toxic species of mercury in the brain is methyl mercury or inorganic or mercuric mercury (ATSDR, 1999; NAS/NRC, 2001).

9. INPUTS TO THE REGULATORY FRAMEWORK

The background information provided in this issue paper has a number of specific implications when considered in the context of a Framework for Metals Assessment and subsequent program-specific methodologies. Like risk assessments for other substances, metal risk assessments may be conducted at particular locations (small to mid-size site-specific assessments) for purposes such as contaminated site remediation or development of a discharge permit. These risk assessments range from simple screening-level exercises to very detailed, data-intensive assessments. Metals-specific issues such as local conditions that affect bioavailability and exposure (see the issue papers on these topics for further discussion) and localized differences in human susceptibility (due to acclimation to naturally occurring higher levels of metal, presence of potential sensitive subpopulations, etc.) can be directly addressed in site-specific assessments.

The Agency conducts national assessments to set criteria (e.g., drinking water standards) or when required to establish controls for environmental releases (e.g., hazardous waste listings under the Resource Conservation and Recovery Act or residual risk determinations under the Clean Air Act). Differing environmental conditions across the country that affect the biogeochemistry of metals make it difficult to set single-value national criteria, and the ubiquity of metals in the environment suggests the need to consider all potential effects in the context of complex mixtures. The topics covered in this issue paper provide some of the necessary background that can be used when determining how to generalize to protect human health throughout the country.

Finally, with over 80,000 chemicals listed on the Toxic Substances Control Act inventory that can legally be used in commerce within the United States, the Agency must set priorities for assessing and regulating substances for the protection of human health. Despite their natural occurrence, many metals can be highly toxic under certain conditions and may be ranked as priority substances. However, consideration should be given to the issues discussed in this paper that may modify the toxicity of metals, as well as to exposure-modifying factors such as relative bioavailability of differing metal ions.

Section 4 of this issue paper elucidates several basic differences between metals and metal compounds from organic compounds that affect the risk assessment process. 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 Exposure Issues

Some relevant exposure issues as they impact health effects are discussed in this report. 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. Exposure issues, including exposure to ambient/background levels, are discussed in the environmental chemistry, bioavailability, and exposure issue papers.

The classification of metals presented in this paper emphasizes the differences in health significance between nutritionally essential metals, nonessential metals, and metals with carcinogenic potential. Separation of metals into these groups impacts all three of the EPA risk assessment scenarios (i.e., site-specific, national, and hazard ranking/prioritization). 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 because of 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, the protection provided by calcium and iron from toxic effects of lead and cadmium, and the protection selenium provides against mercury toxicity. Although standard risk assessment practice estimates total exposure from all potential routes and then addresses all organ-specific effects (e.g., gastrointestinal vs. pulmonary) as having the same degree of significance, Agency policy has dictated that carcinogens are of special concern, as are those chemicals that cause nervous system problems or reproductive development dysfunction or are immunosuppressants.

Recognition of differences in potential toxicity between nutritionally essential metals and nonessential metals should impact EPA risk assessments associated with National Hazard/Risk Ranking Characterization. The implication is that potentially hazardous nonessential 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 (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, as mentioned above, has recently been referred to as the AROI; it is represented by a trough in the U-shaped dose-response curve (IPCS, 2002).

For metals with no known nutritional requirement, concern must be focused on excess exposure, recognizing that the NOAEL 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. Questions might arise on the 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 exclusively for metal exposure assessment, EPA has published guidelines for exposure assessment (U.S. EPA, 1997) 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 exposure to mixtures of metals, there is published guidance for the 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 from deteriorating lead paint. 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. Bioavailability of different metals and metal compounds can be significantly different and this should be borne in mind in comparisons between substances. If two substances were to produce toxicity at comparable levels of systemic exposure, the substance with the higher intrinsic bioavailability would actually be the more hazardous.

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. The toxicokinetic or PBPK/PBPD models are commonly used as predictive models for risk assessment for exposure to lead. 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 RfDs as an expression of risk for non-cancer health endpoints from exposure to potentially 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.

Toxicokinetic issues specific to metals can have the most influence on the regulatory framework at the level of national regulatory assessments for specific metals (e.g., ambient water quality criteria, maximum contaminant level goals, RfDs, or reference concentrations). 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 that one needs to understand 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 as 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, 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 (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 and 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 effective doses and species-specific toxicity of a metal will vary widely depending on its form. This 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, IRIS 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, toxicity data suggest 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 according to 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.

Often, 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

To achieve an adequate internal dose for the study of toxicity, animal toxicologists often use bioavailable forms 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 that are progressively hydrated as the solution stands. However, a site assessor is very unlikely to encounter aluminum in the lactate or maltolate form. Thus it sometimes happens that 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, rarer alternative 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., 1993, 1995; 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

All research reports need to be evaluated for adequacy of design, confounding factors, accurate identification of administered dose, and quality of the study. 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. This often applies 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 (see 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.

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 et al., 2002) and gene transcription (Meplan et al., 2000). Recent data suggest 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 on differences in metabolism and mechanisms of toxicity between metals and organic compounds that might necessitate differences in regulatory policy.

- Research is needed to determine the significance of speciation of metals in tissues in order to evaluate potential toxicity.

- Research should be conducted on mechanisms of toxicity, including carcinogenicity—namely whether carcinogenicity of specific metals occurs as direct or indirect effect and

whether it is a threshold or non-threshold event. For example, some metals are suspected of exerting a carcinogenic effect via indirect processes (e.g., oxygen radicals) as opposed to direct interaction with DNA.

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

- There should be further research into the potential interactions between nutritionally essential and nonessential metals and between nutritionally nonessential metals per se and to assess whether regulation at potentially lower levels for combined exposure may not be warranted due to sparing (protective) effect of certain metals (such as the essential nutrients zinc and copper).

- There should be research into the applicability of toxicokinetic/toxicodynamic 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 biomarkers as endpoints that reflect genetic and protein effects that can be applied to the risk assessment process for regulatory issues.

- Research is needed to meet the needs of sensitive individuals on the basis of age classes and genetic and developmental factors and to better characterize individual sensitivity, e.g., considering genetic and other factors including nutritional status.

- Research is needed to improve characterization of variability in human toxicity and methods for incorporating this information into risk calculations, with associated uncertainty.

- There is a need for methods to link biomarkers of human exposure in order to offer a meaningful predictive tool for ultimate human health significance, including multivariate statistics and visualization tools, approaches for characterizing the severity/functional impairment and recovery/reversibility (including through treatment) of various metal effects in humans across different exposure levels, and methods to identify the effects of key concern for regulatory purposes.

- Research should be conducted on detoxification processes and adaptive response processes in humans, beginning with metals of key concern for regulatory programs.

- Further study should be given to interpreting and applying public health data and information from other health studies (including epidemiological data), considering reporting issues and approaches for addressing variability and uncertainty.

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