4. METAL-SPECIFIC TOPICS AND METHODS

This chapter discusses metal-specific topics and methods to be used in the assessment of risk to humans and ecological entities from exposures to inorganic metals. It applies information and text from the metals issue papers and reflects contributions by EPA scientists and external experts. The final metals issue papers are available on the EPA Web site at http://cfpub.epa.gov/ncea/raf/recordisplay.cfm?deid=86119.

Key topics and tools in this section are presented in subsections on environmental chemistry, exposure pathway analysis, human health effects, and ecological effects. The applications and limitations of the various models and methods for conducting metals assessments are presented to inform the reader. Topics and tools related to bioavailability and bioaccumulation are discussed throughout Chapter 4 because they have far reaching impact that crosses many aspects of metals assessment.

4.1. ENVIRONMENTAL CHEMISTRY

4.1.1. Introduction and Terminology

A general review of factors pertaining to the chemistry of metals in sediments, soils, waters, and the atmosphere is presented in this chapter in the context of risk assessment. Because the behavior of metals defies simple generalities, it is necessary to understand the chemistry of the particular metal and the environment of concern. However, we can generalize factors that control metal chemistry and environmental characteristics where this generalization allows us to progress with estimates of metal fate and effects.

Metal speciation determines the behavior and toxicity of metals in the environment. Speciation refers to the occurrence of a metal in a variety of chemical forms. These forms may include free metal ions, metal complexes dissolved in solution and sorbed on solid surfaces, and metal species that have been coprecipitated in major metal solids or that occur in their own solids. The speciation of a metal affects not only its toxicity but also its volatilization, photolysis, sorption, atmospheric deposition, acid/base equilibria, polymerization, complexation, electron-transfer reactions, solubility and precipitation equilibria, microbial transformations, and diffusivity (Bodek et al., 1988).

The following sections address the application of hard and soft acid and base (HSAB) concepts to metal behavior, including the formation of metal complexes, and the importance of pH and oxidation-reduction reactions to metal mobility and toxicity. The chapter then examines the occurrence and interactions of the metals of concern in natural media, including in surface and ground waters, soils and aquatic sediments, and the atmosphere. Important topics considered in these sections are metal sorption behavior, aging in soils, metal dissolution and transformation and transfer to plants, and methods of determining metal speciation in soils and sediments.

4.1.2. Hard and Soft Acids and Bases: The Stability of Complexes

Complexes are formed between metals (acids) and ligands (bases), both in solution and at the surfaces minerals and organisms. The toxic reaction of organisms to metals can be directly related to the nature of the metal complexes formed in solution and at the surface of the organism.

A useful concept that helps to explain the strength of metal complexing and metal toxicity is that of hard and soft acids and bases (HSAB), which was introduced by Pearson (1973). In this concept, metal cations are Lewis acids and ligands are Lewis bases, with the metal cation and ligand in a complex acting as electron acceptor and donor, respectively. "Soft" implies that the species' electron cloud is deformable or polarizable and the electrons are mobile and easily moved. Soft species prefer to participate in covalent bonding. Hard species are comparatively rigid and nondeformable, have low polarizability, hold their electrons firmly, and prefer to participate in ionic bonds in complex formation (Langmuir, 1997). Hard acids form strong, chiefly ionic bonds with hard bases, whereas soft acids and soft bases form strong, chiefly covalent bonds when they form complexes. In contrast, the bonds formed between hard-soft or soft-hard acids and bases are weak, such that their complexes tend to be rare. Table 4-1 summarizes hard and soft acid and base relationships for the metals of concern. The first text box summarizes the applicability of hard and soft concepts to the formation of metal complexes; the second text box defines ligands.

Hard and Soft Acids and Bases

Hard acids and hard bases. Complexes formed between divalent hard acid cations and monovalent or divalent hard bases are ionic and relatively weak and are often termed "ion pairs." Complexes formed between Be^{2+} or trivalent hard acids and hard bases tend to be ionic and relatively strong.

Soft acids and soft bases. Strong, relatively covalent bonds are formed in complexes between soft and borderline soft acid cations and soft bases. Ligand binding sites on the external or internal surfaces of organisms are often of soft base character and thus bond strongly with soft and borderline soft acid cations.

Ligands

Ligands are anions or molecules that form complexes with metal ions. Depending on whether a ligand shares one, two, three or more electron pairs with metals, it is called a mono-, bi-, tri- or multidentate ligand.

Hard metals (hard acids), which are the least toxic, preferentially bind with hard bases that contain oxygen, forming weaker bonds with soft nitrogen and sulphur species. The strength of binding between hard metals and hard ligands is usually a function of pH. Many of the hard metals are macronutrients. Soft metals (acids) bind preferentially with soft S and N ligands, forming weaker bonds with hard base species such as hydroxide and sulfate. Soft and borderline metals, and Mn^{2+}, which is hard, form bonds of decreasing strength with soft ligands such as sulfide, generally in the following order: $Pb^{2+} > Cu^{2+} > Cd^{2+} > Co^{2+} \cong Fe^{2+} > Ni^{2+} > Zn^{2+} > Mn^{2+}$. The tendency of metals to bind to soft ligands or to organic substrates (which are usually soft) is greatest for soft and borderline metals (soft acids), followed by the hard metals (hard acids), typically in the order $Pb^{2+} > Cu^{2+} > Cd^{2+} > Zn^{2+} > Ca^{2+} > Mg^{2+} >> Na^+$ (Pickering, 1986).

Table 4-1. Hard and soft acids (metal cations) and bases (ligands)

Hard acids	Al^{3+}, Ba^{2+}, Be^{2+}, Co^{3+}, Cr^{3+}, Fe^{3+}, Mn^{2+}, Sr^{2+}, U^{4+}, UO_2^{2+}, VO^{2+}
Borderline acids (between hard and soft)	Co^{2+}, Cu^{2+}, Fe^{2+}, Ni^{2+}, Pb^{2+}, Zn^{2+}
Soft acids	Ag^+, Cd^{2+}, Cu^+, Hg^{2+}, Hg^+, CH_3Hg^+, Tl^{3+}, Tl^+
Hard bases	F^-, H_2O, oxyanions: OH^-, SO_4^{2-}, CO_3^{2-}, HCO_3^-, $C_2O_4^{2-}$, CrO_4^{2-}, MoO_4^{2-} $H_nPO_4^{n-3}$, $H_nAsO_4^{n-3}$, SeO_4^{2-}, $H_2VO_4^-$, NH_3, RNH_2, N_2H_4, ROH, RO^-, R_2O, CH_3COO^-, etc.
Borderline bases (between hard and soft)	Cl^-, Br^-, NO_2^-, SO_3^{2-}, $H_nAsO_3^{n-3}$, $C_6H_5NH_2$, C_5H_5N, N_3-, N_2
Soft bases	I^-, HS^-, S^{2-}, CN^-, SCN^-, Se^{2-}, $S_2O_3^{2-}$, -SH, $-SCH_3$, $-NH_2$, R-, C_2H_4, C_6H_6, RNC, CO, R_3P, $(RO)_3P$, R_3As, R_2S, RSH, RS^-

Source: Modified after Huheey et al. (1993) and Langmuir (1997). "R" refers to an organic molecule.

The tendency of metals to form solid phases, such as sulfides in sediments, is also related to their HSAB qualities. For example, extremely insoluble metal sulfides are formed in anoxic sediments by soft acid metal cations, such as Hg^{2+} ($\log K_{sp} = -57.25$) or Ag^+ ($\log K_{sp} = -49.7$), whereas borderline hard and hard metal cations such as Mn^{2+} ($\log K_{sp} = -19.25$) or Fe^{2+} ($\log K_{sp} = -22.39$) form slightly more soluble, although still highly insoluble, metal sulfides.[1] These

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[1] Solubility products for all sulfides except Ag_2S are from Di Toro et al. (1990). The product for silver sulfide is from Stumm and Morgan (1970).

differences in solubilities are fundamental to the method of acid-volatile sulfide (AVS) normalization of sediment-associated metals (see Section 4.1.5, on Sediment Chemistry). Finally, according to the Biotic Ligand Model (BLM) (see Sections 3.1.4.2 and 3.4), effects of metals are related to or correlated with metal interactions with biological ligands, which are generally soft base species.

4.1.3. Aquatic Chemistry

4.1.3.1. *Speciation and Complexes*

Metal species dissolved in water may occur as free ions, or aquo-ions, or as complexes. Free metal cations are generally surrounded by coordinating water molecules and so have been termed aquocations, although by convention the water molecules are ignored when writing chemical reactions involving metal cations.

The total analytical concentration of a given metal in water is the sum of the concentrations of its free ion and its complexes and any metal associated with suspended solids, whether organic or mineral. For example, the total molal concentration of lead, ΣPb, in a natural water might equal:

$$\Sigma Pb = mPb^{2+} + mPbOH^{+} + mPbCO_3^{o} + mPbHCO_3^{+} + mPbSO_4^{o} + mPb(\text{suspended solids})$$

In most natural waters, the concentration of free lead ion, mPb^{2+}, is less than the sum of the concentrations of its complexes, which in this case are lead complexes with hydroxyl, carbonate, bicarbonate, and sulfate ions. Other metals that are found in natural waters most often as complexes and not as free ions include Al^{3+}, Ag^{+}, Cu^{2+}, Fe^{3+}, and Hg^{2+}. The metalloids As and Se and the metals Cr, Mo, Sb, and V occur most often in aerobic waters and soils as covalently bonded to oxygen in oxyanions. Under oxidizing conditions, these include arsenate, selenate, chromate, molybdate, and vanadate, which themselves are complexes.

Complexes that incorporate metals play a major role in controlling the availability and fate of metals in the environment. Increasing the fraction of a metal that is complexed increases the solubility of minerals of that metal (Langmuir, 1997). For example, the solubility of lead sulfate is related to the molal concentrations of free lead and sulfate ions through the expression:

$$K_{sp} = (\gamma_{Pb}.mPb^{2+})(\gamma_{SO4}.mSO_4^{2-})$$

where the terms γ_{Pb} and γ_{SO4} are the activity coefficients of the ions. The product of the ion activity coefficient and the molal concentration of each species equals the activity of the ion. This equation shows that the activity of free lead ion controls the solubility of lead sulfate. For a given total lead concentration (see the previous equation), the more of the lead that is complexed, the lower will be the concentration of free lead ion. This means that as the extent of lead complexing increases, the total lead concentration must also increase to reach saturation with lead sulfate. In other words, metal complexing increases total metal solubility.

Metal complexing also has a direct influence on metal adsorption to organic matter or mineral surfaces. For example, metal carbonate, sulfate, and fluoride complexes are usually poorly adsorbed, whereas metal hydroxide complexes are strongly adsorbed (Langmuir, 1997). In summary, metal complexing generally increases the solubility and mobility of metals in surface and ground waters.

For many metals, the free metal ion is thought to be the primary metal species that causes toxicity to aquatic organisms. This is consistent with the free ion activity model (FIAM), which assumes that the free or aquo-ion is the most biologically active form of the dissolved metal. Accordingly, the key parameters that can modify the degree of toxicity are those that affect speciation, such as pH and the amount of inorganic and organic ligands (e.g., dissolved organic carbon, DOC) that can form metal complexes and so provide alternative binding sites for the metal ion. Metal toxicity is also affected by other dissolved ions (e.g., Na, Ca) that compete with metals for binding sites on the gills of fish or on respiratory surfaces of other aquatic organisms.

4.1.3.2. *Importance of pH and Redox Conditions*

The pH is probably the single most important variable that influences the behavior of metals in the environment. Thus, metal complexes with sulfate, fluoride, chloride, and phosphate are most stable and important below pH 7, whereas metal carbonate and hydroxide complexes become increasingly more important above pH 6–8.

Also, as discussed in Section 4.1.4, hydrogen ion competes with metal cations for adsorption sites, so that adsorption of metal cations by hydrous ferric oxide (HFO), for example, is low in acid systems but increases with increasing pH. In contrast, oxyanions of As, Mo, Se, and Cr tend to be desorbed from HFO with increasing pH because of competition between the oxyanions and OH^- ion for sorption sites. Furthermore, the solubility of most metal-containing minerals is greatest under acid conditions and decreases with increasing pH.

Figure 4-1 shows the locus of measured values of oxidation potential (Eh) and pH in aquatic systems. The principal controls on Eh are atmospheric oxygen and organic matter. High Eh (oxidizing or aerobic) conditions are maintained in the atmosphere and in most surface waters and shallow soils in contact with atmospheric oxygen. The lowest Eh values and reducing or anaerobic conditions are found in water-logged soils and sediments that contain organic matter, and in ground waters that contain a few milligrams per liter or more of dissolved organic carbon (DOC). Intermediate Eh conditions are found in waters and sediments that are only partially oxidized because of their relative isolation from the atmosphere. Measured Eh values may not agree with Eh values computed from the concentrations of redox-sensitive species. The difference between measured and computed Eh values is discussed at length by Stumm and Morgan (1996) and Langmuir (1997).

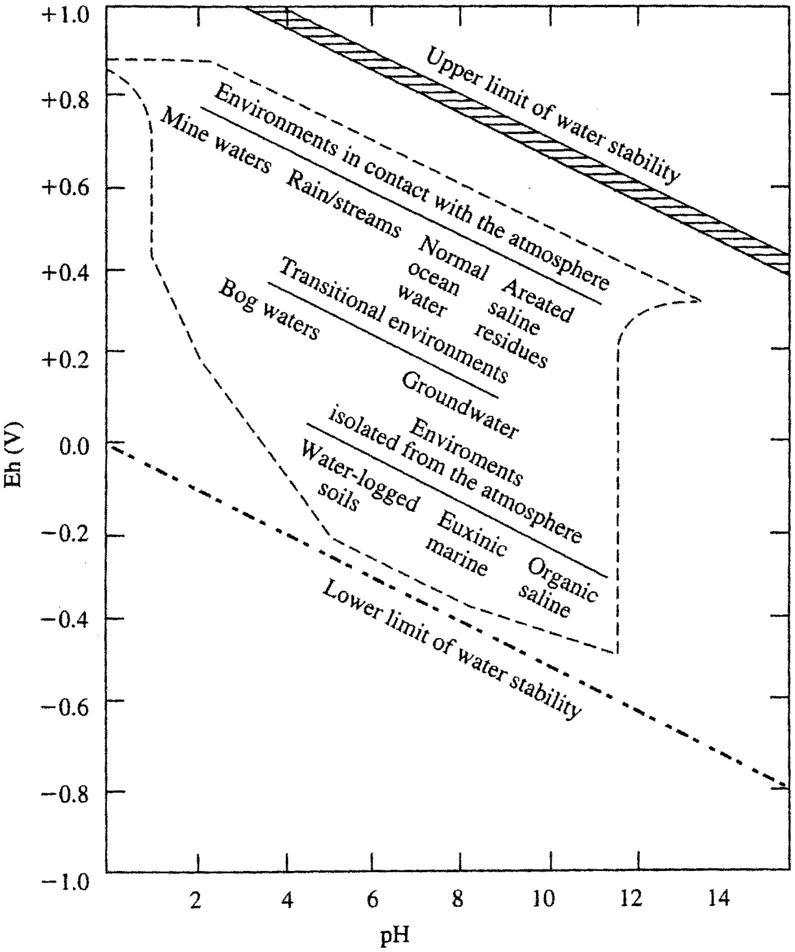

Figure 4-1. Approximate positions of some natural environments in terms of Eh and pH. The dashed line represents the limits of measurements in natural environments, as reported by Baas-Becking et al. (1960).

Microorganisms play an essential role in defining the redox conditions in aquatic systems. The effect of the common sequence of microbially mediated redox reactions on Eh when conditions in a water or sediment become more reducing (depleted in oxygen) or oxidizing is shown in Figure 4-2. These reactions importantly affect or are affected by the redox behavior of the major elements C, S, N, Fe, and Mn and also affect the mobility of most of the metals of concern, which have multiple redox states (Sb, As, Cr, Co, Cu, Mn, Hg, Mo, Ni, Se, and Ag). Thus, Cr(VI) (oxidized) in chromate ion is highly mobile in aqueous environments compared to Cr(III) (reduced). Cr(VI) is considered a known human inhalation carcinogen, whereas Cr(III) is generally considered to have low human toxicity. Among the microbially mediated reactions in Figure 4-2, the one that most affects possible metal toxicity is sulfate reduction, which requires the presence of organic matter. Sulfate reduction produces hydrogen sulfide, which reacts with

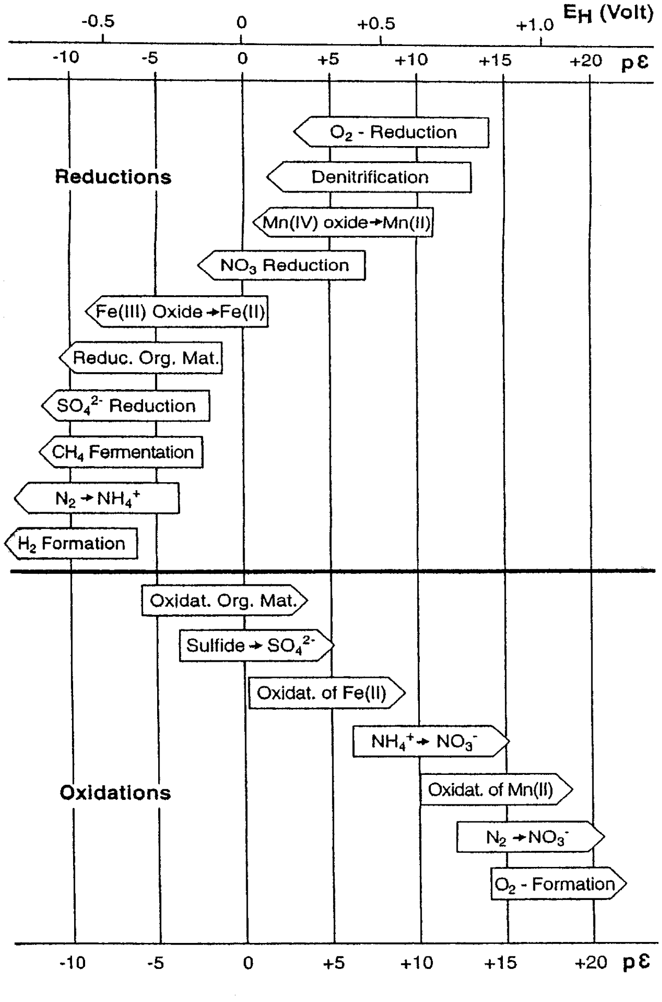

Figure 4-2. Sequence of microbially mediated oxidation-reduction reactions.

metals such as Sb, As, Cd, Co, Cu, Pb, Hg, Mo, Ni, and Ag and can cause their almost quantitative precipitation as insoluble metal sulfides. This immobilizes these metals and so makes them unavailable to plants and animals.

Much of the preceding discussion of the importance of metal speciation, hard and soft acids and bases, oxidation potential, and metal sulfides, is summarized in Table 4-2. The table shows, for example, that the hard acid metals Al, Ba, Be, and Sr have but one oxidation state and do not form insoluble sulfides; thus, their solubilities and mobilities are not directly affected by redox conditions, although they are strongly affected by changes in pH. Also indicated are forms of the metals that occur in soils and waters, with As, Mo, Se, and sometimes Cr and V occurring as oxyanions, and most of the other metals occurring usually as metal cations. Eh-pH diagrams for the redox-sensitive metals of concern and of major elements such as Fe, S, and C are given in Langmuir et al. (2004). These show the detailed occurrence of the metal species under oxidizing and reducing conditions. In simplified terms, the Eh-pH diagram and Table 4-2 show that Fe, Mn, and Tl are most mobile under reducing conditions, whereas S and the remaining metals of concern are usually most mobile under oxidizing conditions.

Table 4-2. Important properties of trace metals as they occur in natural waters and mineral systems: oxidation states, redox sensitivity, tendency to form sulfides at low Eh, dominant chemical species of metals in soils and natural waters not considering most (especially weak) metal complexes

Metal	Symbol	Hard or soft	Oxidation states	Oxidized forms	Reduced forms[a]	Can form sulfides at low Eh	Soils	Waters
Aluminum	Al	H	3+	Al^{3+}	Al^{3+}	no		
Antimony	Sb	H/S	5+, 3+, 0	$Sb(OH)_6^-$	$Sb(OH)_3^o$/Sb(s)	yes	$Sb_{III}O_x$?	$Sb(OH)_6^-$
Arsenic	As	H/S[b]	5+, 3+, 0	$HAsO_4^{2-}$	$H_3AsO_3^o$/As(s)	yes	AsO_4^{3-}	AsO_4^{3-}, AsO_3^{3-}
Barium	Ba	H	2+	Ba^{2+}	Ba^{2+}	no	Ba^{2+}	Ba^{2+}
Beryllium	Be	H	2+	Be^{2+}	Be^{2+}	no	Be^{2+}, $Be_xO_y^{2x-2y}$	Be^{2+}
Cadmium	Cd	S	2+	Cd^{2+}	Cd^{2+}	yes	Cd^{2+}	Cd^{2+}
Chromium	Cr	H	6+, 3+	CrO_4^{2-}	Cr^{3+}, $Cr(OH)_3(s)$	no	Cr^{3+}	CrO_4^{2-}, Cr^{3+}
Cobalt	Co	B	(3+), 2+	(Co^{3+}), Co^{2+}	Co^{2+}	yes	Co^{2+}	Co^{2+}
Copper	Cu[a]	B/S[b]	2+, 1+, 0	Cu^{2+}	Cu^+/Cu(s)	yes	Cu^{2+}, Cu-OM[b]	Cu^{2+}, -fulvate
Lead	Pb	B	(4+), 2+	Pb^{2+}	Pb^{2+}	yes	Pb^{2+}	$Pb(OH)^+$
Manganese	Mn	H	(4+), (3+), 2+	(4+), 2+	(3+), 2+	no[b]	Mn^{4+}, Mn^{2+}	Mn^{2+}
Mercury	Hg	S	2+, 1+, 0	Hg^{2+}	Hg^+/Hg(l)	yes	Hg^{2+}, CH_3Hg	$Hg(OH)_2^o$, $HgCl_2^o$
Molybdenum	Mo	H/S	6+, (5.33+), 5+, (4+)	$HMoO_4^-$	MoO_2^+/$Mo_3O_8(s)$/$MoS_2(s)$	yes	MoO_4^{2-}	MoO_4^{2-}
Nickel	Ni	B	(3+), 2+	(Ni^{3+}), Ni^{2+}	Ni^{2+}	yes	Ni^{2+}	Ni^{2+}
Selenium	Se	H/S	6+, 4+, (0), 2-	SeO_4^{2-}	SeO_3^{2-}/Se(s)/HSe^-	no	SeO_4^{2-}, $HSeO_3$	SeO_4^{2-}
Silver	Ag	S	1+, (0)	Ag^+	Ag^+/Ag(s)	yes	Ag^+	Ag^+, AgCl
Strontium	Sr	H	2+	Sr^{2+}	Sr^{2+}	no		
Thallium	Tl	S	(4+), (3+), 1+	(Tl^{4+}), (Tl^{3+})	$Tl_2O(s)$/Tl^+	yes		
Vanadium	V	H	5+, 4+, 3+	$H_2VO_4^-$	VO^{2+}, $V(OH)_3^o$	no	$V_{IV}O_x$?	HVO_4^{2-}
Zinc	Zn	B	2+	Zn^{2+}	Zn^{2+}	yes	Zn^{2+}	Zn^{2+}

Oxidation states in parentheses are found in mineral systems only. Hard (H), soft (S), and borderline soft (B) metals are indicated.

[a] Cu^{2+} is bordeline soft, and Cu+ is a soft cation. Typically, much or most of Cu in soils is complexed with organic matter.

[b] B/S or H/S denotes whether the oxidized species is borderline hard or hard, and the reduced species is soft.

[c] Cu-OM denotes copper complexed with organic matter.

Source: Modified after Logan and Traina, 1993.

4.1.3.3. *Effects Concentration and Bioavailability*

The traditional method of predicting effects of metals on aquatic organisms is to estimate an effects concentration for the water column and the sediment. For the water column, the EPA's Water Quality Criteria (WQC) (U.S. EPA, 1996a; Stephen et al., 1985) or the Predicted No-Effect Concentration (PNEC) derived following the European Union's (EU's) technical guidelines (EU, 1996) are frequently used as default screening values. However, these criteria make only limited corrections for bioavailability, taking into account only water hardness and ignoring other modifying factors such as pH and competing ligands. The BLM was developed to remedy this situation (Paquin et al., 2002a; U.S. EPA, 2000d; Di Toro et al., 2000). It incorporates the WHAM speciation model and it also models the competitive metal binding at the toxic site of action (the biotic ligand). BLMs are currently available for copper and silver (Di Toro et al., 2000; Santore et al., 2000) and zinc (Santore et al., 2002) and are under development for cadmium, nickel, and lead (see www.epa.gov/waterscience). The development and use of the BLM is covered in detail in Section 4.5, Characterization of Ecological Effects.

4.1.4. Ground Water and Metals Mobility

Site-specific risk assessments for EPA programs often need to predict the rate of movement of metals through soils and their subsequent movement and concentrations in ground water. The primary processes governing the environmental fate and transport of metals in the subsurface are advection, dispersion, matrix diffusion, and retardation (U.S. EPA, 1994c). Advection and dispersion are functions of the system rather than of the contaminant. Matrix diffusion, which is a function of the contaminant, is relatively unimportant and is omitted in most model transport algorithms. Retardation depends on a number of factors (Langmuir, 1997; U.S. EPA, 1994c) and may involve or be affected by the following:

- **Sorption.** The attachment of metal species to mineral surfaces or other surfaces.

- **Speciation.** The distribution of a given constituent among its possible chemical forms, including metal complexes, which have differing tendencies to be adsorbed or desorbed.

- **Precipitation.** The process by which dissolved species exceed the solubility limits of their solids, so that some of the species precipitate from solution. When a metal species reaches mineral saturation, addition of further amounts of the species to solution are precipitated, not adsorbed.

- **Colloid formation.** The process of forming colloids and the association of metal species with them. The metals may be sorbed or coprecipitated with colloidal-sized particles.

- **Biofixation.** The binding of metals to solid materials due to the interactions of microorganisms or plants.

- Natural organic matter (NOM) interactions.

- Other important processes, such as changes in pH, oxidation potential, salinity, concentrations of competing ions, the nature of sorbent phases and their surface areas, and surface site densities.

4.1.4.1. *Metals Sorption*

4.1.4.1.1. ***Introduction.*** The ability of a trace or minor metal such as a metal of concern to sorb to a substrate is usually the determining factor in its mobility. Physical adsorption, which is important for molecular organic compounds, is largely inapplicable to the sorption of the trace metals, which are usually adsorbed as ionic species. In fact, trace metal adsorption is ofen relatively independent of the concentrations of the major ions. *Trace metal adsorption is strongly pH-dependent and a function of ionic strength, metal complex formation, competitive ion sorption, redox conditions, and the amounts and reactive surface areas of specific sorbing solids.* The most accurate and mechanistic approach to modeling and predicting trace metal adsorption is *surface complexation modeling*, using a model such as the *Diffuse Layer (DL) model*, which ideally can account for all of these variables (Langmuir, 1997; Stumm and Morgan, 1996).

The degree of mobility of organic contaminants is often expressed by means of a single *partition coefficient* (K_d) value that describes the distribution of a species between sorbed and dissolved forms (U.S. EPA, 1995a). Mobility is then calculated from the partition coefficient. Such an approach is applicable to metal adsorption only when the conditions listed above are practically constant and are the same in the environment as in the laboratory where the K_d was determined, which is rarely the case. In fact, when metal adsorption is described using partition coefficients, the value of such coefficients typically should be varied by two or more orders of magnitude to reproduce metal adsorption behavior at a specific site. Therefore, single partition coefficients for metals are of little value except in broad regional surveys when a large uncertainty in a K_d value may be acceptable.

4.1.4.1.2. ***Surface complexation models: Diffuse Layer model.*** It has been observed that in many soils and sediments low in organic matter OM), HFO is the most important metal sorbent and the only sorbent that needs to be considered in predictions of metal sorption behavior (Benjamin and Leckie, 1981). The DL model (also called the Generalized Two-Layer Model, or GTLM) in MINTEQA2 has been extensively applied in aquatic environmental studies of metal transport and attenuation. Loux et al. (1989) used the DL model and MINTEQA2 to predict the adsorption and precipitation behavior of eight metals in an oxidized, sandy aquifer as a function of pH. Assuming that HFO was the only sorbent, DL model adsorption adequately described changes in the concentrations of Ni, Pb, and Zn in the sediment. Cadmium behavior was better understood, assuming its precipitation in $CdCO_3$ (otavite). Changes in Cu, Ba, Be, and Tl were not simply explained. Copper may have been adsorbed by organic matter, which was not considered in the modeling.

More recently, adsorption of metals by OM and Al oxyhydroxides as well as HFO has been included in DL modeling with MINTEQA2 (Paulson and Balistieri, 1999). These authors studied neutralization of acidic ground waters by ambient surface and ground waters using a mixing model approach. Particulate organic matter (POM) and HFO were the chief metal sorbents. In pristine systems, Cu is usually the chief metal associated with particulate organic carbon (POC); in the Paulson and Balistieri study, Zn and Cd were mostly adsorbed by POM, and Cu was mostly absorbed by HFO.

It may be possible to estimate metal adsorption with acceptable accuracy without having to measure it, depending on the information available on a specific soil, surface water, or ground water system. What is needed minimally is the weight and surface area of potentially sorbing materials (e.g., metal oxides, clays, and organic matter) in a volume of soil or sediment, or in suspension in a stream. Literature information can then be used to estimate the sorption properties of these materials for use in a sorption model. For example, as noted above in the discussion of the DL adsorption model, where HFO is the dominant sorbent and the amount suspended in a stream is known, estimation of metal adsorption can be accurate to within 10–20%, as shown in Table 4-3 (Smith et al., 1998). As a general observation, other factors being equal, it has be found that the surface charge density—and thus the metal adsorption capacity—of most minerals is largely a function of their surface areas exposed to water (Pabalan et al., 1998). Thus, the adsorption of metals by Al and Fe(III) oxyhydroxides in a system at a given pH may be assumed to be the same if they have the same surface areas.

Table 4-3. Comparison of model predictions[a] and measured values of percent metals associated with the suspended particulate fraction of mine drainage waters from select sites

	Argo-3 (pH 5.6, HFO = 0.007 g/L)		Rawley-3 (pH 6.2, HFO = 0.11 g/L)		Leadville drain (pH 7.2, HFO = 0.001 g/L)	
Metal	**Predicted**	**Measured**	**Predicted**	**Measured**	**Predicted**	**Measured**
As	–	–	98	<78[b]	–	–
Pb	82	<71[b]	80	<93[b]	86	<71[b]
Cu	18	27	60	63	–	–
Zn	<1	0 to 8	<1	0 to 9	2	3
Ni	<1	<1	<1	1	–	–
Cd	<1	<1	<1	6	<1	<1

[a] Model predictions made with the Diffuse Layer model and MINTEQA2.
[b] Dissolved concentration was below the detection limit; value was computed using the limit of detection for the dissolved concentration.

Source: Smith et al., 1998.

Cederberg et al. (1985) and Yeh and Tripathi (1991) considered surface complexation modeling of metal adsorption and metal transport in ground water. Parkhurst (2002) developed a computer model called PHAST,[2] which is a three-dimensional reactive transport model that combines PHREEQC, which has the DL metal adsorption model, with HST3D, a ground water flow and transport model.

Several recent studies have measured and modeled trace metal adsorption and metal transport in streams using a surface complexation approach to adsorption. U.S. Geological Survey researchers of the Toxic Substances Hydrology Program have published a number of papers using the OTEQ and OTIS models. OTEQ is a one-dimensional model for studying the fate and transport of metals in streams and rivers. The model couples the OTIS transient storage model with MINTEQ, which includes DL model adsorption of metals by HFO (Ball et al., 1999; Runkel et al., 1999). Runkel et al. (1999) considered in-stream metal transport, metal oxide precipitation-dissolution, and pH-dependent sorption of copper and zinc.

If greater accuracy or site specificity is required, it may be necessary to measure metal adsorption in laboratory experiments. Such measurements can be performed on pure minerals or on whole (usually sieved) soils. The sorption results may be used to develop DL model

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[2] See http://wwwbrr.cr.usgs.gov/projects/GWC_coupled/.

parameters for metal adsorption (Stumm, 1992; Dzombak and Morel, 1990). For example, model results may then be used to compute partition or distribution coefficients as a function of pH.

Several chemical mass transport codes are available that can model metal transport through porous media using the DL adsorption model. These models include PHREEQC (Parkhurst and Appelo, 1999) and CHMTRNS (Noorishad et al., 1987) and, for transport by streams, OTIS/OTEQ (Runkel et al., 1999). Even if model parameters are estimated on the basis of literature values, such models will generally predict metal adsorption and retardation more accurately than is possible when using single or linearly varying distribution coefficient values.

4.1.4.1.3. ***Application of partition coefficients to metal mobility in ground water.*** Owing to the complexity and multiplicity of the processes involved, recourse is often made to the use of a single partition or distribution coefficient that describes the degree to which the contaminant's transport is retarded relative to water. This approach starts with defining the retardation factor:

$$R_f = \frac{\nu_p}{\nu_c}$$

where R_f is the retardation factor,
ν_p is the velocity of water through a control volume, and
ν_c is the velocity of contaminant through a control volume.

The retardation factor is related to the distribution coefficient through the expression:

$$R_f = 1 + \frac{\rho_b}{n_e} K_d$$

where ρ_b is the porous media bulk density, and
n_e is the effective porosity at saturation given as a volume fraction (Langmuir, 1997).

Laboratory adsorption studies often find that, in simple systems, the value of log K_d for metal adsorption increases linearly with pH. For example, for Zn^{2+} adsorption by HFO, Langmuir (1997) noted that adsorption followed the log equation:

$$\log K_d = -5.48 + 1.77\ \mathrm{pH}$$

The properties and applications of partition or distribution coefficients for metals are comprehensively reviewed in U.S. EPA (1999a). These coefficients may be obtained from the literature, estimated using mathematical models, or measured. In batch tests, vessels are filled with a known amount of sorbent solid mixed with a known volume and concentration of a sorbing metal species (the sorbate). Often the approach is to systematically vary sorbent or sorbate concentrations and pH is a series of centrifuge tubes, for example. After centrifugation, the amount of sorbed concentration is determined by difference from the total sorbate added. The value of K_d, usually in mL/g, is then computed from the expression:

$$K_d = (\text{wt. adsorbed / wt. sorbent}) / \text{solute concentration}$$

In column test experiments, K_d may be computed from test results with the expression:

$$K_d = [(C_i - C_f)V] / C_f * M]$$

where C_i and C_f are the initial and final concentrations of sorbate,
V is the volume of soln passed through the column, and
M is the mass of solids in the column.

Properly designed column experiments generally give results more representative of the in situ behavior of soils and sediments than do batch tests (Langmuir, 1997).

Retardation coefficients tabulated as a function of pH by EPA (U.S. EPA, 1998a) are listed in Table 4-4 for several important metals of potential concern. EPA (U.S. EPA, 1999b) has also presented non-pH-dependent values for lead (900), mercuric chloride (58,000), and elemental mercury (1,000), which for reasons just discussed should be used with considerable caution.

Values for Lead

Values for lead are not tabulated because of their large variability and the difficulty of selecting values that would be representative or meaningful.

4.1.4.1.4. ***Limitations.*** The use of single K_d values for a given pH contains potentially large errors. The values in Table 4-4 will apply only to a specific sorbing phase, amount of sorbent and its reactive surface area, solution-to-solid ratio, and water chemistry. For example, on a weight basis, clays are far less sorbing for most metals than are the FHO or OM. Single K_d

values should be limited to regional studies and should be adjusted to account for regional variations in soil properties.

Table 4-4. Partition coefficients as a function of pH for several important elements of potential concern

Element	pH 4.9	pH 6.8	pH 8
Arsenic(V)	25	29	31
Barium	11	41	52
Beryllium	23	790	100,000
Cadmium	15	73	4,300
Chromium(III)	1,200	1,800,000	4,300,000
Chromium(VI)	31	19	14
Nickel	16	65	1,900
Selenium(VI)	18	5	2.2
Silver	0.1	8.3	110
Thallium(I)	44	71	96
Zinc		62	

Source: U.S. EPA (1998b).

The distribution coefficient approach may apply if fluid flow in the porous media (soil or sediment) is isotropic and adsorption is fast, reversible, and linear (Freeze and Cherry, 1979). These assumptions are often not valid for metal adsorption. Some transport models assume a constant partition coefficient or linearity of the partition coefficient over all concentration ranges. To the extent that sorption is not constant and follows a nonlinear isotherm (which is the usual case for metals), these models will be inaccurate. The best that can be hoped for when single partition coefficients are used to describe metal adsorption is that they represent bounding values in a given application.

4.1.4.1.5. ***Use in decision making.*** U.S. EPA (1999a) discusses the advantages and disadvantages of several methods for measuring partition coefficients, including laboratory batch testing, in situ field batch testing, flow-through testing, and field modeling. In many national assessments, EPA has used the MINTEQ model and its subsequent versions to generate generic partition coefficients that may be applied to regional or national mobility evaluations (http://www.epa.gov/ceampubl/mmedia/ minteq/index.htm or http://www.lwr.kth.se/english/ OurSoftware/Vminteq/).

For screening assessments:

- For screening assessments, partition coefficients have been tabulated as a function of pH by EPA (U.S. EPA, 1998b), and the Agency has also presented non-pH-dependent values for lead (900), mercuric chloride (58,000), and elemental mercury (1,000) (U.S. EPA, 1999b).

- In simple systems, the value of log K_d for metal cation adsorption usually increases linearly with pH, whereas the value of log K_d generally decreases with pH for anion adsorption.

For definitive assessments:

- It may be possible to estimate metal adsorption with some accuracy without having to measure it, depending on the information available on a specific soil, surface water, or ground water system. What is needed minimally is the amounts and surface areas of the potentially sorbing materials (e.g., metal oxides, clays, and OM) in a soil or sediment or in suspension in a stream, and the detailed chemical composition of the water, especially its pH and metal concentration. Literature information can then be used to estimate the sorption properties of these materials for use in the DL sorption model, for example.

- If greater accuracy or site specificity is required, it may be necessary to measure metal adsorption in laboratory experiments designed to parameterize the DL model for application to a specific study area. The experiments could be batch tests that attempt to reproduce the composition of waters and sorbing solids in the study area.

4.1.5. Sediment Chemistry

In addition to the challenges posed by metal chemistry, the sedimentary environment is complex and often highly heterogeneous. Fortunately, we can generalize about the sedimentary environment and the main controlling factors to progress toward a method for risk assessment. This brief review summarizes information on the composition of sediments; processes that act on sediments and their metal burden; and the chemistry of the sedimentary environment that influences the fate, bioavailability, and effects of metals. It is important to consider these factors in light of the aim of estimating potential biological effects of metals in sediments.

Sediment solids can hold up to a million times more metal than an equivalent volume of water. The exact proportions of a chemical held by sediment relative to water is a function of a metal's chemistry as well as the chemistry of the sediment solid and the surrounding environment, and this distribution is dynamic (Diamond and Mudroch, 1990). Because of their large capacity to "hold" metals, sediments have been characterized as "sinks." Although this is largely true, sediments are now appreciated to be temporary sinks, wherein some of the metal can enter ecological and human food webs through several routes (e.g., Diamond, 1995), primarily through accumulation in benthic organisms. These organisms include those that fully or partially live in the sediments (e.g., tubificids, chironomids, trichopteran larvae) or those that feed from the sediment bed (e.g., suckers, carp). Some organisms obtain their chemical dose from both pelagic and benthic routes (e.g., lake whitefish, walleye), but because of high chemical concentrations in sediments, the benthic route can be the dominant route of uptake (Morrison et al., 2000).

Sediments

Bed or bottom sediments are found at the bottom of lakes, rivers, and estuaries. Sediments have several sources that influence their composition and chemistry. The type and chemistry of sediments is also determined by their location in the water body as well as the characteristics of the water body. At any given site, metals can be associated with solid-phase minerals, organic matter, colloids, and pore water. The solid phase can vary from sand (>63 µm), to silt (2–63 µm), to clay (<2 µm). Because clays have more active binding sites than do the other grain sizes and because of their high surface area-to-volume ratio, fine-grained particles are of greatest significance in terms of metal binding.

For humans, the route of entry of metals from the sediments is through water used for drinking, bathing, and swimming. The availability of these metals is mediated by sediment-water exchange processes that can result in the release or remobilization of chemicals from the sediment bed. However, due to the ability of Hg to bioaccumulate in its monomethyl form, fish consumption is the critical route of exposure to this metal for humans.[3]

Many important chemical reactions involving the metals of concern occur in the fine-grained materials that accumulate in the deep parts of water bodies. The controlling factors or master variables that influence metal chemistry are redox potential and pH. A depth profile of the sediments will reveal decreasing sediment porosity and concentrations of dissolved oxygen because oxygen is consumed as organic matter decomposes. pH is often relatively constant or may decrease with depth, but alkalinity may increase owing to mineralization of organic matter (Stumm and Morgan, 1996). As dissolved oxygen is consumed, anaerobic microbes use other electron acceptors in redox or oxidation-reduction reactions in the order of nitrate, ferric iron, ammonium, sulfate, and bicarbonate to produce carbon dioxide, ammonia, sulfide, and methane.

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[3] Marine biota can also be a significant route of exposure to arsenic in its organic forms, such as methylarsonic acid; however, these arsenic species are significantly less toxic than the inorganic forms (Fowler, 1983).

The sequence of microbially mediated redox reactions that produce these reduced species are given in Figure 4-2.

The redox status of the sediments can be assessed by measuring the concentration of dissolved oxygen or other redox-sensitive species, by calculating pE (a measure of electron availability in solution), or by measuring Eh (millivolts) or the electromotive force of the pore water solution. The zone of transition from oxic to anoxic conditions is the redoxycline, which can migrate vertically, depending on the mixing of the overlying water column (e.g., Diamond and Mudroch, 1990). For example, the redoxycline may be 5 to 10 cm below the sediment-water interface in a well-oxygenated oligotrophic lake or river, but it may be above the sediment-water interface in a thermally stratified eutrophic lake or river.

4.1.5.1. ***Metal Chemistry in Sediments***

In this discussion, two pools of metals should be considered. The first pool consist of metals that exist as aqueous (or dissolved) species bound to colloids or DOM and those bound to sediment particles through an exchangeable binding process. This pool is often referred to as the exchangeable or labile pool. The second pool consists of metals found within the mineral matrix of the sediment solids. This pool is largely unavailable to biota, and its release will occur over geologic time scales through diagenetic processes. Because the latter pool is largely unavailable, we will consider only the exchangeable pool of metals. Note that the exchangeable pool will be composed of naturally occurring metals that are released into solution due to weathering and diagenetic processes as well as metals released into the environment due to anthropogenic activities.

The exchangeable pool of metals is subject to speciation in the aqueous phase (e.g., within the pore water) and sorption to solid phases, where sorption is a general term that includes adsorption (the accumulation of matter at the solid-water interface or a two-dimensional process) and absorption (inclusion in a three-dimensional matrix) (Stumm and Morgan, 1996). Here, speciation refers to the distribution of metal species in a particular sample or matrix or species distribution (Templeton et al., 2000). In the aqueous phase, metal will react or bind with dissolved ligands according to the pH, Eh, ionic strength, and abundance of ligands (see above discussion on aquatic chemistry).

The concentration of metal in the dissolved phase is controlled by sorption to the solid phase. Although sorption can occur rapidly, desorption or dissolution of metal from the solid phase may be a two-phase process, where the second phase is rate limiting (e.g., Nyffeler, 1986; Santschi et al., 1986). If we neglect the kinetic limitation of reactions, the distribution of metals among aqueous species and between the aqueous phase and the solid phase can be estimated. Several speciation/complexation models are available to perform this calculation, such as MINEQL+ (Schecher and McAvoy, 2001), the Windermere Humic Aqueous Model (WHAM) (Lofts and Tipping, 1998), and MINTEQA2 (Allison et al., 1991). These models work well under oxic conditions, but estimates of metal binding are less reliable under anoxic conditions, where metal concentrations are most often controlled by the solubility of metal sulfides. Furthermore, in some circumstances equilibrium may not be achieved, particularly when the redoxycline moves more quickly than the rate of metal reaction or when the reaction is governed by microbial processes, as occurs with the methylation of mercury or arsenic.

In oxic sediment pore waters (above the redoxycline), metals will exist as aqueous species, that is, as freely dissolved ions or metal complexes (e.g., phosphate, sulfate, or carbonate complexes), and associated with colloids. Solid-phase reactions are controlled by iron oxyhydroxides and manganese oxides that may exist as colloids, sediment particles or surface coatings of particles, OM that may also exist as colloids or coat sediment particles, and clay colloids and particles.

As Eh declines, the solid-phase manganese oxides are the first to be reduced and thereby dissolve, which releases metals that have been sorbed or coprecipitated with these minerals. Some of the metals released into the pore water may then be adsorbed by iron(III) oxyhydroxides, which are the next to dissolve as the Eh continues to drop. Under reducing conditions, particularly as sulfate is consumed and the sulfur is converted to sulfide, metal concentrations in pore waters again drop as solid-phase metal-sulfides are formed (see discussion below about the role of AVSs in regulating toxicity).

As a result of redox chemistry, metals can undergo seasonal redox-driven cycling between the water column and sediments or within the sediments, depending on the position of the redoxycline. The stages in the cycling are, first, the adsorption or coprecipitation of metals with iron and manganese hydroxides under oxidizing conditions; then with the development of moderately reducing conditions, the reduction and dissolution of the manganese and iron oxyhydroxides, and consequent release of the associated metals into the water or pore water; followed by their diffusion upward toward the zone of low metal concentrations under oxidizing conditions. It is also possible for dissolved metals to diffuse downward toward the zone of low metal concentration owing to their precipitation as sulfides. As a result of this vertical cycling, the depth profile of metals in pore water may not match that of the solid phase (e.g., Carignan and Tessier, 1985). Moreover, it is possible, but less usual, that the cycling can occur relatively rapidly and involve a significant portion of the solid-phase metal. Under these conditions, the solid-phase sediment profile reflects this reworking rather than the historical record of metal loadings (MacDonald et al., 2000).

pH controls metal speciation and binding by affecting the species distribution of dissolved ligands (e.g., phosphate, sulfate, carbonate, humic substances) and the surface charge of binding sites on DOM and solid phases such as iron oxyhydroxides. Generally, at low pH, when surface sites are protonated, the sorption of cationic metals decreases, and, hence, metal mobility increases. The converse occurs at high pH, which results in low metal solubility and greater sorption. The patterns of dissolution and sorption are reversed for metalloids, such as arsenic, that exist as anionic species.

4.1.5.2. *Methods of Estimating Metal Distribution in Sediments*

4.1.5.2.1. ***Application.*** A main objective in terms of assessing the hazard or risk posed by metals is estimating metal in the dissolved phase that is potentially bioavailable. Accordingly, several methods have been developed to estimate the distribution of metals among dissolved and solid phases in sediments. These methods have been thoroughly reviewed by Mudroch et al. (1999, 1997). Although bioavailability is also a function of aqueous phase speciation (see Section 4.1.3, Aquatic Chemistry), limited research has been conducted to estimate metal speciation in pore waters. Generally, ecological risk assessments assume that the exposure of benthic organisms to sediment-associated metal is proportional to the metal concentrations in interstitial water, although some studies indicate that uptake from overlying water (Hare et al., 2003; Roy and Hare, 1999) or ingested sediment may be a significant source of body burdens of metals (see Section 3.2, Metals Risk Assessment Recommendations for Aquatic Environments, for more discussion of this topic).

Distribution of metals in sediment pore waters may be determined by field measurements, experimental methods, and mathematical modeling, with the latter also requiring some field measurements. Concentrations of metals in pore waters may be determined in the field by use of pore water dialysis chambers or peepers and by methods that separate the solid phase from the pore water, although the latter have been shown to be less reliable (Mudroch et al., 1997). Several extraction schemes have been developed to determine the distribution of metal among operationally defined fractions (e.g., Forstner, 1995a; Tessier et al., 1979). It is well known that sequential extraction methods do not cleanly distinguish the occurrence and speciation of different forms of metals in sediments and soils (Tye et al., 2003; Verloo, 1999). Other experimental methods include leaching tests (e.g., Reuther, 1999). The results of any of these methods are concentrations of metals in pore water, which can be related to toxicity benchmarks.

Because of the need to develop Sediment Quality Guidelines (SQGs) for metals that explicitly address toxicity and are based on readily measured parameters, several methods have been developed. For oxic sediments, Tessier and co-workers (Tessier et al., 1993, 1989, 1984; Tessier, 1992) compiled partition coefficients of metals that were derived from field studies of freshwater sediments. The partition coefficients are dependent on pH (because Eh is held constant) and are generally linear over a range of pore water pH values (see above discussion under ground water chemistry for the theoretical basis for development of partition coefficients). Speciation/complexation models also may be used to estimate fractions of dissolved and bound metal species. These models rely on measurements of pH, dissolved oxygen, or Eh to establish redox conditions. The models assume that solid-phase binding is governed by sorption to iron and manganese oxides. Model estimates are less reliable when other solid-phase substrates are dominant (e.g., clay minerals) and are a function of the availability and accuracy of the stability constants for the metal-ligand reactions that are used in the calculations.

For anoxic sediments, the availability of sulfide controls metal distribution and solubility. Operationally, AVSs—mainly iron monosulfide—have been considered as a measure of reactive sulfides (Forstner, 1995a). Studies have demonstrated an inverse relationship between sediment toxicity and AVSs for marine and freshwater sediments (Di Toro et al., 1992, 1990; Ankley et al., 1991) as well as between pore water concentrations and AVSs (Brumbaugh et al., 1994; Casas and Crecelius, 1994). As a screening-level tool, the toxicity of anoxic sediments can be assessed by determining the ratio of AVSs to simultaneously extracted metal (SEM). Low sediment toxicity is indicated when AVSs are in excess (AVS > SEM), which implies sufficient capacity of the AVS to bind essentially all free metal. This topic is further discussed in Section 4.5.

For estimating effects concentrations in the sediment, there are many different SQGs, which vary in their derivation and the degree to which they incorporate bioavailability considerations. Many of the published SQGs are based on empirical relationships between biological effects and the total (dry weight) concentrations of sediment contaminants (e.g., McDonald et al., 1996; Long and Morgan, 1991). Although these empirically based guidelines do show general relationships between the degree of sediment contamination, they do not explicitly account for site-specific differences in bioavailability of contaminants. Although EPA has not formally adopted any single SQG approach as an Agency standard, it has been active in developing the “equilibrium partitioning” (EqP) approach to SQG development. The EqP approach considers effects of sediment chemistry on bioavailability by comparing the concentrations of AVSs, SEM, and organic carbon (U.S. EPA, 2000c; Ankley et al., 1996, 1993; Di Toro et al., 1992, 1990). This approach is reviewed in more detail in Section 4.5, Characterization of Ecological Effects.

4.1.5.2.2. ***Limitations.*** Model estimates are less reliable when other solid-phase substrates are dominant (e.g., clay minerals), and they are a function of the availability and accuracy of the stability constants for the metal-ligand reactions that are used in the calculations.

4.1.6. Soil Chemistry

The cationic metals occur naturally in soils as oxides and hydroxides (Fe, Mn, Al); to a lesser extent as carbonates, phosphates, and sulfates; and in reducing (usually wet or waterlogged) soils as sulfides, which are highly insoluble. The soil parameters important in affecting sorption and precipitation reactions and the extent of their influence—and thus contaminant bioavailability—depend on the intrinsic properties of the contaminants. In the soil environment, metals can exist as cations, anions, or neutral species. Their form significantly affects their sorption, solubility, and mobility. For example, most soils are chiefly negatively charged; thus, metal cations have a higher propensity to be sorbed by soil particles than do metal anions (U.S. EPA, 2003e).

Cationic metals can react with inorganic soil constituents (e.g., carbonates, sulfates, hydroxides, sulfides), forming aqueous complexes, which may be adsorbed or precipitated in mineral form. Most complexation and precipitation reactions are pH dependent (U.S. EPA, 2003e).

Arsenic, chromium, selenium, and vanadium complex with oxygen and typically exist as anionic species under most environmentally relevant scenarios (Bohn et al., 1985; Lindsay, 1979). The most common forms of arsenic are arsenate (arsenic(V)) and arsenite (arsenic(III)), which are present in soil solution in the form of AsO_4^{3-} and AsO_3^{3-}, respectively. The chemistry of arsenic resembles that of phosphate (Barber, 1995; Bohn et al., 1985). Chromium can exist as chromate (chromium(VI) or CrO_4^{2-}), which is usually considered more soluble, mobile, and bioavailable than the sparingly soluble chromite (chromium(III)), which is normally present in soil as the precipitate $Cr(OH)_3$ (Barnhart, 1997; James et al., 1997). Similarly, selenium can be present as selenates (SeO_4^{2-}) and selenites (SeO_3^{2-}). For vanadium, vanadate (VO_4^{3-}) is the most common form (U.S. EPA, 2003e).

Metals can exist in the pore water as aquo-ions or soluble complexes. The bonding of metal species to soil particles can range from ionic to covalent. For most soils in the United States, negatively charged sites are more plentiful; less than 5% of the total available charge on the soil surface is positively charged. Metals existing as cationic species have a greater propensity to associate with such soils. This makes them less bioavailable, but it also results in greater loading of metals into the soil ecosystem. Anionic metals generally move into pore water—and so are more bioavailable—but leach out of the system much more rapidly. In summary, soil pH and availability of charged sites on soil surfaces are the primary soil factors controlling release of metals to pore water and, subsequently, bioavailability (U.S. EPA, 2003e).

4.1.6.1. *Key Parameters Affecting Metal Bioavailability in Soils*

From the preceding overview of how the metals and metal compounds interact with soil constituents, it is clear that soil plays a very significant role in reducing the potential bioavailability of metals in the environment. Given the types of contaminant-soil interactions presented, the primary soil factors controlling the potential bioavailability of metals are soil pH, the availability and character of sorption sites on soil surfaces, the content of Fe and Al oxyhydroxides and soil organic matter, and least important, the soil clay mineral content. The following discussion briefly details the key soil parameters affecting the various contaminants availability to the pore water.

Soil pH is often termed the master soil variable because it controls virtually all aspects of contaminant and biological processes in soil. These processes include dissolution and precipitation of metal solid phases, complexation and acid-base reactions of metal species, and metal sorption as well as microbial activity. Increasing soil pH also results in an increase in the number of negatively charged soil sites, with a concomitant decrease in the positively charged sites. Therefore, increasing the soil pH increases the sorption and removal from pore water (Bohn et al., 1985).

4.1.6.2. *Adsorption Behavior of the Metals of Concern*

4.1.6.2.1. ***Surface area and surface density.*** In porous media, the most important sorbent solids for metals are oxyhydroxides of Fe and Mn. Their important surface properties are discussed in Langmuir et al. (2003). For a given weight of sorbent, metal sorption capacity is proportional to surface area and surface site density. The greatest surface site densities (positively or negatively charged sites) are those of organic material and the oxyhydroxides. These phases are the strongest and most important sorbents of trace metals. Except for kaolinite, the clays (0.02–2 mmol sites/g) have a surface charge that is largely independent of pH, whereas the surface charge of organic matter and the oxyhydroxides is strongly pH dependent.

4.1.6.2.2. ***Importance of pH.*** The pH at which a solid surface changes sign is referred to as the zero point of charge (ZPC). The ZPC of organic matter and kaolinite are below the range of usual pH and while their surface charge decreases with decreasing pH, their surfaces remain negatively charged above pH 2–3. In contrast, Fe(III) and Al oxyhydroxides, such as goethite (α-FeOOH), have ZPC values near pH 7–8 and so have a positive surface charge at low pH and a negative surface charge at high pH. Thus, the negative surface charge of the oxyhydroxides, and their sorptive capacity for metals, increases with increasing pH. Conversely, the positive surface charge of the oxyhydroxides increases as the pH drops, making these phases more effective sorbents for anions under low pH conditions.

These effects are shown in Figure 4-3, which is a plot of percentage sorbed versus pH for metal adsorption by ferrihydrite, or HFO. The curves are called *sorption edges* for each metal. The diagram shows that the oxyanions are strongly adsorbed by HFO under acid conditions but are desorbed (become mobile) with increasing pH. Based on Figure 4-3, combined with adsorption data assembled by Dzombak and Morel (1990), the order of desorption from HFO with increasing pH is selenate, antimonate, molybdate, chromate, vanadate, arsenate, and phosphate. Selenate desorbs between pH 3 and 8, whereas arsenate is strongly held at lower pH values and desorbed between pH 9 and 11. Also based on Figure 4-3 and Dzombak and Morel (1990), with increasing pH, HFO preferentially adsorbs metals in the order $Hg^{2+} > Be^{2+} > Ba^{2+} > Cr^{3+} > Pb^{2+} > Cu^{2+} > Cd^{2+} \approx Zn^{2+} > Ni^{2+}$.

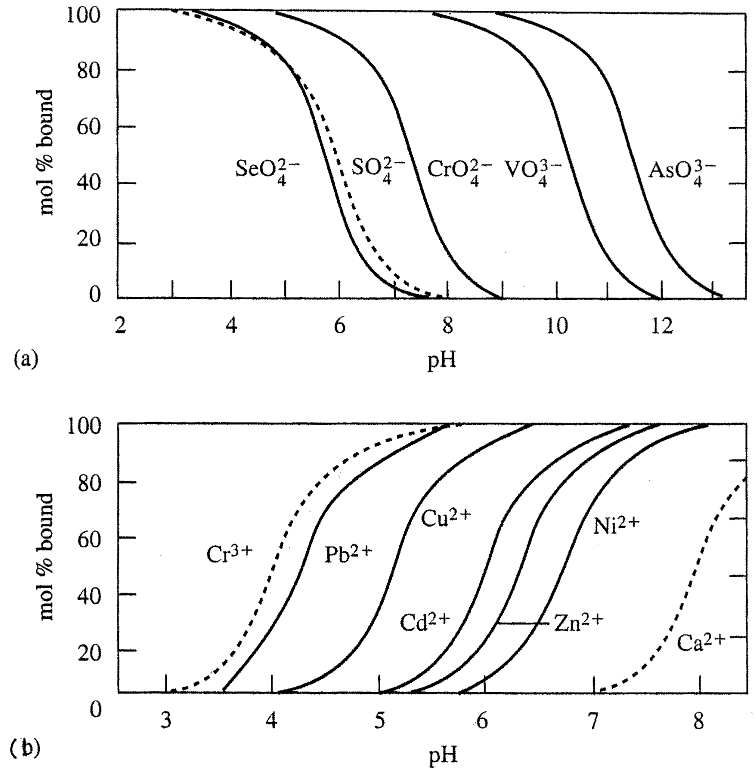

Figure 4-3. Adsorption of various metal cations and oxyanions, each at 5 × 10^{-7} M, by ferrihydrite (ΣFe[III] = 10^{-3} M) as a function of pH at an ionic strength of 0.1 mol/kg. There are 2 × 10^{-4} M of reactive sites on the oxyhydroxide. The dashed curves are calculated after Stumm (1992).

4.1.6.2.3. ***Organic matter (organic carbon) content.*** Organic matter includes plant and animal remains in various stages of decomposition, cells and tissues of soil organisms, and substances exuded from plant roots and soil microbes (Sumner, 2000). Organic matter is primarily composed of carbon, oxygen, and minor amounts of nitrogen and phosphorus. On average, approximately 58% of organic matter is organic carbon. Soils encompass a range in organic matter, from <1% for a sandy soil to almost 100% for a peat soil, with most soils having organic matter contents <10% (Bohn et al., 1985). Also, organic matter content is usually higher in surface soils or in the root zone and decreases with depth in the soil profile. The organic acid functional groups typically present in organic matter have a high affinity to attract metal cations.

4.1.6.3. *Aging of Metals in Soil*

A distinction should be made between persistence of total metals in soil and persistence of bioavailable forms of the metal. As metals age in soils, they decrease in bioavailability over time. It has been well documented that metal chemistry in solutions freshly added or spiked into soils vary from metal forms in field-contaminated soils. Typically, the metal contaminant pool requires time to diffuse into micro- or nanopores and to be absorbed onto organic matter and soil particles. These slow reactions are attributed to micropore diffusion, occlusion in solid phases by (co)precipitation and (co)flocculation, and cavity entrapment. Although the slow reactions play a key role in metal bioavailability, their rates, mechanisms, and controlling factors have not been comprehensively elucidated. Evidence of aging processes is provided by studies of metal extractability and lability (Young et al., 2003; Hamon et al., 1998). It has been frequently observed that easily extractable pools revert with time (~1 year) to more strongly bound forms. Isotopic dilution provides a useful way to quantify changes associated with progressive attenuation of metals in soil. Aging reactions are almost over after about 1 year and are reversible. At present, information regarding the aging reactions of different metals and metalloids, and sorbing solids, is very limited, so it is not possible to generalize which metal(s) age the fastest or with greater/less reversibility.

4.1.6.3.1. *Steady-state calculations for metals in soils*

4.1.6.3.1.1. *Application*. Aging reactions can be determined empirically by calculating partitioning through measurement of the soil pore water concentration of a metal in well-equilibrated soils. If K_ds are calculated from adsorption isotherms, aging should be considered separately. This is related to the high affinity of metals for soil solid phases. In fact, for most metals, metal losses by leaching, erosion, or removal by a crop that is harvested are small when compared with the total metal concentration. However, for some elements, such as Se, the half-life in soil is significantly shorter. Critical factors that affect the mass balance of metals are the anthropogenic and natural inputs and outputs via leaching to ground water, the removal through surface erosion, and crop harvesting. Excluding erosion processes, the elimination half-life of metals in soil ($t_{1/2}$) can be predicted from a soil mass as follows:

$$t_{1/2} = \frac{0.69 \times d \times 10000}{y \times TF + \frac{R}{K_d}}$$

where d is soil depth in meters,
y is annual crop yield (t ha^{-1} y^{-1}),
TF is the ratio of the metal concentration in plant to that in soil,
R is the net drainage loss out of the soil depth considered (m^3 ha^{-1} y^{-1}),
K_d is the ratio of the weight of metal adsorbed to the weight of the sorbent, divided by the metal concentration in a volume of soil solution (L Kg^{-1}).

The time required to achieve 95% of steady state is about four half-lives. This is shown in Table 4-5 for select metals and metalloids. Selenium approaches steady state after only 1 year. As a consequence, Se soil concentrations after 100 years and at steady state are identical. In contrast, concentrations of Cu, Cd, Pb, and Cr(III) are still well below steady-state values after 100 years and, consequently, their concentrations in soil are very similar. The time necessary to approach steady state is a function of the loading rate and K_d.

Table 4-5. Time to achieve 95% of steady-state metal concentration in soil and total soil metal concentrations after 100 years and at steady state[a]

Metal	Loading rate (g ha^{-1} y^{-1})	Kd (L kg^{-1})[b]	T (years)	Soil metal concentration (mg added metal kg^{-1})	
				Steady state	After 100 yrs
Se	100	0.3	1.3	0.01	0.01
Cu	100	480	1860	16	2.4
Cd	100	690	2670	23	2.4
Pb	100	19000	73300	633	2.6
Cr III	100	16700	64400	556	2.6

[a] Assumes a soil depth of 25 cm and a net drainage loss of 3000 m^3 ha^{-1} y^{-1}; background was zero at the start of loading.
[b] Mean K_d (ratio of total metal concentrations in soils to that in pore water) of 49 Dutch soils (De Groot et al., 1998).

Source: Adapted from Smolders et al. (2004).

4.1.6.3.1.2. ***<u>Limitations</u>*****.** It should be noted that the time needed to approach steady state for all the metals considered, except Se, is in the order of thousands of years, and it is difficult to envision that soil conditions would not change in this time frame. However, the concept is useful because it provides a time frame within which to consider risk as a function of loading capacity of the soils and the potential for continued exposure even after cessation of anthropogenic inputs of metals to soils. Limitations on the application of K_d are discussed in Section 4.1.4.

4.1.6.3.2. *Laboratory methods to simulate aging in soils*

4.1.6.3.2.1. *Application*. The aging effect requires laboratory studies on soils to apply a time-dependent weathering or aging treatment of spiked soils. Critical toxicity values generally are based on toxicological tests performed during the period of relatively fast metal adsorption that follows metal addition to soil. Such values would be lower than those derived from a similar study conducted with soils a year or more after addition of the metal. McLaughlin et al. (2000) proposed that toxicity thresholds be set using a sequential testing procedure. Tests would be conducted within 2 to 7 days following incorporation of the test substance to generate an estimate of acute hazard. Another set of soils would be tested 60 days after mixing, and a third would be subject to a leaching process and also tested after the 60-day period. It has been estimated in preliminary studies that toxicity is reduced up to 10-fold owing to aging of metals in soils. However, further studies are warranted and standardized processes should be agreed upon before metals aging can be properly accounted for in soil toxicity testing.

4.1.6.3.2.2. *Limitations*. The leaching and equilibrium times are limited for practical reasons and are meant to simulate some degree of aging and dissolution as a result of weathering. If aging occurs at faster rates than does dissolution, then toxicity will decrease with time. Conversely, if dissolution occurs at a greater rate, then toxicity will increase.

4.1.6.4. *Dissolution and Transformation of Metals*

4.1.6.4.1. *Application*. The dissolution and transformation of a metal compound in soil is related to a series of chemical and physical properties characteristic of the compound itself and of the soil. Environmental parameters such as temperature and humidity have a strong influence on the rate of transformation. When assessing the transformation of a compound in soil, it should be remembered that aging reactions may take place at the same time as transformation and dissolution. When metal salts are added to soil, the form of the salt dictates the rate and amount of soluble metal that will form in the pore water. Insoluble forms of metals (e.g., vanadium pentoxide [V_2O_5]) will transform to soluble free ion (V) at a slower rate than will soluble metal salts (e.g., Na_3VO_4). However, the rate of formation of the free ion is not proportional to the dissolution rate of the salt because aging reactions will remove the free ion from the pore water. The relative rates of dissolution and aging should be considered simultaneously to accurately predict pore water concentrations.

4.1.6.4.1.1. ***Solution speciation (computer-based models).*** Computer-based models employ either equilibrium constants or Gibbs free energy values to determine metal speciation from solution chemistry conditions (concentration, pH, Eh, organic complexes, adsorption/desorption sites, and temperature). Both approaches are subject to mass balance and equilibrium conditions that should be defined. In recent years, as more accurate thermodynamic data have become available, the models have undergone extensive development and can provide useful predictive estimates of metal behavior. A good review of these models and their applications is provided by Lumsdon and Evans (1995). Examples of computer-based speciation models include MINTEQL, REDEQL2, ECOSAT, MINTEQA2, HYDRAQL, PHREEQC, and WATEQ4F.

Both MINTEQA2 (U.S. EPA, 1991b) and VMINTEQ (Gustafson, 2003) contain subroutines that allow estimates of the importance of metal-organic complexing if the concentration of DOC is known. Perhaps more useful in studies of metals in soil solution are programs such as WHAM (Tipping, 1998, 1994) and the Non-Ideal Competitive Absorption (NICA) model (Gooddy et al., 1995). Application of the chemical speciation model WHAM has been discussed by Tye et al. (2003), who successfully predicted Zn^{2+} and Cd^{2+} activities in soil pore water by assuming the metals were adsorbed by soil humus according to a pH-dependent Freundlich isotherm model. Competitive adsorption between Ca^{2+} and Zn^{2+} and Cd^{2+} could be ignored because it did not improve model fits.

4.1.6.4.2. ***Limitations.*** In some instances, metal speciation is controlled by simple reactions. However, in many cases (particularly in contaminated media), the state of equilibrium and the reversibility of metal reactions are unknown. In addition, mathematical thermodynamic equilibrium models suffer from other limitations, such as lack of reliable thermodynamic data for relevant species, inadequacies in models to correct for high ionic strength, poorly known reaction kinetics, and complex reactions and lack of models for co-precipitation/adsorption. The first limitation is perhaps the most significant for contaminated media. As an example, none of the models can predict the behavior of the common, anthropogenic lead phases in paint, solder, or slag.

4.1.6.5. *Soil Metal Transfer to Plants*

The "soil-plant barrier" concept was introduced to communicate how metal addition, soil chemistry, and plant chemistry affect risk to animals from metals mixed in soil (Chaney, 1980). Reactions and processes that take place at the soil-plant barrier are influenced by the following factors: (1) soil solid phases (e.g., Fe, Al, and Mn oxyhydroxides and organic matter) may have adsorptive surfaces that influence soil chemistry; (2) adsorption or precipitation of metals in soils or in roots limits uptake-translocation of most elements to shoots; and (3) the phytotoxicity of Zn, Cu, Ni, Mn, As, B, Al, and F, for example, limits residues of these elements in plant shoots. More recently, inductively coupled plasma-mass spectrometry (ICP-MS) and other very sensitive analytical methods have been used to examine soil solution and soil-plant transfer of 60 elements as a function of soil pH (Tyler and Olsson, 2001a, b). These studies provide evidence that further supports the concept of the soil-plant barrier

For strongly adsorbed metal cations, the pattern of plant response to metals contained in biosolids is strongly curvilinear (i.e., plant metal concentration approaches a plateau with increasing soil metal concentration), rather than being linear with increasing concentration. Several areas for potential errors in the research methodology should be avoided when making these comparisons:

- First, comparison of application rates is valid only after the system has equilibrated utilizing accepted methods.

- Second, soil pH levels should be equal across rates studied; co-variance of soil pH should be used to correct for unequal soil pH (Bell et al., 1988).

- Third, the metal concentration in the source applied affects the slope of metal uptake: higher metal concentration in the source means higher phytoavailability at equal metal applications (Jing and Logan, 1992).

4.1.6.5.1. ***<u>Application/limitations</u>.*** Strongly acidic soils increase plant uptake of Zn, Cd, Ni, Mn, and Co and increasl the potential for phytotoxicity from Cu, Zn, and Ni. In alkaline soils, the high pH increases uptake of Mo and Se. Lead and Cr are not absorbed by plants to any significant extent at any pH (Chaney and Ryan, 1993). However, each element should be considered separately because of its unique chemistry. For example, arsenate is more strongly adsorbed than is arsenite; when a soil is flooded to grow rice, soil microbes can reduce arsenate to arsenite, and the higher concentration of dissolved arsenite can be phytotoxic to rice in more highly contaminated soils. Most other elements have little potential for redox change with change in the redox status of soils. Reduced soils can form sulfide, and sulfide forms low-solubility compounds with most of the metals of concern in soils, including Pb, Zn, Cd, Cu, and Ni (see above discussion on sediment chemistry). For essential elements (e.g., Zn, Cu, Ni), low-solubility species can result in deficiency syndromes. Upon oxidation of the soil, sulfide is quickly oxidized, and the metals are returned to more normal equilibrium reactions of aerobic soils.

4.1.7. Atmospheric Behavior/Chemistry

Most metals and metal compounds exist in the solid phase under ambient conditions and thus occur almost exclusively in the particle phase of the atmosphere, where they are ubiquitous. Anthropogenic sources include combustion from fossil fuels, and metal industries, as well as industrial sources employing specific metal compounds in specific processes. Some airborne metals (e.g., Mn and Ni) may derive largely from crustal sources (U.S. EPA, 1996b). Richardson (2002) included volcanic eruptions and emissions, entrainment of soil and dust, entrainment of sea salt spray, and natural forest fires as significant metals emission sources.

For purposes of risk assessment, particle size is important. The aerodynamic size and associated composition of particles determine their behavior in the mammalian respiratory system. Furthermore, particle size is one of the most important parameters in determining the atmospheric lifetime of particles, which may be a key consideration in assessing inhalation exposures, as well as exposures related to exposure pathways involving deposition onto soil or water (U.S. EPA, 1996b). Metals emitted by combustion processes (e.g., the burning of fossil fuels or wastes) generally occur in small particles or the fine fraction, which is often characterized by particles less than 2.5 microns in diameter ($PM_{2.5}$). In contrast, the larger sized, course mode particles result from mechanical disruption, such as crushing, grinding, evaporation of sprays, or suspensions of dust from construction and agricultural operations. Accordingly, metals in course mode particles (i.e., those larger than approximately 1–3 microns) are primarily those of crustal origin, such as Si, Al, and Fe (U.S. EPA, 1996b). It is noted that the fine versus coarse distinction simply differentiates two relatively distinct size distributions of particles, the separation point of which occurs in the range of 1 to 3 µm. The distinction does not refer directly to particle sampling methods or size fractionations particular to risk assessment (U.S. EPA, 1996b).

Fine and coarse particles typically exhibit different behavior in the atmosphere; fine mode particles exhibit longer atmosphere lifetimes (i.e., days to weeks) than coarse particles and tend to be more uniformly dispersed across a large geographic region (U.S. EPA, 1996b). Relatively lower dry deposition velocities of fine particles contribute to their persistence and uniformity throughout an air mass (U.S. EPA, 1997c). The larger coarse particles (i.e., greater than 10 µm) tend to rapidly fall out of the air and have atmospheric lifetimes on the order of minutes to hours, depending on their size and other factors (U.S. EPA, 1996b).

In most cases, metals do not undergo transformation while in the particle phase; thus, their removal from the atmosphere is regulated by the rate at which the particles themselves participate in wet and dry deposition processes. For example, metals such as As, Be, Cd, Pb are generally found in airborne compounds with a single predominate oxidation state (As(III), Be(II), Cd(II), Pb(II)). Some metals (e.g., the transition metals Cr, Mn, and Ni) present the possibility of changing oxidation state in situ in the particle, although little is known of these processes (U.S. EPA, 2003d). This is an important consideration for risk assessment as the different oxidation states also differ in toxicity (such as for Cr).

For metals that can change oxidation states, much of the atmospheric chemistry takes place in the aqueous phase, such as cloud droplets or water films on particles. Metal salts and oxides that dissolve in water can undergo several reversible reactions, including hydration, hydrolysis, polymerization, and reaction with other anions. The equilibrium between these forms depends on the atmospheric conditions, the equilibrium and solubility constants, and the concentrations of other chemicals. Transformations between oxidation states can occur either to increase the oxidation state (such as oxidizing Cr(III) to Cr(VI)) or to reduce it. These oxidation or reduction reactions can occur through reaction with other species, such as dissolved metals, reduced sulfur species, and organic compounds (Seigneur and Constantinou, 1995). Although models exist that can be used to estimate metal speciation in aerosols with liquid water, the reactions are still highly uncertain.

Mercury is an exception among the commonly occurring metals; it exists primarily in the vapor phase under ambient conditions but can also occur in particle and aqueous phases. At least three species of mercury should be considered: elemental (Hg(0)) mercury, which is largely present as a gas; divalent (Hg(II)) inorganic mercury compounds, which are more water soluble; and particulate-phase mercury (Shroeder and Munthe, 1998; U.S. EPA, 1997b). The behavior of mercury in the atmosphere depends strongly on the oxidation state. Elemental mercury is capable of being transported long distances, even globally; divalent mercury deposits within a few hundred kilometers of sources; and particulate mercury is deposited at intermediate distances, depending on the particle size (Shroeder and Munthe, 1998). Elemental mercury that is deposited can be reemitted from the surface, as can divalent and particulate mercury after biological or chemical reduction to the elemental form.

In the gas phase, elemental mercury can be oxidized to divalent mercury by O_3, OH, H_2O_2 and molecular chlorine, although other halogen atoms might also be important (Shroeder and Munthe, 1998). In the aqueous phase, elemental mercury can be oxidized by OH, O_3, and dissolved chlorine, and divalent mercury can be reduced by processes such as reaction with HO_2 and S(IV). Both vapor-phase and aqueous atmospheric chemistry may involve multiple phases.

EPA has made a substantial effort to evaluate the atmospheric fate of mercury as a result of the requirements of the Clean Air Act. U.S. EPA (1997b) contains a comprehensive evaluation of mercury's atmospheric fate, but this is an area of ongoing research and controversy. EPA continues to be active in investigating mercury behavior in the atmosphere (e.g., Landis and Stevens, 2003; Jaffe et al., 2003; Bullock and Brehme, 2002; Bullock, 2000a, b; U.S. EPA, 2001a).

4.1.7.1. *Application/Limitations*

Most sampling and analytical techniques published by EPA for metals in air are oriented toward evaluation of particular-phase total metals rather than metal species (U.S. EPA, 1999a). These methods involve collection of a particular size fraction of particles (e.g., $PM_{2.5}$, PM_{10}, TSP), with subsequent analysis by x-ray fluorescence, atomic absorption, inductively coupled plasma, proton-induced x-ray emission, or neutron activation analysis gamma spectroscopy techniques. The one notable exception is a method for mercury (Method IO-5) that speciates vapor and particulate forms. To the extent that metals are sorbed to particulate phases, analysis of individual metal species can, at least theoretically, be accomplished by the same techniques used to analyze those species in other solid media.

4.1.8. Metals Speciation Techniques

A wide variety of analytical and chemical techniques have been used to characterize metal speciation in various media (Isaure et al., 2002; Manceau et al., 2000, 1996; Welter et al., 1999; Szulczewski et al., 1997; Charlatchka et al., 1997; Lumsdon and Evans, 1995; Ma and Uren, 1995; Hunt et al., 1992; Gupta and Chen, 1975). These techniques provide information on speciation, particle size, and the source of the metal and also quantitatively determine the metal level present. Of the techniques tested (physicochemical, extractive, and theoretical), the tools that have been used most often to evaluate speciation include the following.

4.1.8.1. *Particle-Bound Metal*

For particle-bound metal, tools include x-ray absorption spectroscopy (XAS), x-ray diffraction (XRD), particle-induced x-ray emission (PIXE and µPIXE), electron probe microanalysis-scanning electron microscope (EPMA-SEM), secondary ion mass spectrometry

(SIMS), x-ray photoelectron spectroscopy (XPS), sequential extractions, and single-chemical extractions.

Over the past decade, numerous advances in materials science have led to the development of a wide range of analytical tools for determining metal concentrations, bonds, and valences of individual particles on a scale that can be considered useful for the speciation of environmentally important materials (soils, wastes, sediments, and dust). Although most of these tools are scientifically sound and offer important information on the mechanistic understanding of metal occurrence and behavior, only a few provide currently useful information on metal bioavailability for use at a "site" level (see Table 4-9 at the end of this section). However, other techniques still may be essential for conducting a detailed characterization of a selected material to describe the chemical or kinetic factors controlling the release, transport, and/or exposure of a metal.

An indirect approach to speciation, in contrast to the direct methods previously described, includes functional or operational extraction techniques that have been used extensively (Tessier and Campbell, 1988; Tessier et al., 1979; Gupta and Chen, 1975). These methods use either a single or a sequential extraction procedure to release species associated with a particular metal within a medium.

4.1.8.2. *Single-Chemical Extractions*

These methods generally are used to determine the bioavailable amount of metal in a functional class (e.g., water soluble, exchangeable, organically bonded, Fe-Mn bound, or insoluble). In a similar approach, sequential extractions treat a sample with a succession of reagents that are intended to specifically dissolve different and less available phases. Many of these techniques are a variation on the classical method of Tessier et al. (1979), in which metals associated with exchangeable, carbonate-bound, Fe-Mn bound, organically bound, and residual species are determined. A number of excellent reviews on the use and abuse of extraction techniques are available (Beckett, 1989; Kheboian and Bauer, 1987; Forstner, 1987). These techniques can be useful in a study of metal uptake by plants and soil invertebrates, where transfer takes place predominantly from a water solution phase. However, these methods are not "selective" for metal species, and these leachable fractions have never actually been correlated to bioavailability.

4.1.8.3. *Plants*

When considering the bioavailability of a metal to plants from soil and sediments, it is generally assumed that both the kinetic rate of supply and the speciation of the metal to either the root or the shoot are the most important factors. In soils and sediments, there is generally a small volume of water in contact with the chemical form of the metal. Although the proportion of soluble metal in pore water is small when compared with the bulk soil/sediment metal concentrations, it is this phase in pore water that is directly available to plants at the root tips. Therefore, understanding pore water chemistry is critical; that is, measuring metal concentrations as simple inorganic species, organic complexes, or colloid complexes is most important. Tools currently used for metal speciation in plants include the following:

- *In situ* measurements using ion selective electrodes (Wehrli et al., 1994; Gundersen et al., 1992; Archer et al., 1989).

- *In situ* collection techniques using diffusive equilibrium thin films and diffusive gradient thin films followed by laboratory analyses (Zhang et al., 1995; Davison et al., 1994, 1991; Davidson and Zhang, 1994).

- Equilibrium models (WHAM/FREEQC/MINTEQA2).

4.1.9. Organo-Metals/Metalloids Transformation Processes

Metals/metalloids can exist in the environment in several valence forms and as organometallic compounds. *Organometallic compounds* (referred to in this section collectively as *"organometallics"*) are compounds that have a metal/metalloid-carbon bond. The bonds in organometallic compounds are generally covalent and between soft metals and soft ligands. Metal/metalloid transformation processes, such as metal methylation, occur through interactions with other chemicals and biota in the environment. Cycling and distribution of organometallic compounds between terrestrial, water, and atmospheric phases may be physically, chemically, or biologically mediated. Examples of some commonly occurring environmentally stable organometallic compounds are shown in Table 4-6.

Table 4-6. Some stable organometallic compounds

Metal/metalloid	Stable organometallic compound
Arsenic	Methylarsenic acid, dimethyl arsenic acid, trimethyl arsine, trimethylarsine oxide
Lead	Tetramethyl/ethyl lead, trimethyl/ethyl lead, dimethyl/ethyl lead
Mercury	Methyl mercury, dimethyl mercury
Selenium	Dimethyl selenide, dimethyl diselenide, seleno-amino acids
Tin	Tributyltin, bis(tributlytin) oxide

Environmental methyl-metal concentrations reflect the net methylation rather than simple rates of methyl-metal synthesis. Metal methylation and demethylation rates in ecosystems are influenced by the speciation and biochemical availability of the metal. Metals involved in abiotic or biotic methylation/demethylation processes are presented in Table 4-7. With the exception of arsenic and selenium, the metals listed in the table form stable complexes with either methyl or ethyl groups. In addition to methyl/ethyl compounds, stable organometallic compounds such as lipids and arsenic and amino acids and selenium are incorporated biochemically.

Table 4-7. Metals/metalloids involved in methylation processes

Process	Metals affected
Environmentally stable organometallics	Si, Ge, Sn, Pb, Hg, As, Sb, Se
Abiotic chemical methylation	Hg, Pb, Sn
Abiotic demethylation	Sn, Pb
Biotic methylation	As, Cd, Hg, Pb, Se, Sn, (others? Sb, Pt,)
Biotic demethylation	As, Hg, Sn, Pb

Source: Bodek et al. (1988).

Organometallic environmental transformations may affect both the mobility and the toxicity of these metals. *The rates of transformation and the organometallic products are dependent on environmental conditions and the population of microorganisms available.* For example, methylation/demethylation rates are dependent on the speciation of the metal, the microbial community, the environmental variables (e.g., pH, temperature, reduction oxidation potential, organic matter, dissolved oxygen, nutrient availability, salinity, complexing agents) and the distribution of the metal between compartments (sediment, water, gaseous). The interrelatedness of these processes has made research into unraveling the factors controlling net methylation difficult and, to date, incomplete. However, some general trends can be predicted with some certainty, and these are discussed in this section.

4.1.9.1. *Abiotic Transformations*

Organometallic compounds that are composed of metals with electronegativities >1.7 are the most stable under environmental conditions. Carbon-metal bonds with more polar (metal electronegatives <1.7) bonds will undergo hydrolysis (reaction with water). *Abiotic chemical methylation* can occur by three mechanisms: transmethylation reactions between mercury and tin/lead alkyls, humic/fulvic substances, and photochemical reactions.

4.1.9.2. *Biotic Methylation Transformations*

Biotic methylation occurs when organisms, primarily microorganisms, transfer alkyl groups to bioavailable metals. In general, it is thought that anaerobic sulfate-reducing bacteria are the principal methylators in freshwater and estuarine environments. However, methylation rates are not always correlated with sulfate-reducing bacteria. Not all sulfate-reducing bacteria are capable of methylating, and efficiency of methylation is dependent on the activity and structure of the bacterial community. Other bacteria may be involved in methylation. Biotic methylation occurs predominantly in the sediment column; however, because the water column by volume is much larger, water column methylation is important.

Maximum methylation rates typically occur at the redox boundary, which varies seasonally and frequently coincides with the sediment-water interface (Ullrich et al., 2001). Methylation rates decrease with increasing sediment depth, probably due to a decrease in biotic habitat. Microorganisms may also demethylate (or dealkylate) organometallic compounds. Microbial-mediated transformations are frequently the most important environmental organometallic processes. Generally, as the amount of organic material increases in a system the microbial populations also increases. Examples of typical bacterial populations in natural waters and sediments are shown below (Ullrich et al., 2001).

High temperatures and anaerobic conditions generally favor metal-methylation formation, and demethylation processes are generally favored under low temperatures and/or aerobic conditions. Studies on the effects of pH are not consistent. Interconnected parameters include pH effects on the microbial communities and effects on the speciation distribution of the metals/metalloids in the water and the sediment as well as adsorption rates. Organometallic compounds appear to increase in the water column in low pH environments, but this may be due to release of methylated metals from the soil and subsequent depletion of organometallic compounds in the soil. Therefore, pH effects on net methylation in a system are not fully understood. In freshwater ecosystems, where sulfate concentrations are typically low, increase in sulfate concentration increases methylation rates. However, in reducing environments, increasing sulfide concentration decreases methylation rates.

The inhibitory effect of sulfide is probably not due to metal sulfide formation but, rather, to the formation of less bioavailably charged metal-sulfur complexes. High organic matter may increase abiotic methylation through humic/fulvic metal reactions; however, this mechanism is poorly understood and confounded because biotic methylation rates may increase in environments with high organic matter. In ecosystems with high DOC concentrations, DOC may bind with metals/metalloids, rendering them unbioavailable and thereby reducing biotic methylation rates.

4.1.9.3. *Organometallic Transformation*

Organometallic methylation and demethylation rates are influenced by both speciation and bioavailability of the metal, the microbial community, and a large number of environmental factors, many of which are interrelated. Sulfide and organic matter are important environmental variables that significantly affect methylation; however, their effect on methylation/demethylation is as yet poorly understood. Which variables dominate differs among locations and between seasons, although it is clear that methylation is predominantly a biologically mediated process. Methylation/demethylation rates are strongly influenced by the metal/metalloid speciation and bioavailability. General trends in methlyation/demethylation rates are outlined in Table 4-8.

Table 4-8. General trends of environmental factors affecting rates of methylation/demethylation

Organometallic transformations	Temperature		pH		SO_4^{2-}	Organic matter	Redox		Salinity
	High	**Low**	**High**	**Low**	**High**		**Oxic**	**Anoxic**	**High**
Net methylation	↑	↓	?	?	?	?	↓	↑	↓
Methlyation aq	↑	↓	↓?	↑?	↓	↓↑	↓	↑	↓
Methylation sed	↑	?	↑	↓	↓	?	?	?	?
Demethylation	↓	↑	↓	↑	?	?	↑	↓	?

↑ indicates an increase in rate.
↓ indicates a decrease in rate.
? indicates conflicting data or insufficient data to indicate a likely trend.

4.1.9.4. *Atmospheric Transformations*

4.1.9.4.1. ***Abiotic chemical methylation/demethylation transformations.*** Boiling points for organometallics for a given metal/metalloid decrease with increasing alkyl substitution and with shorter alkyl chains. For example, the boiling points of organotin compounds decrease with dimethyltin dichloride > trimethyltin > tetramethyltin. Fully methylated metals such as dimethyl mercury may be transported great distances in the atmosphere owing to the combined low boiling point and low water solubility. Methylation of Se, Hg, Pb, and As volatilizes these compounds, contributing to their air concentrations.

Demethylation of organometallic compounds in the atmosphere occurs by primarily by photolysis, such as:

$$(CH_3)_2Hg(g) \xrightarrow{light} Hg^0 + 2CH_3$$

Demethylation may also occur by reaction with ozone, hydroxyl radicals, nitrate radicals, and sorption to particulate matter. Organometallic compounds are also removed from the atmosphere by wet/dry deposition.

4.1.9.5. *Aquatic Transformations*

4.1.9.5.1. ***Abiotic chemical methylation/demethylation transformations.*** In stratified aquatic systems, methyl-metal/metalloid formation occurs predominantly at the oxic/anoxic interface. Organosiloxanes and other silicone-related substances have been considered as possible abiotic methylating agents. *Overall, abiotic methylation is probably of minor importance except in ecosystems with high organic matter*.

4.1.9.5.2. ***Biotic Methylation/Demethylation Transformations.*** Microbial methylation processes play the major role in methylation of Hg, Sn, and As, with methylcobalamin the most likely environmental methyl donor. Metal speciation is a prime factor regulating the methylation potential in a system. Until recently, Hg^{2+} was considered the main mercury species methylated by bacteria; however, current research indicates that uncharged Hg complexes are more likely the principal species methylated (Ullrich et al., 2001). Arsenate can be reductively methylated (via arsenite) under anoxic conditions to dimethylarsine. Selenium and selenite can be methylated via microorganisms; demethylation of organoselenium via other biotic processes is also known.

4.1.9.5.3. ***Environmental Factors affecting methylation.*** Seasonal variation of methylated mercury is thought to be related to temperature, redox potential, and productivity. Seasonal variation for organic arsenic (dimethylarsenic) has also been reported, with organoarsenic species decreasing in late fall and winter.

4.1.9.6. *Terrestrial Transformations*

4.1.9.6.1. ***Abiotic chemical methylation/demethylation transformations.*** Methylation and demethylation of organic mercury compounds in soils appear to be mediated by the same types of abiotic and microbial processes that occur in aquatic systems. The frequency and magnitude of soil moisture play an important role in availability and transformation processes. Because soils are primarily oxygenated systems, particularly in the root zone, conditions favorable to sulfide formation and bacterial methylation occur infrequently. With the exception of peat bogs and similar anoxic, highly saturated soils, methylation generally occurs only at very low rates in soils.

4.1.9.6.2. ***Biotic methylation/demethylation transformations.*** Plants have the capacity to transform metals and metalloids that are taken up from the soil. The most notable example is selenium. Soluble inorganic oxyanions of selenium are readily taken up by plants and converted to organoselenium compounds, such as selenomethionine, selenocysteine, dimethyl selenide, and dimethyl diselenide. Demethylation/dealkylation of organoselenium (e.g., trimethylselenonium), organomercury, organoarsenic, and organotin can occur in soil.

4.1.9.6.3. ***Environmental factors affecting methylation.*** Formation and degradation of organometallic compounds in soils appears to be mediated by many of the same types of microbial and environmental processes as in aquatic systems. Speciation of metals/metalloids dominates the methylation and/or uptake. Methylation/demethylation rates are affected by soil moisture: low moisture decreases biotic processes in soils. Soils high in iron and aluminum oxide, silts, and clay minerals interfere with methylation of metals/metalloids owing to the reduced bioavailability of metals/metalloids. Plants methylate selenium, predominantly to selemethionine and some selenocysteine. Plant uptake and methylation of selenium or arsenic is specific to plant species. Many soil organisms are capable of converting arsenate/arsenite to volatile methylated arsines. Losses of 15–30% per year due to volatilization of arsenic in soil have been reported (ATSDR, 2003). Organolead complexes, on the other hand, are thought to be relatively stable in soils.

Table 4-9. Metal speciation techniques for solids and their associated pore waters

Tools	Species lability	Species particle size	Species valance state	Species bonding	Species composition	Species abundance	Element specificity	Isotopic character	Element sensitivity	Resolution	Availability	Cost
XRD	No	No	No	No	No[a]	No	No	No	3–4 vol%	Bulk	1	$
EMPA/SEM	Yes	Yes	Yes[b]	No	Yes	Yes[c]	B-U	No[d]	100 ppm	0.5–1 μ	2	$$
SIMS	No	Yes	No	No	Yes[e]	Yes[f]	Li-U	Yes	1 ppb	10 μ	4	$$$
XPS	No	No	Yes	Yes	Yes[e]	Yes[f]	H-U	No	wt.%	100 μ	2	$$
XAS	No	No	Yes	Yes	Yes[e]	Yes[f]	He-U	No	ppb	2 μ	5	$$$$
PIXIE	No	No	No	No	Yes	Yes[f]	B-U	No	10 ppm	4 μ	4	$$$$

[a] Identifies crystalline compounds and stoichiometric compositions only.

[b] Valence determined by charge balance of complete analyses.

[c] Technique has limitations based on particle-counting statistics.

[d] Limited when combined with ICP/MS/LA.

[e] Technique requires that each element be tuned and standardized, requiring unreasonable time limits.

[f] Techniques designed and tested only on simple systems. Multiple species require lengthy analytical and reduction.

4.2. HUMAN HEALTH EXPOSURE PATHWAY ANALYSIS

4.2.1. Introduction

This section and Section 4.3 discuss issues of importance when performing human health risk assessments for metals and metal compounds. The information provided will complement other general Agency guidance on the risk assessment process (e.g., Carcinogen Risk Assessment (U.S. EPA, 2003b), Exposure Assessment (U.S. EPA, 1992b), Developmental Toxicity (U.S. EPA, 1991b) and focuses on the unique and specific characteristics of metals and metal compounds that might be applied in metals risk assessments for human health. This section provides some of the scientific basis that underlies metal-specific characteristics of human health effects assessment, but it is not intended to be comprehensive. Appropriate and sufficient reference material provided in this framework document will guide readers to additional details on any of the topics addressed.

4.2.2. Human Exposure

Assessment of human exposures to any chemical agent includes (1) identifying how people come into contact with metals in the environment; (2) determining the concentrations of specific forms (speciation) of the metal in specific media (e.g., soil, water, air, and biota); (3) identifying the pertinent exposure metric (via consideration of dose-response assessment); (4) estimating the exposure metric (e.g., oral intake, inhalation exposure concentration, blood concentration), which may involve quantifying relationships between exposure concentrations and intakes; and (5) identifying sources of uncertainty and natural variability and, where possible, quantifying these in estimates of exposure. Although these components are common to exposures to human and nonhuman receptors, and to metal as well as nonmetal toxicants, some specific aspects of human metal exposure assessment are discussed below.

4.2.2.1. *Environmental Background Concentrations*

For assessments performed to assess impacts associated with particular human activities (e.g., hazardous waste sites, environmental releases), the term “ambient background” generally refers to all other sources of the metals of interest. The contribution of the background to human metal exposure may be significant. For example, metals are natural components of the environment and are repeatedly cycled throughout the biosphere (this component of the ambient background is referred to as “natural background”). Metals are also present as background from persistent anthropogenic activities. During the early 1970s, for instance, industrial sources accounted for more than 90% of the airborne lead, the deposition of which supplied a large fraction of the lead in many ecosystems (Nriagu and Pacyna, 1988). Various strategies are available to estimate background metal concentrations in environmental media (soil, water, and sediment) (U.S. EPA, 1995a, b; 1989b), and a number of documents dealing with the background concentrations of metals in soils have been published by the U.S. EPA (2002b, c, d; 2001b, c).

Dietary pathways account for the major impact of background sources of metals. A number of sources provide information on dietary exposure background for metals (Capar and Cunningham 2000; Schoof et al., 1999a, b; Thomas et al., 1999; Bolger et al., 1996; Dabeka and McKenzie, 1995; Gunderson 1995; Tsuda et al., 1995; Dabeka et al., 1993). The prominence of diet in exposure assessment is discussed by Thomas et al. (1999). The United Kingdom also has extensive archives of metal content of beverages and infant foods (http://archive.food.gov.uk/maff/archive/food). Additional human dietary exposures may occur secondary to commercial processing of foods (e.g., use of preservatives, emulsifiers, taste enhancers, and packaging products that contain metals). Products such as liquid diets for weight loss, infant formula, and supplements for geriatric patients contain metals.

Exposure to metals in the diet can be high enough to approach or even exceed occupational or other well-known exposures to the metal (e.g., arsenic in drinking water). Thus, biomarkers (e.g., urinary arsenic, blood mercury) should take diet into account when estimating exposure. For example, in the 24 hours following ingestion of a seafood meal, the urinary arsenic concentration can often rise to 1 mg/L (WHO/IPCS, 1981). In contrast, persons living in Taiwan in an area with endemic arsenic contamination of the water supply of 50–300 µg/L had urinary arsenic concentrations of 140 µg/L (WHO/IPCS, 2001). Workers at a copper smelter where there was considerable arsenic exposure were found to have urinary arsenic concentrations in the range of 200–600 µg/L (WHO/IPCS, 2001). In a study of 380 American dentists, Brady et al. (1980) reported a mean concentration of 8.5 µg per litre, 7.4% of the participants having blood mercury levels greater than 15 µg/L (WHO/IPCS, 1991). In contrast, median cord blood mercury concentrations in a cohort with high fish consumption in the Faroe Islands was 24 µg/L (NRC, 2000). Mercury concentrations in the blood of people with long-term exposure to methyl mercury from fish were reported to be as high as several hundred µg/L (WHO/IPCS, 1990a).

Lifestyles expose people to metals in many different contexts and contribute to ambient background and total exposures. These exposures occur in the workplace, in nutritional supplements, in pharmaceuticals taken by the oral and injection routes, in medical diagnostic procedures, in cosmetics, in recreational drugs (e.g., smoking), in folk medicines, and in paints and pigments. For example, cadmium from tobacco smoking can significantly affect a person's total exposure.

4.2.2.1.1. ***Application.*** Depending on the scope, purpose, and approach employed for the exposure assessment, background may be considered in different ways. For example, in the case of predicting exposure estimates from information on environmental releases of interest, background estimates may be included as well, and depending on the methodology, the risk estimates may be developed for the aggregate exposure as well as for the different sources of interest, including background. In this approach, differences in bioavailability among the various metal sources should be considered and incorporated into the calculation of total exposure. Alternatively, biomarkers may be used for exposure assessment, which already account for bioavailable fractions. Therefore, while it may not be feasible to partition out contributions specific to the pollutant source or exposure pathway of interest, biomarkers will provide an estimate of aggregate exposure.

4.2.2.1.2. ***Limitations.*** The approach taken for background may depend on the dose-response relationship for the health endpoint of interest. In the case of cancer assessments, where the Agency has derived a probabilistic relationship and the risk management decision framework is particular to the incremental risk of the pollutant source or human activity of interest, the basic background evaluation can simply focus on providing the foundation for that incremental context. Yet for many other health endpoints, the Agency's traditional approach for toxics involves the Reference Dose (RfD) or Reference Concentration (RfC) approach, which may be conceptually described as an uncertainty bound on a No-Observed-Adverse-Effect Level (NOAEL). The NOAEL by itself provides no information about the slope of the dose-response relationship or, therefore, the incremental change in risk expected for an incremental increase in exposure above the background level. Thus, in those cases, it will be more important for the exposure assessment to consider estimates of total, rather than incremental, exposures.

4.2.2.2. *Air Pathways of Exposure*

Air pathway inhalation is the major pathway for human intake of metals in which air serves as the primary medium of contact. Indirect pathways in which air serves as an antecedent medium also include the following: deposition of metals to surface dusts and subsequent intake from ingestion or inhalation; deposition to surface water and sediment and intake from ingestion; and uptake of deposited metals into or onto aquatic and/or terrestrial biota, entrance into the human food chain, and intake from ingestion. Both systemic bioavailability and local actions on nasal mucosa, airways, and lung tissue should be taken into account when considering human health effects incurred via the air pathway.

4.2.2.2.1. ***Applications.*** Most airborne metals, with a few important exceptions (e.g., mercury and arsine) occur in particulate form, which necessitates certain considerations for inhalation exposure assessment. For example, particle size affecting respirability (i.e., how much of the pollutant enters the respiratory system). Additionally, inhalation dosimetry for particles involves some distinctly different processes than for gases (i.e., deposition, clearance, dissolution, etc.), which are also influenced by particle size (U.S. EPA, 1997c, as revised in 2004b). Particle size is thus an important factor in assessing metals exposure, with the focus generally being on particles less than or equal to 10 microns (μ) in diameter (PM_{10}). Larger particles do not generally penetrate far into the respiratory tract and can be cleared to the ingestion route. They may play a larger role in irritant and other effects on eyes and nasal passages, and if deposited in the uppermost reaches of the respiratory tract may be transferred to the digestion tract. Thus, for exposure assessments involving measurements (e.g., using area or personal samples), the size of particles sampled is an important consideration.

For metals for which the Agency has developed RfCs and IURs, the exposure estimate should be for the form of metal used in the dose-response assessment that established the reference values (e.g., exposure concentrations, usually with focus on the respirable fraction) (U.S. EPA, 2004c). For more information on the consideration of particle size in the dose-response assessment for RfCs and IURs, refer to U.S. EPA, 1990. For metals for which the Agency has developed alternative dose-response metrics (e.g., blood lead concentration), respirability, deposition, and clearance as well as absorption into the circulatory system may need to be addressed as part of the exposure assessment.

4.2.2.2.2. ***Limitations.*** In developing inhalation exposure estimates, attention should be given to the form of the metal pertinent to the dose-response assessment (e.g., RfC, IUR). Simply measuring the total amount of a metal without regard to speciation may introduce uncertainties into inhalation exposure estimates, as it can with all exposure routes. Metal speciation affects a range of processes that change how the metal is deposited in the respiratory tract and subsequently distributed throughout the body and, consequently, its potential toxicity (Bailey and Roy, 1994; Oberdoerster, 1992). For example, in assessing the risk of inhaled chromium, the assessor should consider speciation (e.g., Cr+3 vs. Cr+6), as the dose-response assessment includes that specification. The bioavailability of metals via inhalation can be much higher than that of other routes of intake. This may result in relatively high internal doses, even when intakes are similar to those from other routes. An example is the large contribution made by cigarette smoking to the body burden of cadmium (e.g., Friis et al., 1998; Ellis et al., 1979). Variations in airway structure and respiratory conditions (e.g., as with age) may alter the deposition pattern of inhaled particles and contribute to variations in bioavailability (James, 1994; Xu and Yu, 1986; Phalen et al., 1985).

4.2.2.3. *Dust and Soil Pathways of Exposure*

Surface dusts and soil are particularly important media of human contact with metals. Both serve as long-lasting repositories for airborne metal particles, and soils also contain metals due to direct contamination from runoff or mixing with solid wastes. Humans are exposed to metals in surface dust and soil primarily through incidental ingestion or inhalation of suspended dust particles. Dermal contact with metals in soil represents another potential route of exposure, but the relatively low lipid solubility of most metals generally limits absorption through the skin (Paustenbach, 2000; Hostynek et al., 1998). Few studies have actually attempted to quantify the extent or kinetics of dermal penetration of metals deposited on the skin, and the applicability of these studies to metal species and complexes that occur in surface dust or soil is highly uncertain. Therefore, this exposure route is of lesser importance for most metal assessments than the ingestion and inhalation exposure routes.

Infants and children are particularly vulnerable to exposures to metals through the surface dust pathway because (1) their crawling and play activity put them in close proximity to surface dust, and (2) they often mouth their hands (e.g., finger sucking) and objects in their environment. This causes intakes of surface dust that are generally greater than those normally found in adults (e.g., Barnes, 1990). The amount of soil ingested by children can be expected to vary with numerous factors, including age, activity patterns, and accessibility to soil and dust. Data are limited with regard to distinguishing between the quantity of dust ingested and the quantity of soil ingested. This parameter is important in connecting measured soil metal concentrations with surface dust ingestion that occurs in the indoor and outdoor environments (U.S. EPA, 1994b).

Exposure assessment methods for direct soil ingestion is described in the Risk Assessment Guidance for Superfund (RAGS) (U.S. EPA, 1989b; http://www.epa.gov/superfund/programs/risk/ragsa/index.htm). Additional guidance with respect to children (e.g., amount of soil a child may ingest) may be found in the *Child-Specific Exposure Factors Handbook* (U.S. EPA, 2002e).

Few studies of soil ingestion in adults have been conducted; however, the estimates support the general assumption that average daily soil ingestion rates of adults who do not participate in activities in which intensive exposure to surface dust and soil occur (e.g., occupational gardening, construction work) are lower than those of children (Calabrese et al., 1990; Hawley, 1985). Because concentrations of the metal contaminants in soil can be expected to vary with depth, exposure assessments should consider soil metal concentrations at the depth appropriate to the metal(s) of concern and human behaviors and activities. The size of metal-bearing particles also varies with depth. For example, higher concentrations of lead and smaller particles are found near the soil surface (Duggan and Inskip, 1985; Duggan et al., 1985; Fergusson and Ryan, 1984). For review, see Chaney et al. (1988). Because bioavailability of lead and other metals increases with decreasing particle size (U.S. EPA, 2004a; Barltrop and Meek, 1979), both particle size and depth of contamination become very important considerations in metal exposure assessments.

4.2.2.4. *Dietary Pathway*

Food can be a major contributor to human metal exposures from contaminant point sources as well as containing trace amounts of naturally occur metals. Failure to accurately account for the dietary contribution can result in significant errors in exposure and risk estimates for metals (Choudhury et al., 2001). Human dietary exposures to metals may occur through various pathways. Livestock grazing in metal-contaminated areas can take up metals from soils or surface water. Metals also can migrate into surface water and sediments and can be taken up by aquatic organisms that are consumed by humans. Human food crops also can take up metals from soils and surface water or become contaminated with metals through deposition of airborne particles. Metals can enter food during harvesting or processing of produce and livestock, during food storage from metals in food containers, and during preparation of foods for meals.

4.2.2.4.1. *Application*. Estimation of intakes of metals in food requires information or estimates on the levels of the metal in food and the amount of food consumed. Although large-scale surveys of the metal contents of foods and food consumption patterns have been conducted (e.g., Egan et al., 2002; Ryan et al., 2001; U.S. FDA, 2001; O'Rourke et al., 1999; Thomas et al., 1999; U.S. DHHS, 1996), these surveys have several limitations for applications to risk assessment at a contaminated site. Analysis is often conducted with "market basket" samples of packaged processed foods. With few exceptions, such applications have not been empirically evaluated against biomarkers of exposure (Clayton et al., 2002, 1999; Choudhury et al., 2001). For some risk assessments, multimedia fate and transport models may be used to predict concentrations in locally grown or raised foods or wildlife consumed by the population of interest.

4.2.2.4.2. ***Limitations.*** Because some metals (e.g., cadmium and lead) could have long residence time in the body, reconstructing historic dietary exposures can be a challenge. Food consumption surveys generally are limited to short-term consumption (e.g., 1 to 3 days) and do not capture intra-individual variability that would affect long-term averages. Furthermore, food consumption patterns can be expected to change over time; thus, patterns discerned at any given time may not accurately represent historical exposures. An additional challenge is the integration of data from separate metal residue surveys and food consumption surveys (e.g., Tomerlin et al., 1997). This leads to considerable uncertainty in estimates of metal exposure through the dietary route.

Furthermore, estimates of dietary intakes of metals in food based on national or regional data cannot be expected to accurately reflect intakes of metals in locally harvested foods owing to differences in natural background levels of metals. This can be a particularly important limitation when the receptors of concern are subsistence fishermen or hunters. These surveys also may not accurately reflect the amount of metals that enter the food pathway during local food preparation or storage (e.g., in the home).

4.2.2.5. *Water and Sediment Pathway*

Part of the human population obtains drinking water from untreated sources, such as wells. However, people also consume water specifically treated for human consumption. Treatment of ambient water for human consumption removes DOC and suspended organic sediments that can form complexes with metals (AWWA, 1999). Thus, the exposure context of metals in human drinking water will be very different from that of ambient water. That is, inorganic forms of metals in treated drinking water will consist of the more bioavailable, water-soluble species. Treatment also removes bacteria that can participate in organification reactions of toxicological significance to humans (e.g., methylation of inorganic mercuric mercury).

4.2.2.5.1. ***Applications*****.** Estimation of the intake of metals in drinking water requires information about or estimates of concentrations of metals in the water and the amount of water consumed. Data on the metal content of tapwater can be obtained from EPA's Office of Drinking Water (U.S. EPA, 2000d). EPA's *Exposure Factors Handbook* contains exposure information on daily drinking water ingestion and incidental ingestion of water during swimming and showering (U.S. EPA, 1997h). People also can be exposed to metals dissolved in ambient surface water or in association with suspended sediments; the latter can serve as a long-term repository for waterborne metal particles. Such exposures occur during swimming or other recreational activities as a result of incidental water ingestion or during occupational activities in which the sediments are disturbed or resuspended in the water column. Metal bioavailability in ambient surface water can be expected to be much more diverse than in treated drinking water or in ground water because of the presence of organic carbon, inorganics, and suspended organic material that can serve as ligands or reactants for metals. Speciation and concentration will also vary with pH of the surface water. Therefore, estimation of intake of metals from surface water will require appropriate adjustment for relative bioavailability.

4.2.2.5.2. ***Limitations*****.** Metals can enter treated drinking water at various stages of water treatment, distribution, or delivery. Generally, water metal concentrations are measured at the distribution point for municipal water delivery systems. Distribution systems within homes (pipes, storage containers, etc.) and, in the case of lead, glassware, can contribute metals to the water (Graziano et al., 1996). The contribution of metals from pipes (either from the distribution system to the home or within the home) is rarely assessed. This can be highly variable, both within the system and temporally; water that remains in pipes overnight frequently has a higher metal load than water used during the day. Furthermore, the concentration of organic material and other ligands in the water may vary across drinking water sources and can affect the bioavailability of the metal. There is also wide variability in the form of metals in ambient surface water. These factors can be incorporated into site-specific assessments, but local data should be collected on a case-by-case basis. Inhalation of inorganic metal contaminants in water can result from aerosolization, or in special cases, from volatilization (e.g., Hg^0). The aerosolization pathway can be a major source of intake of inorganics under certain conditions.

As an example of aerosol exposure, sea spray can be a significant contributor to iodide intakes in populations that live near the seashore (Whitehead, 1984). A pathway more typical of human exposure to contaminated water is showering, in which areosolization can occur at the water tap. Although models have been developed to predict human inhalation exposures to volatile organics from showering (e.g., Moya et al., 1999; McKone, 1987) (see Guo, 2002, for review), comparable models do not exist for aerosolized metals (Wilkes, 1998), and the magnitude of exposure from showering is unknown.

4.2.3. Routes of Entry

The most frequently encountered routes of entry of metals into humans are ingestion and inhalation. The dermal route is of less concern for most metals but can be important for skin toxicity of some metals (e.g., nickel, chromium). Other routes can be important in specific circumstances, for example, explosions of metal-bearing materials can result in intra- or trans-dermal exposures (Robinson et al., 1983). It is also important to remember that metals can produce toxicity at the point of entry. While metal absorption from skin may be minimal, dermal irritation and sensitization can occur without absorption. Similarly, human health effects such as lung disease and lung cancer can occur from inhalation exposures that do not result in substantial systemic uptake. Routes of entry do not necessarily correspond to the expected exposure pathway. For example, uptake of airborne metals associated with larger particles can actually be attributed to ingestion of surface dust rather than absorption from the respiratory tract. The significance of this process relative to other pathways will depend on the exposure scenario generally being less significant than diet or soil ingestion pathways (see sections 4.2.1 and 4.2.2.2). Thus, uptake in the lung can be the source of exposure by two routes.

Although dermal contact with metals occurs through soil, air, and water pathways, the relatively low lipid solubility of most metals limits absorption through the skin (Paustenbach, 2000; Hostynek et al., 1998). An exception is Hg^0, which is dermally bioavailable (Hursh et al., 1989). In general, empirical information on dermal absorption of metals should be consulted when available (Stauber et al., 1994; Hostynek et al., 1993; Wester et al., 1992; Hursh et al., 1989; Ilyin et al., 1975).

4.2.4. Integrated Exposure Approaches

Approaches to integrating exposure across pathways and physiological routes of uptake include modeling, relative bioavailability estimates, and biomarker assessment.

4.2.4.1. *Modeling*

Few specific exposure models have been developed for metals with the exception of lead. The Integrated Exposure Uptake Biokinetic (IEUBK) model for lead in children (White et al., 1998; U.S. EPA, 1996c, 1994b) was specifically developed for translating exposure measurements into risk estimates at sites contaminated with lead. The IEUBK model and background documentation are available on line at http://www.epa.gov/superfund/programs/lead/ieubk.htm. The IEUBK model assumes that 10 µg/dL is a no effect level, while current science indicates there may be no safe level of lead exposure. The model is not readily generalized to other metals because blood lead levels are used as the dose metric, and dose-response assessment for most other metals use estimates of oral or inhalation intake rates.

A stochastic human exposure model for lead that is linked to a lead pharmacokinetics model (O'Flaherty et al., 1995) has also been developed (Beck et al., 2001). Less complex models linking adult exposures and blood lead concentrations are available as well (Carlisle, 2000; Stern, 1996, 1994; U.S. EPA, 1996c; Bowers et al., 1994; Carlisle and Wade, 1992). These models have not been reviewed by the Agency authors. An exposure model for arsenic has also been reported (Cohen et al., 1998).

Other general exposure models used in risk assessment can be potentially applied to metals, including population-based models. The EPA's Stochastic Human Exposure and Dose Simulation (SHEDS) model is a probabilistic, physiologically based model that simulates aggregate human exposures and doses (i.e., via inhalation, dietary, dermal, and nondietary routes) for population cohorts and multimedia, multipathway chemicals of interest (Zartarian et al., 2000). EPA recently has applied the SHEDS model to estimate arsenic exposure of children from chromated copper arsenate (CCA)-treated wood (Dang et al., 2003). EPA has also developed a Dietary Exposure Potential Model (DEPM) that links national food consumption and chemical residue data to allow estimates of average dietary intakes of metals and other food contaminants (Tomerlin et al., 1997). EPA's Total Risk Assessment Methodology (TRIM) is also being developed for multipathway risk assessment for air pollutants, including metals (See http://www.epa.gov/ttn/fera/trim_gen.html). A generic exposure model, RESRAD, developed by the U.S. Department of Energy for risk assessment of radionuclides (U.S. DOE, 2001; LePoire et al., 2000), includes an extensive human exposure module applicable to other metal contaminants.

4.2.4.2. *Relative Bioavailabililty*

Evaluating metals bioavailability can significantly reduce uncertainty in human health risk assessments and more accurately characterize potential risks to exposed populations.

4.2.4.2.1. ***Discussion***. Evaluating bioavailability is important, because a given dose of a metal in an environmental medium may be absorbed to a different extent than the same dose administered in the study used to derive a toxicity value (e.g., oral RfD or cancer slope factor (CSF)). In addition, it is usually assumed that the bioavailability of all metal species is the same, regardless of exposure media. Studies clearly show that the bioavailability of metals does vary by environmental medium and the species present. Therefore, bioavailability information can significantly reduce uncertainty in risk assessments and more accurately characterize potential risks to exposed populations.

The EPA Office of Solid Waste and Emergency Response (OSWER) has used bioavailability information in making quantitative adjustments. In 1989, OSWER published a risk assessment guidance for use in human health risk assessments at Superfund sites and has updated this guidance periodically (U.S. EPA, 1989b). This guidance recognizes that the toxicity of an ingested chemical depends on the degree to which it is absorbed from the gastrointestinal (GI) tract into the body. Thus, adjustments to bioavailability assumptions were developed to account for differences in absorption efficiencies between the medium of exposure and the medium from which the toxicity value was derived. Further, because RfDs and CSFs are generally expressed in terms of administered dose rather than absorbed dose, it also discusses the need to adjust for differences in the expression of dose between the exposure and toxicity value (e.g., absorbed vs. administered dose). The Agency guidance recommends that the relative bioavailability adjustment (RBA) of a chemical should be assumed to be equal in food, water, and soil in the absence of data to the contrary.

Estimating bioavailability of metals is particularly difficult because it is dependent on many variables, including the physical and chemical form of the metal, the physical and chemical characteristics of the association between the metal and soil particles, particle size of metal species, and the metal source. In addition, metal species continuously undergo reactions in soil, referred to as “aging or weathering,” that affect bioavailability.

4.2.4.2.2. ***Current Practice***. The Agency currently addresses bioavailability through the use of default values and, in some cases, through the development of site-specific and medium-specific values. To date, the most common treatment of bioavailability for human health assessments is to assume that the bioavailability of the metal exposure from the site is the same as the bioavailability of the source used to derive the toxicity value (RfD or CSF). The RfD and CSF are typically developed from laboratory toxicity tests using highly bioavailable forms and are usually based on administered rather than absorbed doses. This is true for all chemicals, but it is of special importance for ingested metals because metals can exist in a variety of chemical and physical forms, and not all forms of a given metal are equally well absorbed. For example, a metal in contaminated soil may be absorbed to a lesser extent than when ingested in drinking water or food.

It is important to recognize that a default RBA value of 1 (100%) is not necessarily conservative (i.e., more protective of human health). The bioavailability of the metal in the exposure medium of concern at the site may actually be greater than in the exposure medium used in the critical toxicity study that formed the basis of the RfD or CSF. If this is the case, assuming RBA of 1 for the medium of concern would result in an underestimate of risk at the site. The Agency recognizes that some cases may exist where sufficient data are available to support development of medium-specific default absorption factors for a particular chemical. The purpose of these medium-specific and chemical-specific default values is to increase the accuracy of exposure and risk calculations even when site-specific studies are not available.

Lead is an example of a chemical for which the Agency has established medium-specific default absorption factors for both children and adult populations. The IEUBK model for lead in children predicts PbB concentrations for a hypothetical child or population of children (birth to 84 months of age) resulting from exposure to environmental sources of lead, including soil, dust, air, drinking water, and diet (U.S. EPA, 1994b; White et al., 1998). An assumption in the model is that the ABA of lead in soil and dust, at low intake rates, is 0.3 (30%) and the ABA of soluble lead in water and food is 0.5 (50%). This corresponds to an ABA of 0.6 (60%) for lead in soil (or dust) compared to lead in water or food. The model also allows for the input of site-specific values.

The Agency has developed the Adult Lead Methodology (ALM) for assessing lead risks in adult populations (U.S. EPA, 1996c). An assumption in the ALM is that the ABA of lead in soil is 0.12 (12%). This value is based on assumptions that the ABA of soluble lead in water is 0.2 (20%) and that the RBA of lead in soil, compared to soluble lead, is 0.6 (60%).

The Agency has also derived RfDs that are specific for an exposure medium, based on consideration of bioavailability or other factors that might suggest unique dose-response relationships in that medium. For example, separate RfDs for cadmium in food and drinking water have been derived through the rationale that the bioavailability of cadmium in water is greater than that of cadmium in food by a factor of 2 (i.e., 5% vs. 2.5%, respectively [U.S. EPA, 2003f]). Similarly, the Agency recommends that a modifying factor of 3 be applied to the chronic oral RfD for manganese when the RfD is used to assess risks from drinking water or soil to account, in part, for potential differences in bioavailability of manganese in water and soil compared to that in food (U.S. EPA, 2003g). Therefore, use of default values should not substitute for site-specific assessments of bioavailability, where such assessments are deemed feasible and valuable for improving the characterization of risk at the site.

As described in the NRC report (NAS, 2002), a variety of tools are available to attempt to measure bioavailability. The approaches include biomarkers of exposure (e.g., ALA activity from lead exposure), cell culture studies, isolated GI tract tissue, whole-animal approaches, and clinical studies. Of these options, the use of whole animals is most feasible (Weis and Lavelle, 1991); clinical studies offer desirable advantages but present many obstacles (Maddaloni et al., 1998). The following discussion focuses on the oral route of exposure.

While a variety of tools are available to attempt measurement of bioavailability, the use of ***whole-animal approaches*** are the most feasible.

4.2.4.2.3. *Animal Models.* Historically, a variety of experimental animal models (in vivo) have been used to evaluate bioavailability, including rats, rabbits, monkeys, guinea pigs, and swine. Within an animal model, evaluation of bioavailability has included measuring the amount of metal in blood, body tissues, or excreta (e.g., feces and urine). The appropriate study design is dependent on the pharmacokinetics of the metal in the animal model and differences between the selected animal species and humans. In other words, it is important to consider how soluble forms of the metal are absorbed, how the metal is excreted, and whether there are any tissues where the metal might accumulate. The most common methods for measuring bioavailability in vivo are blodd, urine, fecal and tissue measures.

The principal advantage of whole-animal oral chemical absorption studies is that they measure bioavailability in its most clinically relevant form, that is, from the GI tract and into the systemic circulation. This integrates all of the relevant biological components related to systemic absorption, including pre-systemic elimination, if present. By using the animals as surrogates for humans, these studies avoid the experimental and ethical problems associated with the use of human subjects. Currently, certain in vivo bioavailability studies conducted with an appropriate species are considered the gold standard for developing bioavailability information suitable for use in quantitative human health risk assessments, and they are often used to validate other bioavailability tools. For example, the young swine model for lead bioavailability has been used to validate in vitro extraction tests.

The ***juvenile swine model*** is presently being used as the preferred animal model by EPA for making site-specific bioavailability adjustments for humans exposed to lead in soil. Site-specific bioavailability adjustments based on successful application of the model have been accomplished at several sites across the US.

4.2.4.2.3.1. *Animal Model Applications*. Animal models have served as the basis for making site-specific bioavailability adjustments at several Superfund sites. Scientists from EPA Region 8 sponsored the development of whole-body *in vivo* bioavailability studies in juvenile swine as a model of young children who were exposed to lead in soil contaminated with various forms of mine wastes (Lavelle et al., 1991; Weis et al., 1992, 1993a). The results of these efforts were subjected to outside peer review and found to be valid and acceptable for use in adjusting the RBA for lead in human health risk assessments. As a result, the juvenile swine model is presently being used as the preferred animal model for lead (U.S. EPA, 2004a). Site-specific bioavailability adjustments based on results from the juvenile swine model have been accomplished at several sites across the country, including the Murray Smelter in Colorado; Palmerton, PA; Jasper County, MO; Smuggler Mountain, CO; and the Kennecott site in Salt Lake City, UT.

Interim draft guidance has also been developed by EPA Region 10 for making bioavailability adjustments with arsenic-contaminated soil (U.S. EPA, 2000a). Recommendations are based on literature data on arsenic bioavailability and the results of a Region 10 animal study in which immature swine were dosed with arsenic-contaminated soil derived from the Ruston/North Tacoma Superfund site, which was a former smelter site (U.S. EPA, 1996d). This interim guidance recommends default values of RBA for arsenic in soil ranging from 60 to 100%, depending on the source of contamination (e.g., mineral processing, fossil fuel combustion, pesticides/wood treatment processes). As with lead, the juvenile swine is the recommended animal model for supporting departures from the default RBA assumptions.

Animal models are generally complex, time consuming, and more resource intensive than other tools, which may limit their feasibility to large sites where it is difficult to adequately characterize variability in bioavailability across the site.

4.2.4.2.3.2. *__Animal Model Limitations__*. Currently, bioavailability studies conducted with an appropriate animal species are considered the most reliable for making quantitative bioavailability adjustments in human health risk assessments. They are also used as a means to validate other tools, such as physiologically based extraction tests. However, animal models are generally complex, time consuming-, and much more expensive than other tools. As a result, use of animal models is usually limited to large sites where it is difficult to adequately characterize the variability in bioavailability across the site. An investigator must ensure that the study design and animal model selected are appropriate for the metal being tested. The best measure of bioavailability (blood vs. tissue vs. feces vs. urine) is dependent on the pharmacokinetics of a particular compound. As discussed above, each measure has its own inherent limitations. Further discussion of the limitations of animal models are discussed elsewhere (NAS/NRC, 2002; NFESC, 2000a, b).

4.2.4.2.4. *In vitro Methods.* Recently, significant effort has been expended on developing *in vitro* methods for assessing the RBA of metals, due to their ease of use and potential cost savings when compared against more traditional *in vivo* methods using laboratory animals. Several researchers have investigated *in vitro* models that attempt to simulate the conditions in the GI tract (Drexler et al., 2004; Medlin, 1997; Rodriquez et al., 1999; Ruby et al., 1993, 1996, 1999) and which are often referred to physiologically based extraction tests. These methods are based on the concept that the rate and/or extent of metals solubilization in GI fluid is likely to be an important determinant of metals bioavailability *in vivo*. These assays provide a measure of bioaccessibility or the amount solubilized in the GI fluid and available for potential absorption (Ruby et al., 1993).

Model development has focused on the simulation of complex physiological and biological functions within the GI tract, including considerations of pH, solids:fluid ratios, motility/transit, and solution chemistry. The most common approach has been the two-solution method, which addresses pH changes in the GI tract by providing an exposure to both the low pH of the stomach (1.3 to 3) and the higher pH of the small intestine (5.5 to 7). Solution pH is usually maintained either by titrations with drop-wise addition of acid or base while solutions are continuously monitored or by using buffers.

The solids:fluid ratio used for *in vitro* models has ranged from 1:10 to approximately 1:150 (g/mL). None of these ratios reflects the 2:1 ratio observed in adults (3000 g daily food intake vs. the 1500 mL stomach volume) (Washington et al., 2001). It is recommended that this ratio be dictated by practical considerations. Therefore, a sample mass that can be accurately weighed and is representative should be provided, along with a volume that can help maintain good particle-to-solution contact and minimize any unusual kinetics.

Motility and transit time within the GI tract are difficult to model with standardization. Both processes vary greatly and can be affected by diet and daily cycles. Historically, investigators have used either diffusers, stirrers, or rotation devices to mimic these factors. All methods are adequate; however, the diffuser system is difficult to control and clean. Also, rotation mechanisms are not favorable to techniques that require constant pH monitoring.

Variations observed in extraction fluid chemistry are by far the greatest source of method deviations. Although most methods have the gastric solution dominated by HCl, other acids, proteins, and peptides have been added, with extraction times of about 1 hour. Intestinal solutions have their pH adjusted by addition of sodium bicarbonate and/or other biological salts and are extracted for 3 to 5 hours. Most systems were maintained at a temperature of 37 °C, and some methods have used argon to maintain anaerobic conditions, even though the GI tract is aerobic in humans.

4.2.4.2.4.1. ***Applications.*** Physiologically based extraction tests have been conducted for a variety of metals, including lead, arsenic, cadmium, nickel, and mercury. Model results are currently being used as a screening tool only until adequate model validation has occurred. To date, EPA has not endorsed the use of *in vitro* techniques for making site-specific bioavailability adjustments.

4.2.4.2.4.2. ***Limitations.*** In vitro methods are clearly significantly cheaper and less time consuming than standard *in vivo* studies. However, these extraction techniques are based on the premise that solubility or bioaccessibility is the primary factor controlling bioavailability, which is not necessarily the case. In addition, these tests cannot reflect the complex physiological or pharmacokinetic aspects of human absorption.

One of the key limitations of this approach is adeptly defined in NAS/NRC (2002):

> Regulatory acceptance of the tools used to generate bioavailability information in risk assessment is expected to be influenced by several factors, including the relevance of the tools to the site conditions and the extent of tool validation. Validation variously refers to the performance of a tool or approach in terms of reproducibility, reliability, and multi-lab calibration. An appropriate body of experimental work to validate a tool would (1) clarify where and when a tool yields a definitive response; (2) clarify that the tool can be linked to a biological response of a similar magnitude, and that the linkage stands up across a range of conditions in the type of environment that is being managed; (3) test the prediction of bioavailability using different types of experiments and field studies; (4) clarify which types of biological responses are best predicted by the approach; and (5) include critiques of the best applications and the limits of the tool, especially compared to alternatives. A tool that is well accepted and validated should be given greater weight than one that is new or experimental.

OSWER is currently developing a bioavailability document on metals to advise risk assessors and managers on whether to collect site-specific information on the bioavailability of metals in soil and how to evaluate bioavailability data for use in human health risk assessments. The document will outline a decision framework that explains how to use bioavailability data consistently as part of a human health risk assessment. The decision framework will consist of a two-tiered approach. The first tier presents general guidelines for determining whether bioavailability is worth considering at a particular site. The second tier involves an ordered process for the actual collection and analysis of bioavailability data.

4.2.4.3. *Biomarkers*

Integration of exposures across media, route, and time of exposure can be reflected in biomarkers of exposure. The World Health Organization (WHO/IPCS, 1993) defines a biomarker of exposure as "an exogenous substance or its metabolite or the product of an interaction between a xenobiotic agent and some target molecule or cell that is measured in a compartment within an organism." Ideally, 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 also can 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.

4.2.4.3.1. ***Application*****.** In the case of metals, urinary cadmium and blood lead are examples of exogenous substances used as biomarkers of exposure. The measurement of metals in biological fluids has been used as 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) fall into the area of transition in the continuum from exposure to effect.

For many of the metals of interest, biomarkers of exposure and effect are used as basic tools for population or molecular epidemiology studies of effects of exposure to humans of various metals. The Centers for Disease Control and Prevention (CDC) conducts an extensive biomonitoring program of human blood and urine that includes lead, mercury, cobalt, uranium, antimony, barium, beryllium, cesium, molybdenum, platinum, thallium, and tungsten (CDC, 2003). The data are summarized in age, gender, and ethnicity categories.

4.2.4.3.2. ***Limitations*****.** A biomarker of exposure is a measure of cumulative exposure to a metal and also of metal actually existent in the body, 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. Failure to consider background dietary sources of metals may result in a misinterpretation of the exposure. For example, arsenobetaine is a nontoxic 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 correct frequency and timing of sampling of biological fluids and tissues, as well as the correct interpretation of the results, depend 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 whether an acute exposure to lead occurred.

The validity of a biomarker is supported by three kinds of relevance: analytical, toxicokinetic, and biological (Schulte and Talaska, 1995; Grandjean et al., 1994; WHO/IPCS, 1993). Key analytical issues include specificity, sensitivity, standardization of methodologies (to reduce intra- and interlaboratory variability), speciation, quality assurance, and the availability of reference samples. Analytical methods for the detection of metals include ICP-MS, hydride generation atomic absorption, and fluorescence spectrometry. When coupled with high-performance liquid chromatography, these methods are enhanced because of the ability to detect speciated parent metal and metabolites. Although these methods can be very reliable for the analysis of metals in biological fluids, using them for tissue analysis is more difficult. Digestion and extraction make it difficult to fully speciate the metal and produce interfering matrix factors. Reference standards for tissues are seldom available. 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 noninvasive in vivo techniques; however, the sensitivity is extremely limited. A disadvantage of any in vivo method is that the metal species in the environmental exposure cannot be estimated correctly.

4.2.5. Toxicokinetics and Toxicodynamics

Several specific properties of metals that affect their absorption, distribution, metabolism, and elimination can be identified. These properties of metals can influence their physiological handling as compared to organics (Table 4-10) and should be taken into account in pharmacokinetic analyses related to human health risk assessment. Properties that affect toxicodynamics should also be taken into account in these analyses.

Table 4-10. Metal (versus organic) compound properties affecting absorption, distribution, metabolism, and elimination

Organic compounds	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 forms.	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 to 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.

The evaluation of toxicodynamics addresses the sequence of biochemical events at the cellular and molecular levels that begin when toxicologically active form of the metal interacts with the target (e.g., from molecule to a protein, enzyme, or other cellular molecule, that leads to a toxic physiological response. Toxicodynamics involves the biological processes that underlie the severity of an effect as well as its reversibility, recovery, and adaptive response. These evaluations are applied for single metals as well as for metal mixtures, recognizing that one metal can induce multiple effects (from critical to secondary and more, as the exposure levels increase) and common effects can be exerted across many metals. To assure that effects are appropriately combined, it is important to reflect the underlying mechanism or mode of action; the potential influence of toxicokinetic processes should be considered (e.g., changes in gastrointestinal absorption and liver and kidney retention can change liver and red blood cell functions).

Two properties of metals, their hydrophilic nature and their characteristic protein binding, are discussed in more detail below, followed by a discussion of available physiologically based pharmacokinetic (PBPK) models.

4.2.5.1. *Hydrophilic Properties of Metals*

Solubility in aqueous media is one of the major factors influencing absorption of metals and metal compounds. The water 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 Section 4.1). 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 when embedded in body tissues, but metallic mercury swallowed into the gastrointestinal tract is not soluble (Goyer and Clarkson, 2001).

The relative hydrophilic nature of metals versus organic compounds influences absorption at different sites. Absorption of metals in the gastrointestinal tract is hindered by the lipid nature of intestinal cell membranes but is favored by solubility 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. Elemental mercury and dimethyl mercury (Siegler et al., 1999) are notable exceptions. When dermal absorption does occur, the mechanism may differ between organic substances and metals. 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).

Hydrophilic metal ions do not readily diffuse into richly lipophilic tissues, such as the brain, liver, and neutral fat stores, where they would be difficult excrete. Although biotransformation of metals occurs, the products typically maintain a hydrophilic profile. Entrance of metals or inorganic metal compounds into lipid-rich tissues like the brain depends on hydrophilic pathways. 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).

Although the low lipid solubility of metal ions 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 should be assessed in terms of its ability to access transporters and of the presence of transporters in potential target organs. Furthermore, complex lipids can offer high-affinity binding sites for metal ions that promote their distribution to lipid compartments, and some metals, such as thallium, have a demonstrated affinity for adipose compartments.

Generally, the primary factor that influences the uptake and distribution of organic substances within the body is the substances' lipophilicity. Passage through cellular membranes and partitioning to organs occurs primarily through passive diffusion through the lipid portions of cellular membranes. Metabolism (or lack thereof) can, of course, influence the distribution and excretion of organic chemicals primarily because of the accompanying changes in chemical structure and, therewith, lipophilicity. The uptake, distribution, metabolism and excretion of an organic chemical can often be predicted by consideration of the substance's chemical structure and lipophilicity (i.e., octanol/water partition coefficient).

In contrast to organic substances, the uptake and distribution of metals in inorganic forms is generally influenced by atomic size and charge, and the availability of active cellular processes that naturally exist for the uptake and distribution of nutritional metals (e.g., calcium, sodium, potassium, magnesium) that can also transport other metals into or across cell membranes. The assumption of linear partitioning in tissues commonly applied to organics, and which is largely lipophilicity-based, is therefore not appropriate for the capacity limited processes that generally control the uptake and disposition of metals. Rather, the uptake, distribution, metabolism, sequestration, and mechanisms of action of metals is generally considered in the context of their kinetic behavior.

Metals can have residence times in the body as long as months or years, particularly when they are bone-seeking elements or are associated with tissue storage proteins. Metals, such as lead, strontium, and uranium, may be incorporated into bone, stored there, and many years later may be released from the bone tissue into the systemic circulation to sites of toxic action, by mechanisms closely linked to bone metabolism. These mechanisms include incorporation and loss with bone formation and resorption and, depending on the ionic radius and charge of the metal, can include migration within the bone complex as well as out of the bone (O'Flaherty, 1998). While long-term deposition of a metal (e.g., lead) in a tissue (e.g., bone) may not necessarily result in toxicity to that tissue, subsequent release of that metal from that tissue enables transportation of the metal to its site of toxic action elsewhere in the body. It is important for the risk assessor to consider the potential toxicological consequences of storage or deposition of metals in body compartments as part of the overall human health assessment.

Modeling the kinetics of metals with extended tissue residence times presents challenges in that anatomical and physiologic functions, along with changes in exposure, need to be characterized as a function of age and clinical status. For example, bone turnover is rapid in children and relatively quiescent in adults, but is increased in adults during conditions such as menopause, pregnancy, and lactation, to name a few. Ninety to ninety five percent of the adult human body burden of lead is contained in the bone. However, lead is not permanently fixed in bone tissue, but returns to the blood as bone is resorbed and by return from the bone surface (O'Flaherty, 1998). Gulson et al (1995) observed that between 45 and 75 percent of blood lead in a group of adult women had originated from the bone. Physiologically based kinetic models have been developed or are in the process of development for arsenic, lead, chromium, and mercury (O'Flaherty, 1998).

4.2.5.2. *Metal-Binding Proteins*

Metals react with many different proteins in the body that may modify kinetics. Many metals bind with albumin for purposes of transport in the circulatory system and across cell membranes and within cells. However, research is identifying a growing number of proteins that play specific roles in transport, cellular uptake, and intracellular storage of metals (Goyer and Clarkson, 2001), including the following:

- **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.

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

- **Membrane carrier proteins.** A number of recently discovered carrier proteins transport metals across cell membranes. Many of these carrier proteins are multispecific (e.g., divalent metal transporter 1 and 2, metal transporter protein 1), Some metals are transported as complexes with endogenous ligands; no transport systems are intended for the ligand itself, accepting substrates that vary considerably but are recognized by the attached metal ion (Dawson and Ballatori, 1995).

- **Metallothioneins.** The metallothioneins are a group of low-molecular weight (MW) 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 metals bound to metallothioneins differ depending on the species, the organ, and previous exposures to metals, but most of them contain at least two different types of metals. For example, metallothioneins isolated from adult or fetal human livers contain mainly zinc and copper, and 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). Although 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.

- **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 because it binds a number of metals, including cadmium, zinc, beryllium, and aluminum.

- **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).

4.2.6. Pharmacokinetic/Pharmacodynamic Modeling of Metals

Physiologically based pharmacokinetic/physiologically based pharmacodynamic (PBPK/PBPD) modeling of metals entails the mathematical description and modeling of a substance's behavior in the body (e.g., absorption, distribution, metabolism, excretion, and toxic effects). PBPK and PBPD models are valuable risk assessment tools for interspecies, high-dose/low-dose, route-to-route, and exposure scenario extrapolation (Krishnan and Andersen, 1994). PBPK models consist 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. PBPK models that include a fetal compartment are particularly valuable for human metal risk assessment. In utero exposure can be important for establishing the body burden of certain metals before birth that result from transplacental transfer.

PBPK models have historically been developed and used for risk assessment of volatile organic compounds (VOCs) (e.g., methylene chloride) (Andersen et al., 1987) but have also been applied to many metals (White et al., 1998; Clarke, 1995). Metals differ in their kinetic behavior from VOCs in a number of ways, as discussed by O'Flaherty (1998). Guidelines exist for model evaluation, and these should be used as a framework to determine whether a particular model is appropriate for use in risk assessment (Clark et al., 2003; Andersen et al., 1995).

4.2.6.1. *Application*

Combined use of PBPK and PBPD models provides understanding of the complex relationships between exposure and target organ effects. PBPK models are often capable of predicting aggregate exposures for comparison to exposure models.

For metals that have long retention times in tissues, the maternal tissues can serve as a reservoir for exposures during fetal development. This can be a particularly important exposure pathway for metals that accumulate in the inorganic matrix of bone (e.g., lead, strontium, uranium) because mobilization of bone minerals to develop the fetal skeleton can result in a transfer of maternal bone stores of metals to the fetus (e.g., Gulson et al., 1999a, b; Tolstykh et al., 1998). Transplacental exposures cannot be directly measured from environmental measurements but require the use of PBPK models. A few models of transplacental transfer of lead in humans have been developed; models for other metals are not available for use in risk assessment. The lead models reported to date are limited in that they rely on assumptions of a steady state between maternal and fetal blood lead concentrations (U.S. EPA, 1996c, 1994b; Leggett, 1993; O'Flaherty, 1993). This assumption will be violated if the mother is no longer exposed to lead and if the fetal exposure is due to remobilization of lead from the mother's bones. Furthermore, if the mother experiences lead exposures for the first time during pregnancy, the lead will be partitioned among the various body compartments (bone, hair, blood, fetus) in a dynamic manner.

When using PBPK models or other dosimetric adjustments in the risk assessment process for metals, one should explicitly consider the absorption/distribution and kinetic factors discussed above. There are special considerations for cellular uptake, interaction with nutritionally essential and nonessential metal, protein-binding behavior and function, incorporation into bone, metabolism, and excretion as outlined in Table 4-11.

Table 4-11. Kinetic factors to consider when evaluating the use of PBPK models or other dosimetric adjustments in the risk assessment process for metals in humans

Cellular uptake	‣ Carrier-mediated uptake (e.g., phosphate or sulfate transporters) ‣ Facilitated transport in the form of organic complexes
Interaction with 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 behavior and function	‣ 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
Incorporation into 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)

Pharmacokinetic models for use in human metal risk assessment incluide three models for lead. 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; subsequent exchanges between blood plasma, liver, kidney, and richly and poorly perfused tissues; and excretion from liver and/or kidney (O'Flaherty, 1995). The IEUBK model was developed by EPA for predicting lead levels in children (U.S. EPA, 1994b). The Leggett model allows simulation of lifetime exposures and can be used to predict blood lead concentrations in both children and adults (Leggett, 1993). 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).

4.2.6.2. *Limitations*

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.

In addition to kinetic factors, 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 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 the dose metric selection. A critical consideration will be to match the toxic endpoint with the active form of the metal in cases where sufficient data exist to suggest that there are one or more active forms of the metal or metalloid (e.g., arsenic).

4.3. HUMAN HEALTH EFFECTS

4.3.1. Introduction

Issues of importance when conducting the Effects Assessment (dose-response determination) for human health risk assessments for metals and metal compounds are discussed in this section. The discussion provides some of the scientific basis that underlies metal-specific characteristics of human health effects assessment, but it is not intended to be a comprehensive review. Further detailed discussions on the points raised here can be found in Goyer et al. (2004). Suggested approaches for including metal-specific information in risk assessments are intended to complement other general Agency guidance (e.g., Carcinogen Risk Assessment, Exposure Assessment (U.S. EPA, 2003), Developmental Toxicity (U.S. EPA, 1991).

4.3.2. Essentiality Versus Toxicity

Seven elements are designated as nutritionally essential for humans by the National Academy of Sciences (NAS) (Table 4-12, first column). The categorization as an "essential nutrient" includes the identification of Recommended Dietary Allowances (RDAs) or Adequate Intakes (AIs) by the Food and Nutrition Board (FNB) of the NAS. The FNB has also examined the possible beneficial effects of other elements (Table 4-12, second column), which have not yet been categorized as essential (or not) (NAS/IOM, 2002). The extent to which these elements can currently be considered beneficial to humans and animals varies and is discussed in Goyer et al. (2004) and in NAS/IOM (2001, 2002).

Table 4-12. Metals classified by their known essentiality

Nutritionally essential metals	Metals with possible beneficial effects[a]	Metals with no known beneficial effects
Cobalt Chromium(III) Copper Iron Manganese (animals but not humans) Molybdenum Selenium Zinc	Arsenic Boron Nickel Silicon Vanadium	Aluminum Antimony Barium Beryllium Cadmium Lead Mercury Silver Strontium Thallium Tin

[a] Possible beneficial effects in some physiological processes and some species as reviewed by the Food and Nutrition Board (FNB) (NAS/IOM, 2000). Beneficial effects have been posited for other metals but have not been officially reviewed by the FNB.

Table 4-12 (third column) also lists some metals of interest to the EPA; the FNB has not yet reviewed their status as essential/beneficial nutrients. This category includes the metals lead, mercury, and cadmium. Dose-response curves for essential elements are shown in Figure 4-4.

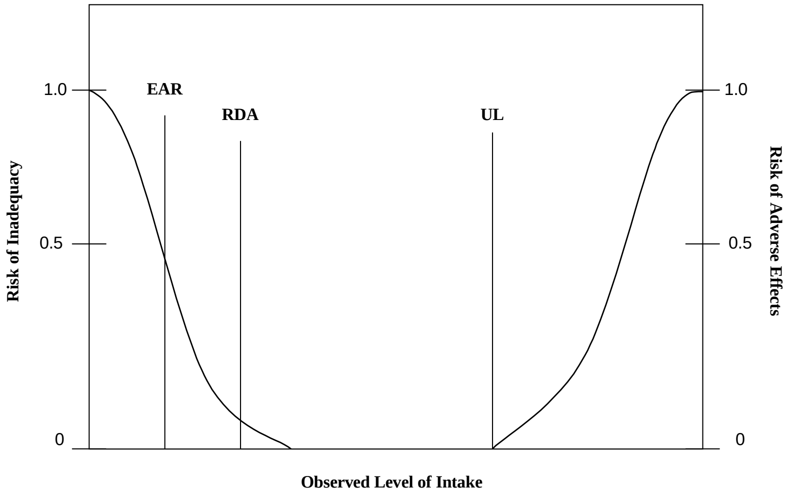

Figure 4-4. Dose-response curves for essential elements. The response of organisms to metals that have both toxic and nutritional properties is conceptualized as having three phases: an area where there is a risk of inadequacy, a neutral area, and an area where there is a risk of adverse effects.
Source: FNB (2000)

Note: This figure simply illustrates basic concepts and does not reflect an exact scale. (That is, although it might appear that the RDA is set at a level associated with a risk of inadequacy above 0.02-0.03, that is not the intent.

Dietary Reference Intakes
Recommended Dietary Allowance (RDA)—The average daily dietary nutrient intake level sufficient to meet the nutrient requirement of nearly all (97 to 98 percent) healthy individuals in a particular life stage and gender group.
Adequate Intake (AI)—The recommended average daily intake level based on observed or experimentally determined approximations or estimates of nutrient intake by a group (or groups) of apparently healthy people that are assumed to be adequate; used when an RDA cannot be determined.
Tolerable Upper Intake Level (UL)—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.
Estimated Average Requirement (EAR)—The average daily nutrient intake level estimated to meet the requirement of half the healthy individuals in a particular life stage and gender group**.**

Considerable concern for human health has been focused on the toxicological aspects of essential metals. Because of this concern, the FNB of NAS has provided Tolerable Upper Level Intake Values and RDAs for these metals where data permit and has provided guidance for assessing risk from dietary exposures to these elements (NAS/IOM, 2001). The World Health Organization (WHO/IPCS, 2002) also has provided guidance on methods for assessing risks from excessive exposure to nutritionally essential metals.

4.3.3. RDAs and RfDs/RfCs

The RDA and Reference Dose (RfD)/Reference Concentration (RfC) differ significantly. The RDA is indexed to a clinical effect of deficiency; and it is used to guide people to take in enough of that material to maintain their health. Conversely, RfDs/RfCs are used to assess the potential for toxic effects from elevated exposures (note that RfDs/RfCs are based on many endpoints, which have differing levels of relevance to whole organism effects). In any case, the RDA is designed to minimize adverse effects associated with nutritional deficiency, so is applicable only to dietary exposures (notably food and beverages). Note that these values are not necessarily synonymous with optimal health (for example, it has been suggested that zinc intakes above the RDA might have certain beneficial/protective effects, including for the prostate in men, for limiting menopausal calcium loss in women, and assisting copper metabolism). RDAs have been identified for different age groups and genders, with RDAs listed for 16 different age-sex and 6 age-pregnancy combinations (NAS/IOM 2003). These RDAs are based on distributions from empirical data or general assumptions that cover 97 to 98% of the population in the given age/gender category, and for women, additional pregnancy/lactational categories. The RDA is given as mass (milligram, or mg) per day.

In contrast, the RfC is used to assess the potential for a noncancer effect. It represents the amount of a given metal or other material that humans can take in every day without appreciable risk of any harmful effect during a lifetime, including for sensitive subpopulations. The RfD is chemical- and route-specific (oral). The RfC represents a similar measure, but for the air concentration to which someone can be exposed (via inhalation). Sensitive subgroups are commonly addressed through the use of uncertainty factors (which also account for variability) incorporated into these values. Dietary exposures to commercial foods (not locally grown) are typically categorized as “background” and are not often included in environmental risk assessments. The The RfD is given as a mass (mg) per kilogram (kg) body weight that can be safely ingested orally every day, and the RfC is given per m^3 of air (i.e., amount safely inhaled).

Both RDAs and RfDs/RfCs have been developed for several metals; for example, values exist for chromium, copper, manganese, molybdenum, selenium, and zinc), and ULs are available for boron and vanadium, and RfDs (but not RDAs) are available for certain compounds of those metals. This highlights the basic concept underlying all of toxicology that *the dose makes the poison* (from Renaissance physician Paracelsus). That is, dose is the key factor in determining whether the effect of a given metal will be beneficial or adverse, considering other exposure conditions.

4.3.4. Biology Relevant to Toxic and Essential Properties of Metals

The bioaccessibility and bioavailability of metals in locally grown food will depend on the species of the metal in the environment and after ingestion; this exposure may be affected by other chemicals such as metal-chelating agents that restrict uptake (e.g., phytates in plant foods) or facilitate uptake (e.g., ascorbic acid). Metals in food can be found in organic forms or complexes not found in soil, sediment, water, or air. For these reasons, the bioavailabilityof a metal within food may differ considerably from its bioaccessibility in an environmental media; to illustrate, different oral RfDs have been developed for cadmium in food and cadmium in water.

The term “molecular or ionic mimicry” has been applied to 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 play an important role in the health assessment for specific metals. One well-studied example is the replacement of zinc by lead in heme-synthesis, which inhibits the function of heme-synthesizing enzymes (Goyer and Clarkson, 2001).

Single nutrient deficiencies, such as iron deficiency anemia or iodine deficiency goiter, are well characterized. Generally, intake of essential elements is sufficient in affluent populations to avoid such diseases. However, mild multiple nutrient deficiencies may occur that may be indistinguishable from the pathophysiological effects of primary deficiency of a single metal. In conducting environmental exposure analyses, it is important to consider whether any nutritional deficiencies exist in the population being assessed, or if intake of all trace elements needed to maintain health is met, so results do not confuse adverse health effects due to nutritional deficiency with those resulting from toxic responses to excessive levels.

The uptake of essential metals from dietary food and water and their subsequent distribution within the body are regulated by homeostatic mechanisms that allow enhanced absorption or excretion at low dietary intakes and diminished absorption/excretion with high dietary intakes. Furthermore, dietary factors can reduce the uptake of essential metals. For example, high amounts of phytates in the diet complex with endogenous and exogenous zinc, thus preventing reabsorption and increasing zinc elimination. Specialized carrier proteins that may originally have developed as a transfer mechanism for the essential elements also are used to sequester or eliminate nonessential metals (see above, Section 4.3.3.3).

However, homeostatic control mechanisms can be overwhelmed (e.g., by ~~the~~ a high dose level) or otherwise circumvented to produce a toxic effect . (Note that actions of metals on skin or pulmonary membranes are not driven by homeostatic controls.) For example, at high doses chromium salts can produce severe effects in the skin or respiratory tract mucosa, and inhalation of zinc oxide fumes can give rise to inflammatory response (per cytokine excretion) that can produce a local tissue effect (unrelated to systemic zinc exposure levels)..

Toxic effects of metals also result from interactions that block the availability or activity of essential metals. For example, lead can block the utilization of iron in heme synthesis by inhibiting the enzyme ferrochelatase, and there is evidence that cadmium can block the entry of zinc into the fetus, thereby causing a variety of developmental defects in the newborn. Cadmium, lead, and mercury, in combinations or by themselves, may reduce the availability of zinc, copper, and selenium when these essential elements are present in marginal amounts in the diet. In addition, a competitive interaction between one or more essential metals could lead to toxic effects, e.g., copper toxicity may be enhanced by reduced levels of molybdenum or vice versa.

4.3.4.1. *Limitations*

The RDAs have been developed for essential metals (as well as other materials, including vitamins) and are designed to provide adequate nutritional intake for 97 to 98% of the population. Should there be a narrow window between the required and toxic amounts of an element (e.g., as for selenium), then it is possible that sufficient amounts for those who need the most may be above toxic levels for the majority of the population. This potentiality should be evaluated on a case-by-case basis using what is known about potential sensitivities to that element. As a note, RDAs have been established for single chemicals, not mixtures, while metal RfDs to date are also for single chemicals. Under this situation, the component-based approach to assess mixtures is commonly applied (see section 4.3.6).

4.3.5. Toxicity

Metals are associated with numerous health effects that are reviewed in detail in reports from EPA (IRIS reports), the Agency for Toxic Substances and Disease Registry (ATSDR) Toxicological Profiles, reports from the World Health Organization's International Programme for Chemical Safety, and toxicology textbooks. 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), and inorganic lead compounds are considered probable human carcinogens by EPA's IRIS program, while IARC (2004) has concluded there is limited evidence of carcinogenicity to humans (See: http://www.epa.gov/iris/subst/0277.htm#carc and http://monographs.iarc.fr/htdocs/announcements/vol87.htm). Other effects of metals are also well documented, including effects on the neurological, cardiovascular, hematological, gastrointestinal, musculoskeletal, immunological, and epidermal systems. For example, following oral exposure, beryllium can cause intestinal lesions and copper can cause intestinal irritation, while nickel can decrease kidney weight and cadmium can cause proteinuria, and both trivalent chromium and nickel can decrease liver and spleen weights. Many metals, including those that are toxic (including carcinogenic), follow the metabolic pathways of similar essential metals, the result of similar binding preferences among various metals (Clarkson, 1986). Carcinogenic 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 for detoxify metals. The body may use other detoxification mechanisms, such as long-term storage (e.g., cadmium) and biliary and/or urinary excretion.

4.3.5.1. *Application*

Many factors related to pharmacokinetics and susceptibility act as determinants of toxicity following exposure to a metal. Short-term exposures may produce target organ effects very different from those produced by a similar dose 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 the renal, cardiovascular, nervous, or hematopoetic systems. Survivors of acute high-dose arsenic ingestion usually experience multiple organ effects, sometimes with long-term sequelae. Long-term, low-dose exposures from ingestion of metals in food and water generally cause an accumulation in target organs over time. Such exposures can involve any organ system 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 (e.g., kidney) but will 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).

Nickel and nickel compounds and chromium and chromium compounds are well-established contact allergens. Other metals that have been cited as contact allergens include copper (WHO/IPCS, 1998), cobalt salts (AIHA, 2003), organomercurials (AIHA, 2003), beryllium (WHO/IPCS, 1990b), palladium (Kimber and Basketter, 1996), and gold (Kimber and Basketter, 1996). Toxic interactions with the immune system that result in exaggerated responses are known as hypersensitivity or allergic reactions. Allergic contact dermatitis (or delayed hypersensitivity) is one such example and occurs as a result of allergy to a substance (antigen) through cell-mediated immunity. In sensitized persons, such reactions can be provoked by minute amounts of the allergen. There are two main phases in cell-mediated immunity, the sensitization phase (in which the person becomes allergic to the antigen) and the elicitation phase. Sensitization usually takes at least 10 days. When sensitization has been achieved and the individual is then re-exposed, a reaction is obvious after a characteristic delay of 12–48 hours (hence the term "delayed") (AIHA, 2003). Although there is some connection between skin and respiratory sensitization, it does not follow exact rules, and the dermal mode is a much more common reaction to metals.

4.3.6. Metal Mixtures

Metal mixtures present interesting challenges for the risk assessor. As described below, certain metals can mimic other metals, and depending on the dose and composition of other the metal mixture can result in either a toxic or protective effect. Because metals are naturally occurring substances, they more frequently occur as mixtures than as single, toxic-level exposures. Thus, it is important to account for multiple, simultaneous metal exposures to realistically assess health risks.

4.3.6.1. ***Molecular or Ionic Mimicry***

A large body of literature provides examples of *molecular* or *ionic mimicry* of metals. This phenomenon is central to aspects of uptake and biokinetics for metals within the body. The term *molecular* or *ionic mimicry* has been applied to 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. A number of reviews discuss this phenomenon, giving examples of the mechanism of toxicity for specific metals (Ballatori, 2002; Clarkson, 1993). 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. For example, the protective effects of zinc against copper toxicity are most likely due to diminished gastrointestinal uptake of copper. Lead replaces zinc in heme synthesis by inhibiting the function of heme-synthesizing enzymes (Goyer and Clarkson, 2001). The substitution of calcium by lead results 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). 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 increases the uptake of lead into the body, presumably as a result of lead uptake via calcium active transport processes. Calcium supplementation then diminish lead uptake via both competitive binding to uptake proteins and down-modulation of active transport activity. Lead is actively taken up into the body and sequestered into bone because of ionic mimicry for calcium. Similarly, cadmium uptake may in large part be related to ionic mimicry of zinc. 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 replace the phosphate in mitochondria, impairing synthesis of ATP and energy metabolism.

As a converse of the enhanced toxicity that can result from nutritional deficiency, the effects of moderate doses of naturally occurring metals that are not required for nutrition can be reduced (or antagonized) by essential metals found in foods. Diet, therefore, can be a major factor in determining whether potential adverse health effects of additional metal exposures are moderated or enhanced . For example, humans can be exposed to mercury by consuming fish that have absorbed mercury from contaminated water, whereas selenium present in the same water can act as a natural antagonist for mercury toxicity, and vice versa (although the protection is inferred through formation of non-reactive mercury-selenide complexes, rather than as result of ionic mimicry); cadmium in soil can be ingested via fruits and vegetables grown on that soil, while zinc in nuts also eaten as part of that diet can limit (or antagonize) cadmium toxicity. 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.

4.3.6.2. *Studies of Metal Mixtures*

Few controlled studies exist on the toxicologic interactions of metals relevant to levels found in the environment. ATSDR has compiled and evaluated interaction studies for various combination of chemicals, including two sets consisting only of metals: (1) arsenic, cadmium, chromium, and lead; and (2) copper, lead, manganese, and zinc. Additional interaction profiles that include two or more metals are: (3) cesium, cobalt, strontium, trichloroethylene (TCE), and polychlorinated biphenyls (PCBs), and (4) arsenic, strontium-90, TCE, hydrazine, and jet fuels (available online at http://www.atsdr.cdc.gov/iphome.html). Few studies quantified the magnitude of the interaction, whether using the authors’ definitions of toxicologic interaction or EPA’s definitions based on dose and response addition. (This same limitation also applies to non-metal mixtures.) The summaries below indicate some of the qualitative conclusions available regarding potential toxic interactions.

A study of a mixture of cadmium, lead, and zinc study in rats found slightly more marked adverse hematological effects with the ternary mixture exposure than with the cadmium-lead, cadmium-zinc, or lead-zinc mixtures (Thawley et al., 1977); inconsistencies in dietary levels of calcium and vitamin D in this study, however, may have made comparisons problematic. A well-controlled rat study has reported significant synergism between cadmium and lead regarding testicular atrophy (Saxena et al., 1989). That study also demonstrated protective effects of high dietary levels of zinc, which effectively reduced the testicular effects of the cadmium-lead mixture to control levels. No studies have been located that would allow extrapolation of those high exposure results to more common, lower environmental levels. Fowler and Mahaffey (1978) investigated a relatively wide range of endpoints 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 for binary mixtures compared with ternary mixtures.

For some endpoints, the data are not sufficiently robust to show even the direction of interaction (i.e., whether the joint action will be dose additive or greater or less than additive). Many animal studies use 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. Experimental efforts to identify and quantify interaction mechanisms among metals are still needed.

4.3.6.3. *Human Health Assessment*

Approaches for assessing joint toxicity following exposure to mixtures have been developed by EPA (U.S. EPA, 2000b, which updates U.S. EPA, 1986a). The selection of risk assessment approach begins with the assessment of data quality. Available data are classified into three categories: data on the mixture of concern, data on a mixture of a toxicologically similar nature, and data on individual effects of each component chemical in the mixture. The first approach—assessment of the data based on the mixture of concern—is the preferred approach when the data allow and is essential when considering a complex mixture (e.g., containing hundreds of component chemicals). Such data include epidemiologic studies on the complex mixture or in vitro data on the complex mixture. The typical example of a complex mixture is coke oven emissions; few metal mixtures relevant to environmental exposures would be expected to fall within this category. The second approach is an assessment of data on a mixture of a toxicologically similar nature to that of the mixture of concern, and a similar limitation applies to the usefulness of this approach. The third approach is based on the toxic or carcinogenic properties of the components in the mixture. In this approach, information on toxicologic interactions of components is incorporated into the assessment. This last approach will likely be most useful for assessing metals in the environment, because this situation is most common (i.e., something is known about the components of the mixture but nothing is known about the mixture itself, or a similar mixture).

When no information on interactions exists (i.e., to indicate higher-than-additive or lower-than-additive results), a dose-additive or risk-additive model is applied as a default. The decision on which of these to use depends primarily on how toxicologically similar the metals in the mixture are. For metal mixtures, a dose-additive approach would be suggested if each metal could be thought of as a concentration or dilution of every other metal in the mixture. Three approaches have been used when the components are considered to be dose additive: the Hazard Index, the Relative Potency Factors, and the Toxicity Equivalence Factors (a special case of the Relative Potency Factors). The choice of approach will depend on the quality of the data. If the metals were assumed to behave independently of each other, then response addition would be the approach applied. This approach is often used for cancer assessments at Superfund sites and other environmental contamination situations; note that response addition is not valid for high exposure concentrations. For mixtures containing metals known to interact, then an interaction-based Hazard Index can be estimated (U.S. EPA, 2000b; Hertzberg et al., 1999).

Dietary information is an important part of evaluating the potential for mixture toxicity and interactions. As described above, some quantitative data exist for some metal combinations, and where sufficient data exist, they can be used to predict the pattern of interactions for various proportions of the mixture components or to quantitatively modify the risk assessment. Although at this time few such data are available, some studies do exist, and in some cases the results can offer at least qualitative insights. For example, *in-vitro* studies showed that chromosome mutagenicity resulting from co-exposure to arsenic and antimony was less-than-additive. That is, less cell damage was observed than would have been expected from an additive effect from the two metals (Gerbel, 1998). However, it may be difficult to validate laboratory data in the absence of comparable field (epidemiological) data (McCarty et al., 2004). Exposures to other types of stressors also must be considered. A recent progress report on a mixtures study of metals and polycyclic aromatic hydrocarbons (PAHs) indicated that environmental metals decreased levels of the enzyme needed to catalyze PAH bioactivation (a precondition of carcinogenesis), affecting induction at both transcriptional and post-translational levels (Kaminsky et al., 2003). (Note that the carcinogenicity of the metals themselves has not yet been assessed.)

A number of in vivo studies of multiple metals and metal compounds were published before 1980. The draft interaction profiles developed by the Agency for Toxic Substances and Disease Registry and EPA include evaluations of these metals and more recent publications for a selected set of metals.

The EPA provisional interaction profile for arsenic, cadmium, lead, and zinc includes information relevant to chronic exposures as well as insights from acute toxicity studies. Some acute studies considered a variety of endpoints – mortality, testicular necrosis, and hepatotoxicity (Hochadel and Waalkes, 1997) – while others focused only on mortality (Yanez et al., 1991; Diaz-Barriga et al., 1990; Yoshikawa and Ohta, 1982). The studies captured in these draft profiles often include measurements of changes in chemical distributions in target tissues, which can be difficult to interpret in terms of potential health risks. Nevertheless, some specific joint toxicity insights can be gained. An example of how this information can be summarized for at least qualitative consideration in a health risk assessments is given in Table 4-13.

Current studies of toxicological interactions are limited, often relying on in vitro assays of bacteria and cell lines. These studies have tended to focus on mechanisms at the cellular and molecular levels, although their implications for health risk assessment on the tissue or whole organism level are not yet clear. For example, Pounds et al. (2004) identified responses in a recent study of interaction toxicity among simple chemical mixtures of cadmium, mercury, methylmercury, and trimethyltin, using cultured murine renal cortical cells that are targets for metal toxicity. Meanwhile, a model for synergistic metal activation leading to oxidative damage of DNA (genetic material, deoxyribonucleic acid) has been developed by Sugden et al. (2004). This model used in vitro assays to assess the oxidative activation of chromate by arsenite. Although toxicokinetics are a common focus of recent work, interesting new studies are also breaking ground in the area of toxicodynamics, including those under way at TNO in the Netherlands (by J. Groten and collaborators). Future studies are expected to produce key insights that will improve the ability to assess human health implications of exposures to multiple metals.

4.3.7. Variations in Susceptibility

Many factors can contribute to human variation in susceptibility to metals. In addition to diet/nutritional status discussed above, these include other lifestyle factors such as smoking or alcohol consumption. Smoking can damage the lung, which can affect the ability to withstand the insult caused by simultaneously or subsequently inhaling metals, particularly those that act directly on the lung such as beryllium, cadmium, chromium, and nickel. Similarly, alcohol damage to the liver can be exacerbated by metals that also cause liver pathology. Other factors influencing susceptibility include age, gender, concurrent damage or disease, and genetic polymorphisms. The discussion below focuses on susceptibility issues considered key for metals; such issues should be at least qualitatively considered in identifying and evaluating potentially susceptible subpopulations for metals risk assessments. Examples for each of these additional factors are offered below.

4.3.7.1. *Age*

Differences in the pharmacokinetic behavior of metals exist at different life stages, particularly for the nutritionally essential metals (WHO, 1996a). During the immediate postnatal 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, numerous studies have been conducted on the effects of lead and 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 were not identified in a study conducted several years ago (WHO, 1996c). For comparison, the RDA for copper for children up to 8 years old is just under half that identified for teenagers to adults (NAS, 2001).

The sensitivity to skin irritants is considered to generally decrease with age, so children could be more sensitive to metal irritants. Meanwhile, the general loss of renal function with age means older adults will be less able to withstand the harmful effects of metals that affect the kidney, (e.g., cadmium; U.S. EPA, 1999). Note that the RDAs specifically tabulated for older adults (FIU 2004) remains similar to those identified by NAS for this population group within the overall tables published in 2001 (FIU 2004). Thus, when assessing metal risks across different population groups, it will be important to review the current status of metal-specific information with age implications for the metal(s) in that study.

4.3.7.2. *Gender*

Pregnancy and lactation increase demand for some essential metals, particularly copper, zinc, and iron (NAS/IOM, 2003; Picciano, 1996). Because of physiological changes that include higher iron (and calcium) requirements, hormonal changes, and susceptibility to respiratory disease, it has been suggested that pregnant women could be predisposed to the toxic effects of beryllium, lead, and manganese (U.S. EPA, 1999f). As a note, the RDAs for copper, iron, molybdenum, and selenium are higher for pregnant and lactating women than for other women or men; for selenium, the RDA for other women is the same as that for men (NAS/NRC, 2000); in contrast, the RDA for zinc is the same for pregnant women 19 to 50 years old as it is for men aged 19 to 70 and older. For additional comparison, the RDA for chromium for pregnant women is roughly the same as that for men, which is higher than for non-pregnant women and lower than for lactating women (NAS/NRC, 2001).

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 basic gender differences independent of pregnancy also may account for differences in toxicokinetics between women and men. Women have only about two-thirds the fat-free body mass of men—so 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, and calcium can also be lost as women age. These differences have an impact on body content of minerals (WHO/IPCS, 2002). Women also have significant loss of iron during menstruation, and it has been shown that absorption and toxicity of cadmium are greater in women, related to decrease in iron stores (Berglund et al., 1994).

4.3.7.3 *Concurrent Damage or Disease*

People with higher-than-average biological sensitivity to environmental stressors include allergics and those with pre-existing medical conditions (e.g., with compromised immune systems as a result of a disease or treatment for it, such as chemotherapy). For example, chemotherapy can damage the kidney over time, and at certain levels other medicines (such as acetaminophen) can damage the liver. This may increase metal sensitivity for those metals that cause liver dysfunction.

Skin abrasions or other irritations also can alter exposures to and subsequent effects of metals (although dermal absorption is not a primary route of metals exposure for intact skin). For example, both nickel and chromium can cause allergic contact dermatitis, so their combined presence could result in joint toxicity with a potential for interaction; broken skin could potentially increase the absorption of other metals (and other toxic agents) across the exchange boundary. Beyond these well-established contact allergens, other metals have also been cited as possible contact allergens, including copper (WHO/IPCS, 1998), cobalt salts (AIHA, 2003), beryllium (WHO/IPCS, 1990b), palladium (Kimber and Basketter, 1996), and gold (Kimber and Basketter, 1996).

With regard to effects associated with high-dose metals exposures, elevated arsenic intakes – from drinking water with levels 17 times above the U.S. drinking water standard – have been linked to blackfoot disease, a vascular complication that represents a severe form of arteriosclerosis (EPA 2004). like many such diseases, the incidence of blackfoot increases with age and thus is also linked to the age factor. Some studies suggest that higher doses of copper and manganese might be linked with Parkinsonism (which could be reversible) (Feldman, 1992;

Gorell et al., 1999). Thus, these studies offer insights for specific metals risk assessments where exposure conditions, including levels and durations, were sufficiently similar.

4.3.7.4. ***Genetic Polymorphisms***

Individuals vary considerably in the nature and severity of their response, and potential repair and recovery, from exposure to metals and metal compounds. Such differences may be due to genetic polymorphisms that can alter the transport and 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) that is believed to be due to impaired biliary excretion of copper, resulting in accumulation in and damage of various tissues, particularly the liver, brain, kidney, and cornea; hemolytic anemia can also result. 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 (WHO/IPCS, 2002).

Hemochromatosis is a common inherited disorder related to 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).

ATSDR (2003) reported that a relationship between human leukocyte antigens (HLA)[1] and nickel sensitivity was observed in patients who had a contact allergy and positive results in a patch test for nickel. The nickel-sensitive group had a significant elevation in HLA-DRw6 antigen compared with normal controls. The relative risk for patients with DRw6 to develop a sensitivity to nickel was approximately 11-fold.

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 did not receive attention until about ten years ago (Smith et al., 1995). Fleming et al. (1998) found that the relationship of bone lead to the cumulative blood index for workers

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[1] The major histocompatibility complex is a group of genes on chromosome 6 that code for the antigens that determine tissue and blood compatibility. In humans, histocompatibility antigens are called human leukocyte antigens because they were originally discovered in large numbers on lymphocytes. There are thousands of combinations of HLA antigens.

with occupational exposure 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.

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 continue to be described. The human MT gene locus is very complex; four individual isoforms and more than ten subforms of the MT1 gene exist, including functional and nonfunctional genes. Because of the protective role that MT plays in metal metabolism, an understanding of the biological role of significant variations in this MT gene locus (both within and among individuals) can offer insights into differential sensitivity or susceptibility and will help characterize variability across a population.

Although all MT proteins are associated in some way with a protective role, each of the multiple isoforms appears to have a unique function, as reflected by their unique tissue distributions, the specific conditions under which each is expressed, and unique properties of the metal binding clusters (Bogumail et al., 1998). The multiplicity of the human MT gene loci makes them potentially important for understanding genetic variability. Emerging technologies are enabling new research in the areas of genomics, proteomics, and metabolomics that will eventually improve human health risk assessments for metals and other stressors, including the role that MT isoforms play in variability in response.

Other creative approaches have coupled human tissue studies with animal toxicology studies. For example, studying lung slices from humans (from transplant or tumor resection) and from rodents exposed to the same metals offers promise for improving our understanding of potential effects from inhalation exposures. Comparison of study results between rats and mice under similar exposure regimes and linking them to similar human exposures will provide insights into whether human responses are better predicted by rats or by mice.

4.4. ECOLOGICAL EXPOSURE PATHWAY ANALYSIS

Risk is a function of both hazard (i.e., the toxicity of a substance) and level of exposure. The route of exposure, as well as other details such as timing and duration, is also important because exposures vary in their effectiveness in delivering a dose across a biological boundary (Ryan, 1998; Ott, 1985). No risk exists unless an effective exposure to a receptor occurs.

Exposure routes include inhalation, ingestion, and dermal or, for plants, root uptake and leaf exposures. *Pathways* describe the specifics of any exposure and include transport of the contaminant in the environment as well as exposure route for organisms of concern (e.g., dietary ingestion of a soil contaminant that has been taken up by plants). *Phase association* and *chemical speciation* influence metal movement through pathways and the availability of a metal for delivery of the dose to receptors of concern.

Metal Exposure Assessment

Metal exposure assessment includes consideration of the exposure routes and pathways specific to metals, the phase associations and chemical forms of the metals, and the expression of exposure and target doses in a manner consistent with defining hazard thresholds for particular organisms.

Major metal sources to waters and lands include diverse manufacturing, mining, combustion, and pesticide activities. Major atmospheric sources are oil and coal combustion, mining and smelting, steel and iron manufacturing, waste incineration, phosphate fertilizers, cement production, and wood combustion (Haygarth and Jones, 1992). Metals from these atmospheric sources can find their way into soils, sediments, and water. Other major sources to aquatic and terrestrial systems include chlor-alkali, acid, pigment, electronics, and copper sulfate production.

Some exposure assessments do not involve anthropogenic releases of metals to the environment. Rather, they focus on changes in exposure to ambient metals that result from other aspects of human activities. For example, acidification of freshwater changes aluminum phase association and speciation, resulting in an increased dose of naturally occurring metals to aquatic biota (e.g., Campbell et al., 1992). Intensive irrigation mobilizes selenium that is naturally present in relatively high concentrations in western soils, and consequent evaporative concentration in wetlands, impoundments, and other low-lying areas in arid regions of the United States can lead to toxic exposures (e.g., Wu et al., 1995).

Methods for assessing transport of metals through various media (water, soil, air) are described in this section. Exposure to aquatic organisms through water and dietary routes (looking again at models to account for site-specific differences in bioavailability) is also discussed. The section concludes with discussions about quantifying exposure to metals for terrestrial organisms.

4.4.1. Aquatic and Terrestrial Transport Pathways for Metals

When an exposure assessment is completed for a site, the available data are usually limited in their coverage with respect to the characterization of exposure levels over both time and space. Depending on the situation, it may be advantageous to use a transport and fate model (i.e., a computational model) to fill in the data gaps, such that an improved characterization of exposure levels is available for usel by the risk assessor. Models are also useful in situations where it is desirable to have an estimate of future exposure levels that are expected to result from the implementation of remediation measures. These results can be used to quantitatively evaluate the effectiveness of alternative remediation scenarios that are being considered. The models can also be used to refine the design of the viable alternatives so that an optimal remediation strategy can be developed.

Although numerous models are available for use, most are based on the same fundamental principles. That is, metals are ubiquitous in the environment, as they are found in the aquatic, terrestrial, and atmospheric compartments. Within each compartment, they are present in association with water (freely dissolved metal or as organic and inorganic metal complexes), particles (sorbed, precipitated, or incorporated within a mineral phase), and air. The evaluation of metal transport therefore requires evaluation of the distribution of the metal among these phases, within each compartment, as well as the movement (i.e., the transport) of each of these within and among the various compartments. It is important to simulate the movement of water and particles explicitly because this provides a way to evaluate differences in the degree to which various chemicals/metals may be transported in association with particles (i.e., via settling and resuspension) or in association with the dissolved phase (diffusive flux of dissolved metal). The analyst represents the environmental setting of interest as a series of discrete, interconnected volumes. Mass balance equations for air, water, solids, and metal are then formulated for each volume to obtain a system of mass balance equations that may then be solved for the concentrations of interest over both time and space. Note that it is not necessary to include all of the compartments in every model. For example, models for a site that is impacted by a smelter might call for use of a model of an atmospheric compartment (e.g., to simulate transport of a release from a stack) and a terrestrial compartment (to simulate fate of atmospheric inputs to the soil). Alternatively, for an aquatic setting dominated by previously contaminated sediments, it may be sufficient to consider water and sediment transport alone and to neglect the atmospheric and terrestrial compartments.

Although the underlying principles of most models are similar, the features included vary widely from one model to the next. The output from a relatively simple model may be adequate for decision-making purposes in some instances. Some relatively simple models are limited in their applicability to steady-state analyses and spatially uniform conditions, but if this will provide a reasonable and/or conservative representation of conditions at a site, it may provide the analyst with a useful and cost-effective modeling alternative. In other cases, the analysis may require the completion of time-variable simulations to properly represent conditions that vary over time, such as daily or seasonal variations in flow and upstream boundary concentrations, point source loads, and pulse exposures. The analyst should select is an appropriate model because not all models will be applicable to every situation. Although in principle the more sophisticated models provide the risk assessor with the capability to complete a more detailed and mechanistically based analysis than will a simple model, successful application of these models will require greater resources (data, time, and funding) than will the use of a simpler model, and that the analyst also must possess a relatively high level of modeling expertise. It is for this reason that the more sophisticated models are usually reserved for use in higher level, definitive assessments.

Many of the models available for use in evaluating the transport and fate of metals were originally developed for application to neutral organic chemicals. As a result, these models frequently include a variety of reactions that are not necessarily germane to an analysis of metal transport and fate (e.g., biodegradation, photo-oxidation, and volatilization). Although these models still may be of use in an exposure assessment for metals (the nonapplicable processes often may be bypassed), a more significant problem is that they often fail to represent some important metal-specific processes. For example, the evaluation of metal speciation and metal partitioning between dissolved and particulate phases will be represented only in a very simple manner in such models. This limitation may be overcome, at least in part, by performing the requisite metal-specific analyses with a stand-alone chemical equilibrium model, but this approach will place an added burden on the analyst to integrate the results of the two models in a technically defensible manner.

An overview of some of the aquatic fate and transport models available for use is presented in the following subsection. Although models that include some metal-specific capabilities will be noted, no single model that is currently available for use includes all of the metal-specific features that would be desirable for use. More detailed discussions of these and other transport and fate models, as well as a number of chemical equilibrium models, may be found in Paquin et al. (2003) for aquatic systems and Allen (2002) for terrestrial systems. Additionally, work is in progress to develop updated models that will offer improved metal-specific capabilities (e.g., see the discussion of the Unit World model in Section 5). As a result, metal fate and transport models should be viewed as an evolving technology, with new models expected to become available in the not too distant future.

4.4.1.1. ***Aquatic Transport Models***

Modeling of metal transport and fate within aquatic systems involves the representation of hydrodynamic transport to simulate movement of water, particulate transport to simulate the movement of particles, and chemical transfers and kinetics to simulate exchange of metal between dissolved and particulate phases and between the water column and benthic sediment (Figure 4-5). The analyst has the option of using independent hydrodynamic transport, sediment transport and chemical fate models, or an integrated model that incorporates all of these processes. Although the models described below are mainly in the latter category, their use does not preclude the use of a stand-alone model (e.g., a hydrodynamic or sediment transport model) as an aid in the development of inputs to the integrated model.

Modeling the movement of metals through an aquatic system begins with a characterization of the movement of water through the system. The time scale for the hydrodynamic analysis should be represented in a way that will satisfy the needs of the sediment transport and chemical fate analyses that are also being performed. For example, low-flow conditions associated with minimum dilution may be judged to be the most critical conditions in a setting involving a point source discharge, while peak flow conditions may need to be simulated in a setting where resuspension of contaminated sediments is the primary concern. A steady-state model might be appropriate for use in the former case, and a time-variable model would likely be needed in the latter case. Thus, the details of the specific problem setting will necessarily have an influence on both model selection and how the model will be used.

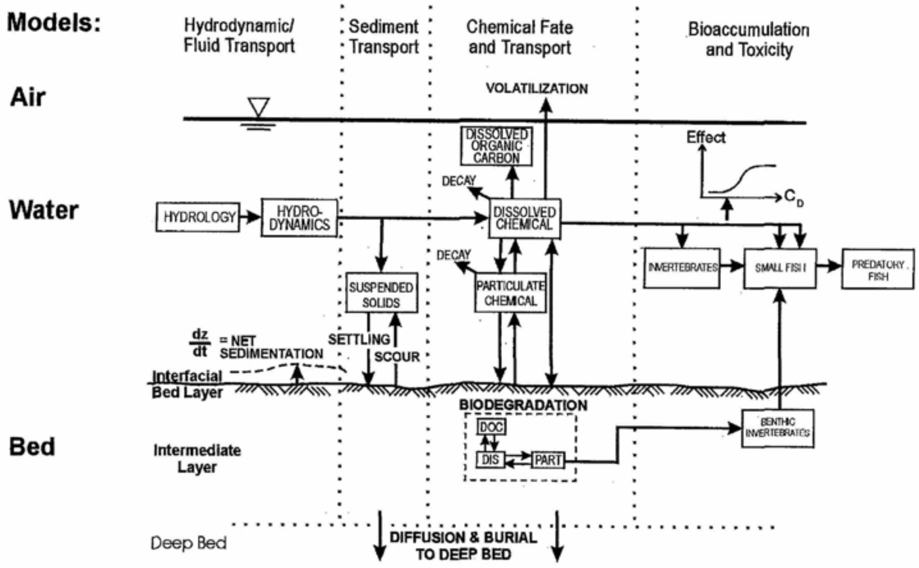

Figure 2-2 Example modeling framework

Figure 4-5. A generalized model framework for chemical fate and transport in an aquatic system.

Source: Paquin et al., 2003.

The reader also should be aware of several reviews of fate and transport models that have been completed since the early 1980s. In earlier reviews, Delos et al. (1984) reviewed models for wastewater load allocations, and Mills et al. (1985) described screening-level analyses for water quality assessments of conventional pollutants. The Agency subsequently prepared an updated summary of the features included in the water quality models that were available for use in exposure assessments (U.S. EPA, 1987). Schnoor et al. (1987) simultaneously published their review and included detailed descriptions of fate and transport models and their required input parameters. Later, EPA published a review describing the use of modeling tools for the development of total Maximum Daily Loads (TMDLs) in watersheds (U.S. EPA, 1997a). More recently, Paquin et al. (2003) completed a review of exposure, bioaccumulation, and toxicity models for aquatic systems, with a focus on their applicability to metals (exclusive of organometallics). Because of the advances that have been made since the mid-1990s with regard to the development of fate and transport models, including some recent efforts to couple these models with metal speciation models and more sophisticated stand-alone hydrodynamic and sediment transport models, the latter reviews by U.S. EPA (1997a) and Paquin et al. (2003) tend to include the most up-to-date information with regard to the availability of models that are appropriate for use. These reviews also include example applications of many of the models discussed.

4.4.1.1.1. ***Applications.*** As noted above, fate and transport analyses may be performed by using an integrated hydrodynamic, sediment, and chemical transport model or by employing what tend to be relatively sophisticated stand-alone versions of these three submodels. The advantage of the former approach is that integration of the hydrodynamic, sediment, and chemical transport results takes place in a seamless manner with limited need for an analyst's intervention. This is in contrast to the use of stand-alone models, where the output of one model should be formatted in a way that ensures it is amenable to use with the other models that are to be applied. A distinct advantage of the latter approach is that it has the potential to reduce the time needed to complete a model run, an important consideration for a multiyear simulation of a large and complex problem setting. For example, it may not be necessary to repeat the simulation of hydrodynamic and sediment transport if the model input being modified affects only chemical transport (e.g., partition coefficient). A similar line of

Integrated Model

The fate and transport of metals in aquatic systems is most reliably predicted using ***integrated models***, rather than stand-alone hydrodynamic or sediment transport models.

reasoning would apply to the use of an integrated transport model that includes metal speciation versus one that uses a stand-alone chemical equilibrium model to satisfy this need.

Analytical Solution Models

Analytical solution models of aquatic system fate and transport are the simplest models with the lowest computational requirements. They are solved analytically to provide concentrations over metal of time or space.

The fate and transport models that may be considered for use in the analysis of an aquatic setting are listed in Table 4-13 and discussed below. The models range in complexity from simple mass-balance calculations that can be performed on a hand calculator, to one-dimensional steady-state models, to multidimensional time-variable models. The models are listed in groups that correspond to the mathematical solution technique used to solve the governing mass-balance equations (analytical solution, steady-state numerical solution, and time-variable numerical solution), a categorization that is approximately in accordance with their ease of use as well. Paquin et al. (2003) describe example applications of many of these models to illustrate how they have been previously applied. Although publications of successful modeling applications serve as useful illustrations of how models may be used, it is important to bear in mind that “past performance should not be viewed as a promise of future returns.” That is, the successful application of any model will depend on many factors other than the model framework itself, including the complexity of the problem setting, the data that available for use, and the experience of the analyst. Another important point to keep in mind is that a complicated model is not necessarily a “better” model to use than a simple one. Models should be selected on a case-by-case basis.

The simplest modeling analysis to consider involves simple screening-level calculations. While clearly applicable to a screening level of analysis, such calculations can frequently provide a quantitative perspective on the severity of a problem, one that will be useful in defining the nature of subsequent modeling analyses that may be required.

Table 4-13. List of fate and transport models

Abbreviation	Name	References
Analytical Solution Models:		
WQAM	Water Quality Assessment Methodology	Mills et al. (1985, 1982a, b)
DJOC-III	Donald J. O'Connor (1988), a triad of papers	O'Connor (1988)
RIVRISK	RIVer RISK	Grieb (1995); EPRI (1996)
SLSA	Simplified Lake and Stream Analysis	Di Toro et al. (1981) HydroQual (1982)
USES	Uniform System for Evaluation of Substances	RIVM et al. (1994), as described in Johnson and Luttik (1995)
QWASI	Quantitative Water Air Sediment Interaction	Mackay (1991); Mackay et al. (1983)
TRANSPEC	Transport and Speciation	Bhavsar et al. (2004)
Steady-State Numerical Solution Models:		
CTAP	Chemical Transport and Analysis Program	HydroQual (1982, 1981)
PAWTOXIC	PAWtuxent TOXICs	Wright (1987)
SMPTOX3 (Version 2)	Simplified Method Program - Variable - Complexity Stream Toxics Model	LTI (1992) Dilks et al. (1995, 1994)
MEXAMS	Metals EXposure Analysis Modeling System Includes EXAMS and MINTE	Felmy et al. (1984) Burns et al. (1982)
Time-Variable Numerical Solution Models:		
EXAMSII	EXposure Analysis Modeling System - II	Burns and Cline (1985); Burns et al. (1982)
RIVEQLII	RIVEr QuaLity II	Chapman (1982)
WASTOX	Water Quality Analysis Simulation of TOXics	Connolly and Winfield (1984)
RCATOX (AESOP)	Row-Column AESOP for TOXics (Advanced Ecological Systems Operating Program)	HydroQual (2003)
WASP5	Water Quality Analysis Simulation Program, Version 5 Distributed w/ DYNHYD5, Dynamic Hydrodynamics 5	Ambrose et al. (1993) Ambrose et al. (1993)
META4	Metal Exposure and Transformation Assessment Model	Medine (1995) Martin and Medine (1998)
DELFT3D	Delft 3D Model	Delft Hydraulics (1998)
MIKE21	MIKE21	Danish Hydraulic Institute
HSPF	Hydrologic Simulation Program - FORTRAN	Bicknell et al. (1993)
RECOVERY	RECOVERY	Boyer et al. (1994) Ruiz et al. (2000)
EFDC	Environmental Fluid Dynamics Code	Hamrick (2002)

Source: Paquin et al. (2003).

Water Quality Assessment Methodology (WQAM) is an excellent resource for use in these types of analyses. WQAM describes a variety of simple screening-level procedures that may be used in water quality assessments. It provides a useful overview of the types of simplified analyses that may be performed and is a generic framework that does not generally attempt to address metal-specific modeling needs. An analyst having limited water quality modeling experience would probably find this to be a useful reference.

A series of three papers by O'Connor (1988) also provide a helpful introduction to the basic concepts and use of fate and transport models. These publications include analytical solutions and example applications for a variety of conditions, including spatially varying suspended solids concentrations. Although these solutions are not currently available in the form of a computer program, they could be readily adapted for this purpose.

Analytical solution models tend to be inherently simple models, a requirement of the solution technique used. As a result, they tend to have modest development and computational requirements (an advantage), but, at the same time, they limit the analyst with regard to the level of detail that can be used to represent the problem setting with the model. For example, these models normally allow the user to represent the system as a one-dimensional, uniform cross-section stream or a completely mixed water body, with constant inputs over time, and particulate and dissolved exchange of chemicals between the water column and sediment. Their simple structure makes these models well suited for use in screening level analyses. Models in this category include the River Risk (RIVRISK) model, Simplified Lake and Stream analysis (SLSA) model, and Uniform System for the Evaluation of Substances (USES) model. Each of these models includes a water column and a single-layer sediment compartment. RIVRISK is a steady-state model that may be applied to a one- or two-dimensional stream setting. It is one of the few models in Table 4-13 that includes a bioaccumulation subroutine. However, it is a proprietary model of the Electric Power Research Institute and is available only to registered users. The SLSA program (HydroQual, 1982; Di Toro et al., 1981) may be used to represent a one-dimensional stream or a completely mixed lake. Its utility in time-variable mode was demonstrated by its success in simulating the long-term (about 5 years) recovery of a quarry that had been dosed with instantaneous releases of the organic chemicals DDE and lindane (Di Toro and Paquin, 1984). Finally, the USES model was developed in Europe to model dissolution and movement of antifoulant paints on boats (Johnson and Luttik, 1995). It provides the analyst with a simplified way to estimate concentrations of organic chemicals and metals in a completely mixed water body, with successful model applications having been reported for metals (Johnson and Luttik, 1995). USES has recently been replaced by a considerably more complex version, the European Union System for the Evaluation of Substances (EUSES) model (Berding et al., 1999).

Another analytical solution model that has been refined to include metal-specific features is the Quantitative Water Air Sediment Interaction (QWASI) model (Mackay, 1991; Mackay et al., 1983). QWASI, developed as a fugacity model for application to lakes and rivers, is used to simulate the exchanges of chemicals with measured vapor pressure between air, water, and sediments (the Unit World Model for organic substances). A numerical solution version was subsequently developed, one that considers multiple species, as occurs with metals (Diamond et al., 1992). More recently, it has been coupled with MINEQL to allow for interconversion of species and consideration of metals in dissolved, colloidal, and particle phases in water. It is known as the TRANSport and SPECiation (TRANSPEC) model (Bhavsar et al., 2004) and is not yet applicable to sediments.

The remainder of the models listed in Table 4-13 and described below are numerical solution models. The first four are applicable to steady-state conditions and the remainder to time-variable conditions. The steady-state models are appropriate for use in a screening level or definitive level of analysis. Their main advantage in comparison to analytical solution models is their capability to represent more complex system geometry and transport regimes (at the expense of an increased level of effort required to set up the model). The Chemical Transport and Analysis Program (CTAP) is essentially a numerical solution version of SLSA, with the added capability of being able to represent two- and three-dimensional systems and multiple-particle size classes. Pawtuxent Toxics (PAWTOXIC) is a relatively simple one-dimensional model for estimating particle deposition or resuspension in river and streams, assuming EqP conditions. PAWTOXIC does not include exchange of dissolved metals between water and sediments, and it can be used only to simulate net settling or resuspension.

The Simplified Method-Program Variable-Complexity Stream Toxics (SMPTOX) is of particular interest for metals because it represents one of the first models to include the simulation of AVS and SEM (see Section 3.4) for copper, cadmium, nickel, lead, and zinc. It does not consider the potential for oxidation of metal sulfides. SMPTOX, another model that has evolved from the SLSA framework, is applicable for use in simulating a one-dimensional river or stream and it also includes both particle settling and resuspension and a diffusive flux of dissolved metals between water column and sediments. As a Windows-based model, it is relatively simple to use.

The last steady-state model to be considered, Metals Exposure Analysis Modeling Systems (MEXAMS), links EXAMS (a widely used fate and transport model developed by EPA) with MINTEQ, a chemical equilibrium model (Felmy et al., 1984) (see Section 4.1, Environmental Chemistry, for further discussion of MINTEQ). MEXAMS has not yet been coupled with EXAMSII (a time-variable model), so it can handle only steady-state conditions. EXAMS can be applied to a one-, two-, or three-dimensional system and includes an interactive bed layer. By transferring total metal concentrations to MINTEQ in an iterative process, EXAMS is able to calculate fate and transport of metal species. It requires the user to specify a bulk exchange coefficient to represent water-sediment exchange.

The remaining models listed in Table 4-13 include the capability to perform time-variable simulations. As a general rule, such models also can be used to simulate steady-state conditions, such as determining simple waste-load allocations during critical low-flow conditions. Input requirements will be relatively high for time-variable models, as the user may need to specify time variable inputs (e.g., upstream and tributary flows and concentrations, point source loads). The Exposure Analysis Modeling System II (EXAMSII, Version 2.97) is a relatively well-known model that was originally developed for steady-state applications. EXAMSII also represents bulk exchange of water and solids between the water column and sediment, rather than separate terms for particulate and diffusive fluxes of metal. It has the advantage of being very flexible with regard to execution of both simplified steady-state assumptions and more realistic (albeit more complex) time-variable analyses. It has also been widely used for many years and is well documented.

River Quality II (RIVEQLII) (Chapman, 1982) was developed specifically for inorganic substances. It incorporates relatively sophisticated chemical equilibrium calculations through linking with MINEQL (Westall et al., 1976) (see Section 4.1.5.1 for further discussion of MINEQL). RIVEQLII is applicable to one-dimensional rivers and streams. It assumes equilibrium conditions and incorporates particle setting to the bed, sorption reactions, and precipitation and dissolution of chemical to and from the sediment bed.

The next four models listed in Table 4-13, WASTOX, RCATOX, WASP5, and META4, evolved from an earlier model, the Water Quality Analysis Simulation Program (WASP) (DiToro et al., 1981). The Water Quality Analysis Simulation of Toxics (WASTOX) (Connolly and Winfield, 1984) incorporates particulate and dissolved transport processes that are very similar to SLSA and CTAP. Row-Column AESOP for Toxics (RCATOX) (HydroQual Inc., 2003) is a more recent implementation of WASTOX, one that has the potential to take advantage of parallel processing capabilities. Since RCATOX is still under development, it has not yet been released for general use.

The Water Quality Analysis Simulation Program, Version 5 (WASP5) (Ambrose et al., 1993) is the version of WASP that is currently supported by EPA. Essentially, WASP5 incorporates many of the EXAMS capabilities, plus refined sediment transport capabilities. WASP5 is distributed with two different subroutines: EUTRO, which is used in eutrophication problems, and TOXI5 for simulating fate and transport. The WASP5 package also includes an associated food chain model. Notably, WASP5 and RCATOX are designed to interface with state-of-the-art hydrodynamic and/or sediment transport models, a feature not shared by any of the other available fate and transport models. This capability would be of use in estuarine and coastal systems having complex hydrodynamic conditions. WASP5 interfaces with the PC-compatible hydrodynamic model DYNYD5 (Ambrose et al., 1993). WASP5 and RCATOX can both interface with the Estuary, Coastal, Ocean Model (ECOM), a family of hydrodynamic models (Blumberg and Mellor, 1987), and with ECOMSED (HyroQual, 1998), a sediment transport model.

The Metal Exposure and Transformation Assessment Model (META4) model is essentially WASP5 combined with MINEQL. It is used for developing TMDL waste-load allocations and evaluating remedial actions and TMDLs (Martin and Medine, 1998). It is applicable to a variety of receiving waters, including ponds, streams, rivers, lakes, and estuaries, and can be run as one-, two-, or three-dimensional systems. META4 addresses some of the shortcomings in WASP4 to more accurately describe metal dynamics by, for example, the recent addition of subroutines to represent the interactions of dissolved metals with iron oxyhydroxides in the water column and sediment under variable pH and the ability of the model to predict future concentrations of the major cationic metals under variable regimes of water chemistry. It also handles numerous point and nonpoint loads and sequential deposition or scouring of sediment bed layers.

The Delft 3D model (DELFT3D), developed by Delft Hydraulics Lab in The Netherlands, is another model that is quite sophisticated with regard to its capabilities. It is a flexible integrated model that includes the following modules: hydrodynamics, water quality (including sediment transport), chemistry, and wave generation. Although the model software is proprietary, the model itself is commercially available.

MIKE21, developed by the Danish Hydraulic Institute (DHI), is another commercially available fate and transport model that is applicable to rivers. This three-dimensional model includes both particulate and dissolved transport processes. One of the modules of a recent release may be used to simulate the fate, transport, and bioaccumulation of metals.

The Hydrologic Simulation Program-FORTRAN (HSPF) (Bicknell et al., 1993) is the most recent version of a family of models originally developed in the 1960s to simulate agricultural runoff. It is unique among the models listed in Table 4-13 because that it includes hydrology and nonpoint-source terrestrial runoff modules, in addition to in-channel fate and transport capabilities. HSPF is frequently used to simulate complex watersheds and provides information for continuous input simulations or for storm events. It models well-mixed reservoirs as well as branched river systems and can include up to three sediment types. It has been widely used throughout the United States, but its high level of sophistication makes it most appropriate for use by experienced modelers.

RECOVERY, a model supported by the U.S. Army Corps of Engineers, has been developed with features that make it amenable to evaluating sediment capping alternatives (Ruiz et al., 2000; Boyer et al., 1994). The model is structured as a well-mixed surface-water layer overlying a vertically stratified but horizontally well-mixed sediment bed. The sediment is defined by three zones (surface, deep contaminated, and deep clean), which can be further subdivided on the basis of porosities, contaminant concentrations, and other factors. RECOVERY is particularly useful for evaluating sediment-capping scenarios and sites with old contamination (where clean sediment has layered over the contaminated bed).

One other model that is nearing release for use as a fate and transport model is the Environmental Fluid Dynamics Code (EFDC) (Hamrick, 2002). EFDC, originally developed as an advanced level hydrodynamic model, was subsequently modified to include sediment transport capabilities. It is a state-of-the-art model that incorporates a variety of options for representing sediment transport. Most recently, it was further refined for use with chemicals, with an initial application to metals in which two-phase linear partitioning was used (Ji et al., 2002); a second application was the ongoing analysis of polychlorinated biphenyls (PCBs) on the Housatonic River in Connecticut. The chemical fate subroutine does not currently evaluate metal speciation. EFDC is scheduled for release in the near future.

Water quality analyses often require probabilistic results, as the WQC in the United States are expressed in probabilistic terms, with an allowable 1-in-3-year exceedance frequency. Steady-state models cannot evaluate a return period for exceedences. When using these models, a Monte Carlo analysis can be conducted to generate a large number of model inputs and subsequent solutions that can then be analyzed probabilistically to obtain a characterization of the probability of exceeding a particlar concentration. Time-variable models that generate long-term simulations (e.g., 20 years) can also be statistically analyzed to evaluate the exceedence frequencies. Several statistical models have been developed specifically for conducting these types of analyses. The Probabilistic Dilution Model (PDM) (Di Toro, 1984) is one such model. Program Monte (HydroQual, 1997) is a Monte Carlo program that generates a time series of daily concentrations. The Dynamic Toxics (DYNTOX) model (LTI, 1994) is an EPA model that uses three statistical methods to predict the frequency of water quality standard violations. RIVRISK, one of the steady-state analytical solution models described above, also contains a built-in Monte Carlo simulator.

Transport and fate models will vary considerably in their ease of use, with the simpler one-dimensional and analytical solution models placing relatively modest demands on the user. Although it may be a simple matter to set up and run this type of model, their proper use requires the user to exercise a considerable amount of judgment. More complex models become increasingly difficult to use and interpret, and errors associated with setting up the model are not always easy to detect. Hence, it is important that the relatively sophisticated modeling analyses be performed by an experienced analyst, one who is familiar with the details of the processes included in the model being used. Owing to the complexity of setting up and parameterizing the more complex models listed in Table 4-13, particularly for analysis of a complex problem setting, their use will often be limited to relatively refined definitive assessments.

Further detailed discussions about features, limitations, and example applications of the models discussed in this section are provided by Paquin et al. (2003). This review also identifies additional models designed for use in specialized regions of water bodies, such as mixing zones or plumes. The appendix includes information about where to obtain many of models discussed

4.4.1.1.2. ***Limitations.*** The partition coefficient, which controls the distribution of metal between the dissolved and particulate phases, is considered to be a key model parameter in chemical/metal fate and transport evaluations (see Section 4.1.4). It is important for several reasons. First, the distribution of metal between the dissolved and particulate phases has a direct bearing on the magnitude of particulate fluxes of metal that occur in association with the settling and resuspension of sorbed metal. Second, it also controls the magnitude of diffusive fluxes of metal between the sediment interstitial water and the overlying water column, as this flux is proportional to the concentration gradient of total dissolved metal (free ionic metal + metal-DOC and metal-inorganic ligand complexes) between these compartments. Partitioning reactions also affect the metal bioavailability and the route of exposure to a metal (food vs. water). Given its importance, it is necessary to recognize that the assumption of equilibrium partitioning is a potential oversimplification in some situations (e.g., near a point source discharge to a receiving water, or immediately following a pulse exposure of such as an overflow from a storm sewer system). Under these conditions, the assumption of equilibrium between the dissolved and particulate phases may be less appropriate than is otherwise the case and additional uncertainty will necessarily be associated with the model results. If this situation exists, consideration should be given to conducting special studies (e.g., measurement of free metal concentrations over time in a water sample) to test the validity of the equilibrium assumption.

For neutral organic chemicals, the magnitude of the partition coefficient is often assumed to be proportional to the dissolved organic carbon content of the particles. Use of linear partition coefficients is likely to be an oversimplification in the case of metals, where a variety of sorption phases may be important. Several models are available that provide a relatively detailed representation of these metal-particle interactions (see Paquin et al., 2003, for a review).

Many models that were initially developed for organic substances assume first-order decay processes. These are of little importance for metals, but they sometimes are used in screening-level assessments to represent removal of sorbed material from the water column. This approach provides a relatively simplistic representation of the underlying processes and should be used with caution. Given the capacity of most computing environments today, the potential exists to use relatively sophisticated metal-specific sorption and water-sediment flux models, even for simple assessments. However, until such subroutines become more widely available, their use will be problematic, especially for an inexperienced user. Modification of existing models may be an option, but this should be attempted only by an individual who is experienced in model development and programming.

Modeling of mercury and metalloids, such as arsenic and selenium, is complicated by transformation processes that change the form of the metal. Methylation of mercury and arsenic or binding of selenium to amino acids (e.g., selenomethioine) changes both their physical and biological properties (see Section 4.1. for a discussion of transformation processes). Similarly, metals that readily change oxidation state (e.g., chromium) also require additional considerations in fate modeling. Many of the same transport models can be used, but input parameters will require modification. Such models are beyond the scope of this framework, which is focused on inorganic forms of metals. See, for example, the Mercury Cycling model by Hudson et al. (1994) for further guidance on fate and transport models for mercury.

All modeling exercises are limited by the validity of the model framework, the accuracy of input parameters, and the experience of the analyst (Dzombak and Ali, 1993). All exposure assessments should include an explicit description of model assumptions and associated uncertainties.

4.4.1.2. *Terrestrial*

Movement of metals through soils is dependent on the chemical properties controlling speciation, the presence of ligands that control complexation of metals within pore water (and ground water) and adsorption onto mineral surfaces, and the rate of water flux through the soil. Metals are lost from the soil primarily by leaching into ground water, although in particular instances uptake by plants can represent a significant loss. Section 4.1.4 reviews the processes and models that predict movement of chemicals through soils or partitioning onto mineral surfaces (i.e., partition coefficients). These serve the same role as the fate and transport models discussed above for aquatic systems and therefore are not repeated here.

4.4.2. Routes of Exposure to Aquatic and Terrestrial Species

4.4.2.1. *Aquatic Species*

Potential exposure routes for aquatic species include inhalation/respiration, dermal absorption, and diet (either food or incidental sediment ingestion). The extent to which a metal is taken up by any one of these exposure routes is difficult to define for all relevant routes. Inhalation/respiration, which for aquatic species means general exchange across respiratory surfaces, can involve diverse gill and lung types. Respiratory surfaces include fish gills, various molluscan and arthropod gill types, the pseudo-lungs of pulmonate gastropods, cells surrounding the sponge spongocoel, mammalian and avian lungs, and plant leaves. Absorption includes movement across the skin; the walls of such diverse structures as spongocoels, the cnidarian gastrovascular cavity, and the echinoderm water-vascular system; the filtration-based feeding structures of many crustacea, insects, and polychaetes; diverse phytoplankton cell membranes; and plant roots and leaves. Ingestion pathways can involve direct consumption or consumption after exchange through a trophic web. The diversity of potential receptors makes definition of exposure pathways more difficult for aquatic receptors than for humans.

The respiration/inhalation route is a particular challenge in aquatic exposure assessments because of the differing types of respiratory organs, the dynamic nature of the respiratory process in water, and the intimate contact between a receptor and metals dissolved in waters.

Further complicating the issue, some respiratory organs can also be involved in locomotion, excretion, ion regulation, and food capture, sorting, and ingestion. The absorption route can involve uptake across a phytoplankton cell membrane, amphibian skin, arthropod exoskeleton, the general integument of an infaunal clam or annelid, or the egg membrane of a bird or amphibian. Absorption can occur from overlying or interstitial waters; these sources have very distinct chemistries that influence metal bioavailability.

Similarly, the ingestion route is difficult to define for aquatic receptors because of the diversity of feeding modes and food sources, such as sediments, suspended solids, microflora, animal tissues, and plant tissues. The combining of respiratory, locomotive, or feeding structures complicates description of the ingestion pathway for some species. The presence of life stages that feed differently also confounds exposure assessment.

Despite the complexities associated with quantifying exposure of aquatic animals to metals from multiple routes of uptake, the relative importance of the different uptake pathways has received considerable attention in recent years (Wang 2002; Hook and Fisher, 2001; Fisher et al., 1996; Bjerregaard et al., 1985). Bioenergetic-based kinetic models used to describe the accumulation of contaminants in aquatic animals have been developed relatively recently and have been successfully applied to a variety of organic and inorganic contaminants. These models provide a broad framework for addressing controls on contaminant bioaccumulation for diverse organisms and can be used for studying contaminant bioavailability and determining the relative importance of different routes of contaminant accumulation, including that of metals (Landrum et al., 1992; Wang et al., 1996). The models are flexible enough to incorporate environmental variability in contaminant sources, contaminant concentrations, food availability, and organism growth rates in their predictions of organism contaminant levels.

Applications of one-compartment biokinetic models using laboratory-based measurements of key model parameters (assimilation efficiency, uptake rates from water and food, elimination rates) have been extended to field situations for populations of marine mussels (Wang et al., 1996; Fisher et al., 1996); Ag, Cd, Co, and Se in clams (Griscom et al., 2002; Luoma et al., 1992); Po in copepods (Stewart and Fisher, 2003; Fisher et al., 2000); Se in fish (Baines et al., 2002); and freshwater mussels (Roditi et al., 2000). Site-specific model predictions for metal concentrations in animal tissues are strikingly close to independent field measurements for diverse water bodies, suggesting that it is possible to account for the major processes governing contaminant concentrations in aquatic animals and that the laboratory-derived kinetic parameters are applicable to natural conditions.

Temporal Aspects of Exposure

Temporal aspects of exposure are important considerations in assessing aquatic risks to metals. Organisms may respond vary different to episodic exposure than they respond to the constant exposures incorporated into classic toxicity bioassays. Sound risk analyses clearly articulate all assumptions about exposure durations.

Temporal aspects of exposure should be considered in assessments of any toxicant. Rapid speciation and phase changes associated with changes in pH/Eh make temporal issues particularly germane to metals. Fluctuating or pulsed exposures occur in situations such as rapid changes in pH/Eh associated with photosynthesis and respiration, hypolimnetic discharge from stratified reservoirs, biocide (e.g., copper sulfate) spraying, ingestion of prey items with seasonally high metal concentrations, surface waters receiving wastewater treatment plant effluent, urban storm water, snowmelt, and acid precipitation runoff. Transient metal concentrations may be orders of magnitude higher than background concentrations but may last for only a few hours. These episodic exposure scenarios have been poorly characterized for metals (Hoang et al., 2005). Any risk assessment for metals should clearly state all assumptions about duration of exposure.

Water pathway and respiratory route. For acute exposure of most water column organisms, binding of metals to the gill (respiratory) surface is the primary route of exposure. Binding of metals to the gill surface is the primary route of exposure for most water column organisms, at least for short-duration exposures. Chemical kinetics play an important role in this context, as gradients in pH and ionic composition exist at the gill surface microlayer due to respiration, excretion, and ion regulation. Dissolved aluminum toxicity to freshwater fish is a good illustration of this point (Playle and Wood, 1990). The deposition of aluminum on gills is determined largely by the rapid shift from dissolved ionic aluminum to an $Al(OH)_3$ precipitate on the gill surface due to the more alkaline state of gill microlayer water. How much aluminum speciation and phase association changes occur is a function of the initial pH of the bulk water. Exposure assessment should address metal speciation kinetics in such situations (see Section 4.1, Environmental Chemistry) or else inaccuracies will emerge in subsequent effects assessment.

Primary Route of Exposure

Binding of metals to the gill surface is the primary route of exposure for most water column organisms for short-duration exposures.

4.4.2.1.1. ***Application*****.** Predicting exposure at the respiratory surface should include analysis of chemical speciation, chemical kinetics, and binding with biological ligands on the gill (or equivalent respiratory surface). The FIAM states that, in general, metal ion availability and effect are correlated with free ion concentration or activity in the water (Brown and Markich, 2000; Campbell, 1995). Consequently, knowledge of free ion concentration or activity is crucial to fully describing exposure. The BLM further suggests that the bioactivity of a metal is a result of its interaction with biological ligands (i.e., biological macromolecules on the surface of the respiratory organ) (see Section 4.5, Characterization of Ecological Effects). It follows that a dissolved metal ion's bioactivity is a function of its complexation with dissolved ligands (which determines how much free ion will be available for binding with biological ligands) and the affinity and stability of the metal complexes with the biological ligands. Further complexation of metals takes place within the cells of the respiratory organ, with the remaining free metal available for binding to transport macromolecules for delivery to the organism's circulatory system; this results in the true delivered dose.

Free Ion Activity Model (FIAM)

The ***FIAM*** states that, in general, metal ion availability and effect are correlated with the free ion concentration or activity in the water. It also recognizes the role of competing ions in reducing metal bioavailability (e.g., calcium reducing zinc bioavailability). The FIAM is a powerful tool as long as it is applied with enough understanding to anticipate or recognize exceptions.

Equilibrium speciation can be used to estimate the aquatic free ion concentration and its resulting activity. In some cases, the free ion can be measured directly during an exposure assessment. Combining insights from the FIAM approach with those from the BLM and HSAB (see Section 4.1, Environmental Chemistry) theories allows general prediction of metal activity on biological surfaces as different as fish gills (Janes and Playle, 1995; Reid and McDonald, 1991; Pagenkopf, 1983), green algae (Parent and Campbell, 1994; Crist et al., 1988), and bacteria (Azenha et al., 1995). Entry across the integument could also be addressed with conceptual tools of the FIAM and BLM (Krantzberg and Stokes, 1988), but this application is not yet sufficiently developed for immediate use.

In the absence of sufficient information about speciation, exposure concentrations for dissolved metals such as cadmium, lead, and zinc can be normalized on the basis of water hardness. Most practitioners fit linear models (log of toxicity endpoint = log a + b [log of hardness] and back-transform them to produce a normalizing function:

$$\text{Toxic endpoint} = 10^{a}\,(\text{hardness})^{b}$$

This power function contains an easily corrected back-transformation bias that should be removed from predicted hardness-adjusted exposure concentrations (Newman, 1991). The use of hardness as a normalizing function should be done only when information on speciation is lacking, as there is greater uncertainty using this method.

4.4.2.1.2. ***Limitations.*** The FIAM is a powerful tool as long as it is applied with enough understanding to anticipate or recognize exceptions. As examples of important exceptions, charged uranium complexes are toxic, in addition to the free ion (Markich et al., 2000), and the neutral mercury complex $HgCl_2^0$ is bioavailable due to its high lipophilicity (Simkiss, 1996). Small organic ligands bind metals forming nonionic complexes that are also exceptions to the FIAM. Copper bound to ascorbate was bioavailable to the green alga, *Scendesmus sp.* (Campbell, 1995). Silver bound to glutathione or cysteine was bioavailable to *Ceriodaphnia dubia* (Bielmyer et al., 2002). In addition, some nonionic metal-inorganic ligand compexes, such as NiCO3, may also be bioavailable (Hoang et al., 2004).

The BLM has been parameterized for copper, nickel, silver, and zinc in fish, algae, and invertebrates (*Daphnia, ceriodaphnia*). Some initial work has been completed for other species and metals, but rigorous parameterization/validation is limited to the organisms and metals listed above. Recent efforts in Europe have focused on extending BLM principles to describe chronic toxicity; the results should become available in the near future. At present, the BLM approach assumes that delivered dose is equivalent to the amount of metal bound to the gill (or other respiratory organ), which may overestimate exposure. Models needed to predict within-cell complexation of metals into available and nonavailable pools have not yet been developed.

4.4.2.2. *Food Chain Pathway and Dietary Exposure*

Defining the particulars of metal exposure by ingestion is complicated by the diversity of feeding modes, digestive systems, and physiology of candidate receptors. That dietborne exposure to metals can result in accumulation in aquatic organisms is well established, although the rate and magnitude vary among organisms. What is less well understood is how best to express dietary exposure in a way that can be linked to potentially toxic effects. For nonionic organic chemicals, evidence is strong that whole-body burdens of chemical (normalized to lipid

> Currently, *no* standard approaches exist to assess dietborne exposures of metals to aquatic organisms in water quality assessments, and consequently, the most significant limitation involves a lack of broad understanding of the mechanisms underlying dietborne metal toxicity.

content) are robust metrics of toxicological dose, and these relationships appear to be independent of whether exposure was via water or diet. In fact, the greatest strength of the body residue approach for organic chemicals is that it effectively integrates different exposure pathways into a single expression of dose and toxicological potency.

For metals (aside from organo-selenium and methyl mercury), the situation is far more complex, and whole-body residue does not appear to be a robust indicator of dose when compared across a range of exposure scenarios and/or organisms. The reason that whole-body residue/effect relationships are not as effective for assessing metals probably stems from the fact that although distribution of nonpolar organic chemicals in organisms is influenced largely by passive partitioning, the uptake, distribution, and disposition of metals is governed by a number of active biochemical processes. For example, some organisms take up metal and sequester it into "storage" compartments in chemical forms that have little toxicological potency, whereas other organisms actively excrete excess metals. Even for a particular organism, uptake and disposition of metal may vary between waterborne and dietborne exposure (e.g., Kamunde et al., 2002; Szebedinszky et al., 2001).

Although these issues confound the development of simple dose/effect metrics based on whole-body metal residues, one should presume that residue/effect relationships could be established if there were better understanding of more specific concentration/response relationships for the site (or tissue) of toxic action. For example, some studies have suggested that the metal concentration in the cellular cytosol (as opposed to that bound to cell walls or sequestered in nonbioavailable metal granules) may provide a better expression of internal metal dose associated with toxic effects (Wallace and Luoma, 2003; Wallace et al., 2003; Wallace and Lopez, 1996). Others have suggested that whole-body residue residue-effect relationships are confounded because the factor that determines the effects is not whole-body concentration per se, but the rate of metal uptake in relation to metabolic capacity for detoxification and storage, and, therefore, effects are governed by factors that influence the rate of uptake. When uptake is elevated, the concentration of metabolically active metal at the site(s) of action increases (e.g., the spillover hypothesis) and effects ensue (Rainbow, 2002).

> Assessment of dietborne metal exposure is best considered in two contexts: (1) dietborne exposure leading to accumulation and exposure to higher levels in the food chain (e.g., humans, wildlife) and (2) dietborne exposure leading to direct effects on exposed organisms.

For these reasons, assessment of dietborne metal exposure should be considered in two contexts: (1) dietborne exposure leading to accumulation and exposure to higher trophic levels in the food chain (e.g., humans, wildlife) and (2) dietborne exposure leading to direct effects on exposed organisms. For the former, the primary challenges are to predict the aggregate uptake of metal from both waterborne and dietborne exposure and to express that accumulation in terms that are appropriate to assessing risk to the consuming organisms. Issues that come into play in this scenario include whether forms of metal that are sequestered and detoxified by the aquatic organism are bioavailable to organisms that then consume those aquatic organisms. The available data to date suggest that metals can be sequestered/detoxified via different mechanisms/forms in an organism, with metals sequestered as inorganic granules having greatly reduced to no bioavailability to consumer organisms, while metals detoxified via metallothioneins have relatively higher bioavailability to consumer organisms (Wallace et al., 2003; Wallace et al., 1998; Mason and Jenkins, 1995; Nott and Nicolaidu, 1994). However, owing to different digestive physiologies and other factors, changes in the bioavailability of metals sequestered/detoxified by these different mechanisms or forms may occur (Wang, 2002). With respect to the second scenario, the literature is mixed on the degree to which this is an important pathway for inducing toxicity to aquatic organisms and how best to quantify exposure in that context.

4.4.2.2.1. ***Application*****.** There currently are no standard approaches to assess dietborne exposures of metals to aquatic organisms in water quality assessments (Schlekat et al., 2001). For the organometallic compounds of organo-selenium and methyl mercury, dietborne exposure has been clearly shown to be a primary route of both uptake and toxic effects and should be considered in assessments of these metal compounds. Beyond those two compounds, the picture is much less clear. Toxicity to aquatic organisms from dietborne exposure to metals has been demonstrated where exposure is sufficiently high, although in some cases these concentrations are extreme (e.g., 10,000 µg/g Cu) (Handy et al., 1993). If the dietborne exposure necessary to elicit effects is exceptionally high, it is not clear that this pathway will drive ecological risk, as the environmental concentrations necessary to produce these exposures may be so extreme that ecological risk will occur via other pathways (e.g., direct toxicity of waterborne metal).

In other studies, however, effects from dietborne exposure have been demonstrated at relatively low exposure concentrations, such as in zooplankton studies (Hook and Fisher, 2002; 2001a, b). This raises much greater concern for metals assessment because it raises the potential for toxicologically significant exposures occurring in cases where risk via a waterborne pathway is low. That potential notwithstanding, other studies evaluating dietborne exposure with the same organisms and metals but somewhat different methods have reached different conclusions regarding the significance of dietborne exposure (De Schamphelaere and Janssen, 2004; Metzler, 2003). Dietborne exposure to metals is an area of active research, and it is likely that new insights will be forthcoming to rationalize what now appear to be conflicting data into a more comprehensive understanding of dietborne metal effects. Until that time, the absence of a standard approach will require that decisions regarding the handling of dietborne metal exposure in aquatic assessments be made on a case-by-case basis. Some bioaccumulation models for metals include consideration of dietborne exposure, although few, if any, link this accumulation directly to effects.

In the context of screening for exposure and potential risks to consumers, the use of whole-body inorganic metal concentrations in prey species may have some utility despite the uncertainties associated with trophic transfer and bioavailability of dietary metals (i.e., in cases where whole-body residues are below dietary toxic thresholds). For more definitive assessments, further research is needed on quantifying the bioavailabilty and effects of inorganic dietary metals (with the exception of certain organometallics where dietary toxicity has been well established).

In cases where trophic relationships are not well understood, stable isotope techniques can aid in defining the trophic status of species of concern and can be used to delineate the food web (i.e., who is feeding on whom). The ^{15}N concentration increases relative to ^{14}N concentration with each trophic exchange, and statistical models can link trophic status to metal concentration (e.g., Cabana et al., 1994). Additionally the potential for using stable (and radio)-isotopes of a metals can serve as a tools to understand dietary versus waterborne uptake, particularly in laboratory studies. Other uncertainties include how behavioral changes induced by dietborne metal exposure may affect the survival of the exposed organism (Irving et al., 2003).

4.4.2.2.2. ***Limitations.*** As indicated above, the primary limitation in assessing dietborne metal exposure for aquatic organisms is the absence of a broad understanding of the mechanisms underlying dietborne metal toxicity and the consequent lack of a standard assessment approach. Establishing rigorous residue/effect relationships that integrate waterborne and dietborne metal exposure is a critical consideration. When assessing dietborne exposure through direct measurement from field samples, there are methodological issues to be resolved, such as whether to depurate the digestive system of prey organisms. Metal contained in food or sediment within the digestive system will increase measured body burdens, but it may not have the same biological availability as metal residing within organismal tissues (Chapman et al., 2003). Fractionating body burdens of metals (e.g., cytosolic metal vs. metal granules) has been suggested as a better means of identifying the most readily accumulated fraction of dietborne metal (Seebaugh and Wallace, 2004; Wallace et al., 2003; Fisher and Reinfelder, 1995; Reinfelder and Fisher, 1994), although the interpretation of this information in the context of risk assessment has not been rigorously developed.

4.4.2.3. *Sediment Exposure*

Most situations involving dissolved metals can be addressed adequately by assuming near-equilibrium conditions between water and sediment concentrations (Hoffmann, 1981), as long as one is sufficiently aware that some conditions can lead to important nonequilibrium dynamics (Paquin et al., 2003). Bioavailability, sediment transport, and chemical speciation all affect exposure of benthic organisms to metals. Additional issues of trophic transfer and routes of exposure (gills or equivalent vs. dietary) should be considered in the same manner as discussed above for water column organisms.

Sediment Exposure Estimates

Not all benthic organisms are exposed to sediments. Some feed and respire in the overlying water column. Sediment exposure estimates apply only to those organisms that extract nutrients or oxygen from sediments and pore water.

The most widely used approach for assessing metal exposure in sediment is based on EqP theory, with sulfides as the primary partitioning phase (Di Toro et al., 1990; 1991). Other approaches include sequential extractions with different media (water, weak acids, strong acids) or normalization to total iron or organic carbon. Each has its strengths and limitations. Detailed discussions are provided in Paquin et al. (2003) and Newman et al. (2004); additional information is provided in Section 4.5.9.

4.4.2.3.1. ***Application.*** Several approaches have been taken for estimating exposures to sediment-associated metals that account for bioavailability differences among various sediments. The EqP approach assumes that chemical activity in the sediment, as indexed by chemical concentration in the interstitial water, is proportional to the chemical's bioavailability to sediment-dwelling organisms. In anoxic sediments, sulfides provide the primary binding phase for many cationic metals. These metal sulfides are highly insoluble and are thought to have very low toxicity. Thus, in sediments where there is more sulfide than metal, most metal should be present as sulfides and therefore relatively nontoxic. The amount of reactive sulfide is quantified by measuring the amount of sulfide liberated when sediment is extracted with 1 N HCl. This procedurally defined quantity is known as acid-volatile sulfide or (AVS). The amount of reactive metal is determined from the same extraction by measuring the metal concentration in the acid extractant. This quantity is known as simultaneously-extracted metal or SEM. The potential bioavailability of metal is determined by comparing the relative molar concentrations of the two. When SEM-AVS < 0, sufficient sulfide exists to bind all SEM and metal toxicity is not expected. When SEM-AVS > 0, metal is present beyond the binding capacity of sulfide, and toxicity may occur if there is sufficient excess metal but not sufficient other binding phases to bind the metal. Use of this SEM-AVS as exposure estimates that are correlated with toxicity of metals in sediment has been explored closely for Class B or borderline Class B metals (Berry et al., 1996; Hansen et al., 1996; Ankley et al., 1996; Ankley et al., 1991; Carlson et al., 1991; Di Toro et al., 1990). See Section 4.5.9 for further discussion.

Although the correspondence of SEM-AVS to toxicity was found to be strong in these studies, some question the applicability of the approach to all benthic organisms because it is based on the chemistry of bulk anoxic sediment, and many organisms live in oxygenated burrows. In addition, several studies have shown some degree of metal accumulation in organisms exposed to sediments where sulfide is in excess and metals are thought to be nonbioavailable (or at least nontoxic). A better understanding of the mechanisms of metal accumulation from sediment and their relationship to toxic effects is needed to help interpret these issues. Until such information becomes available, the SEM-AVS model can be used in exposure estimations as long as its shortcomings are acknowledged appropriately.

Sem-AVS

When the molar concentrations of ***acid-volatile sulfide (AVS)*** in sediment exceed the amount of *simultaneously extracted metal* (*SEM*), the metals are expected to associate with the solid phase and not be bioavailable.

Other tools for determining the exposure concentration of sediment-bound metals include metal concentrations in chemical (Fan and Wang, 2001; Babukutty and Chacko, 1995; Tessier et al., 1984), acid (Langston, 1980; Luoma and Bryan, 1978), or biomimetic (Weston and Maruya, 2002; Mayer et al., 2001; Chen and Mayer, 1998) extracts. However, no consensus yet exists on their best use for different types of metals or metalloids. Several

Exposure Assessments for Benthic Organisms

The following information would enhance exposure assessments for benthic organisms: (1) improved computational or analytical methods for analyzing distribution of metal among components of the sediments, (2) improved computational methods for assessing the influences of metal form in sediments on sediment-water metal exchange, and (3) a better understanding of the processes controlling bioaccumulation of metals from solution and food by metazoan species directly exposed to the sediments.

other methods have been proposed. Based on the premise that iron oxides in oxic sediments lower metal bioavailability, iron in a 1 N HCl sediment extract has been used to normalize metal exposure concentrations (Luoma and Bryan, 1978). Increasing concentrations of organic carbon can decrease metal bioavailability (Crecelus et al., 1982), so normalization of sediment metal concentrations to organic carbon content has been useful in other cases. The more readily extracted metals from sequential chemical extraction schemes tend to be the most bioavailable (Young and Harvey, 1991; Tessier et al., 1984) and can be used as exposure metal concentrations.

4.4.2.3.2. ***Limitations.*** Exposure assessment for benthic receptors could be enhanced if a clearer consensus were reached about the utility of each method for different classes of metals, biological species, and sediment types. Specifically, the following information is needed to improve exposure analysis: (1) improved computational or analytical methods for analyzing distribution of metal among components of the sediments, (2) improved computational methods for assessing the influences of metal form in sediments on sediment-water metal exchange, and (3) a better understanding of the processes controlling bioaccumulation of metals from solution and food by metazoan species directly exposed to the sediments (Luoma, 1989).

EPA (U.S. EPA, 2002f) published a report on the application of solid phase AVS equilibrium partitioning sediment benchmarks (ESBs) and interstitial water ESBs as No-Effect guidelines to predict sediments that are acceptable for the protection of benthic organisms. Details of the SEM-AVS method are discussed in Section 4.5.9 and the metals issue papers. The method has been applied to predict toxicity of metals in sediment for Class B or borderline Class B metals. An ESB based on the difference between the concentration of SEM and AVS is appropriate for protecting benthic organisms from the direct effects of sediment-associated metals, and not for estimating metal bioaccumulation. Chapman (2003) discusses limitations concerning the AVS-SEM approach, including the degree to which the EqP approach adequately represents exposures for organisms living in microenvironments in the sediment and/or who are exposed via ingestion of sediment. Site-specific measurements, where possible, are useful for confirming that generalized approaches like SEM-AVS appropriate for specific assessment scenarios. Furthermore, one should recognize that bioaccumulation of metal may still occur even when sulfides are in excess, and the potential consequences of this accumulation should be considered.

4.4.2.4. *Terrestrial Species*

Pathway of Exposure for Terrestrial Organisms

Pathways of exposure for terrestrial organisms to metals include movement from soils through the food web, and to a lesser extent, air deposition either into soils or directly onto terrestrial receptors (e.g., plants).

Terrestrial wildlife, plants, and invertebrates accumulate metals from direct contact with soil or sediment, from ingestion of contaminated food (plants or other animals), and from incidental soil or sediment ingestion. A conceptual model for direct and indirect exposure of terrestrial receptors to metals in soil is presented in Figure 4-6. Pathways of exposure include movement from soils through the food web, and to a lesser extent, air deposition either into soils or directly onto terrestrial receptors (e.g., plants). Because of significant differences in exposure patterns, it is more convenient to discuss methods by receptor group (invertebrates, plants, wildlife) rather than by pathways or environmental compartments.

4.4.2.4.1. ***Soil invertebrates.*** The soil ecosystem includes a complex food web of soil invertebrates (both hard- and soft-bodied) that feed on each other, decaying plant material, and bacteria or fungi. For risk assessment purposes, however, exposure is described as a function of soil concentration rather than a detailed analysis of movement of metals through the food web. This is a reasonable approximation for soft-bodied invertebrates (e.g., earthworms) whose exposure is primarily through soil pore water (from both dermal absorption and soil ingestion) (Allen, 2002). There is more uncertainty in correlating soil concentrations with effects in hard-bodied invertebrates because they are primarily exposed through ingestion of food and incidental amounts of soil. (Sample and Arenal, 2001). Regardless, for all types of soil invertebrates, exposure estimates should account for differences in bioavailability among soil types, which include differential partitioning to soil particles and pore water, metal speciation, and aging. Each of these processes is discussed in detail in Section 4.1, Environmental Chemistry.

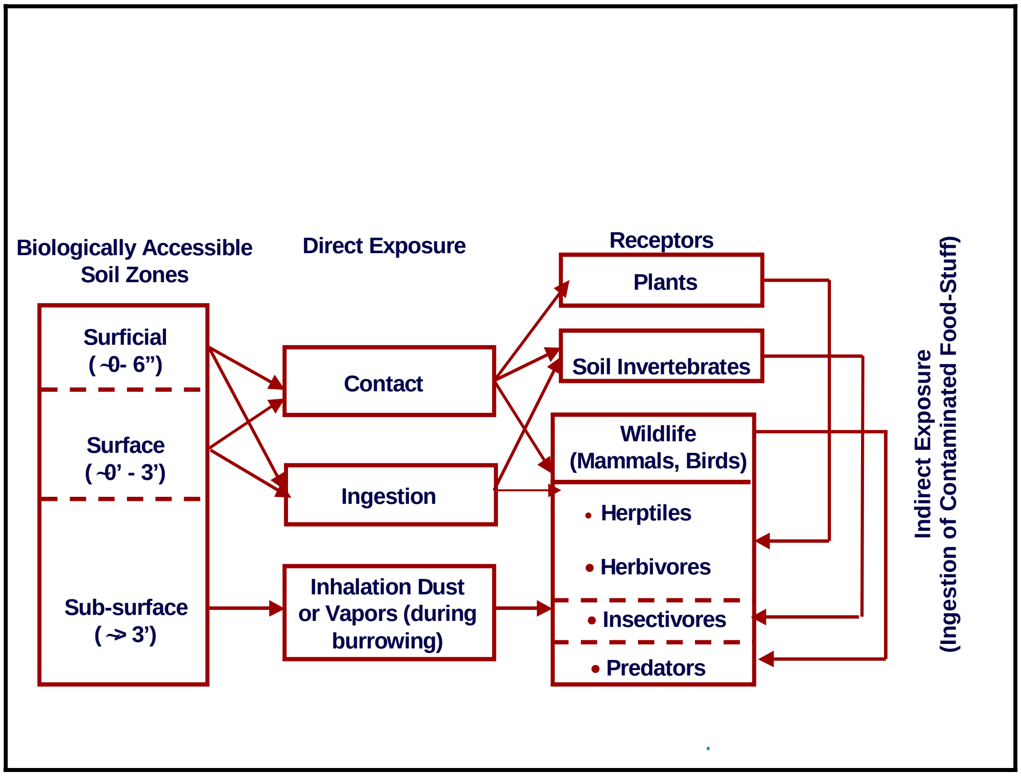

Figure 4-6. Conceptual model for direct and indirect exposure of ecological receptors to metals in soil zones.

Source: Menzie and Little (2000).

4.4.2.4.1.1. *<u>Application</u>*. Currently, soil invertebrate exposure is calculated on the basis of total metal concentration in bulk soils collected in the top 0–12 cm of soil (U.S. EPA, 1989c). In detailed, higher level assessments, the organic matter on top of the soil (the “duff”) may be analyzed separately to provide further detail on exposure to detritivores (such as *Collembola*) and deeper-soil-dwelling organisms (e.g., various species of earthworms). However, such measures of exposure are limited, as they do not account for differences among soils in bioavailability factors.

Cation exchange capacity (CEC) recently has been shown to be an important factor modifying zinc bioavailability in soils for both invertebrates and plants, and presumably it will be important for other cationic metals as well. However, CEC is strongly dependent on the type and amount of organic material and oxyhydroxides present in the soil and is strongly pH dependent. Surface charge on organic material and oxyhydroxides increases with increasing pH, thereby increasing their sorptive capacity for metals (thus decreasing metal bioavailability). Conversely, positive surface charges increase as the pH drops, increasing sorption of anions (e.g., arsenic or selenium) under low pH conditions and decreasing sorption of cation ionic metals. Clays, on the other hand (except for kaolinite), have a surface charge that is largely independent of pH. Therefore, normalization of toxicity data to CEC can be done only within specific soil types and pH ranges, which frequently are not specified either in laboratory bioassays or many field studies. Furthermore, it is important to note that most published values of CEC are measured at pH 7.

Soil chemical models are being developed to predict how aging will modify bulk soil concentrations when soils are amended with soluble salts. Aging reduces the bioavailable fraction of metals over time (see Section 4.1.6.3 for a discussion of aging in soils). Preliminary studies suggest that consideration of aging may result in estimates of the bioavailable fraction as low as 0.1 × bulk soil concentrations. Until the data become available for metals of concern, toxicity values derived from soluble-salt amended soils (which have not simulated aging) cannot be reliably corrected to approximate aged metals in field situations. Appropriate adjustments should be included in toxicity test protocols to simulate aging (McLaughlin et al., 2002), except when assessing acute (short-term) risks of spills.

4.4.2.4.1.2. ***<u>Limitations</u>*****.** Data on CEC for field soils are often available, but similar information from laboratory studies of the toxicity of metal-spiked soil currently in not. Furthermore, the dependency of CEC on soil type (amount and type of organic matter, type of clay, and pH) also complicates the comparison among studies. Therefore, although exposure concentrations can be adjusted across field locations of similar soil type and pH, it is more difficult to make appropriate comparisons of field exposures with laboratory-generated concentration-response functions. Expressing exposure on the basis of pore water concentration is the goal, to reduce the variability in toxicity among sites; however, there are currently significant limitations to collecting and interpreting metal-related data from soil pore waters. Such information is not available in the published literature and, therefore, should be estimated using EqP theory (as with sediment pore water analyses). Published soil binding coefficients (K_ds) can be used, although these values are inherently uncertain as well (published value depends on derivation method, soil type, etc.; see Section 4.1.4 for a discussion on the limitations of K_ds).

4.4.2.4.2. *Plants.* Plants access metals through the pore water, although mycorrhyzae, protons, and phytosiderophores released by the root can significantly influence the microenvironment and change uptake rates of metals (George et al., 1994; Sharma et al., 1994; Laurie and Manthey, 1994; Arnold and Kapustka, 1993). Furthermore, plants have both active and passive mechanisms for taking up or excluding metals, depending on internal concentrations and whether or not the metal is an essential micronutrient, or whether it is mistaken for an essential micronutrient. Plants can be exposed to metals via aerial deposition onto leaf surfaces, trapping metals in hairs or rough cuticular surfaces. This might provide an exposure route for herbivores; it may also provide an exposure route for plants, as there are ion channels through the cuticle that are able to transport ionic metals from the leaf surface to other locations in the plant, depending on the inherent mobility of the metal in the xylem and phloem (Marschner, 1995).

Metal Accumulation in Plants

The highest accumulation of metals in plants generally occurs in the roots.

4.4.2.4.2.1. *Application*. All plant species take up metals from soil through their roots via various mechanisms (Raskin et al., 1994; Cataldo and Wildung, 1978). The default approach to estimating exposure of plants to metal is measuring metal concentrations in bulk soil. However, as with soil invertebrates, this overestimates exposure because it does not account for differential bioavailability that results from complexation. Furthermore, with time, the bioavailability of soil metals may change due to dissolution or complexation; thus, “point-in-time” measurements of a soil may not reflect the future bioavailability. For example, Pb sulfide spilled onto soil (relative bioavailability 1–5%) with time would weather to Pb sulfate (relative bioavailability 50%), which could further evolve to sorb onto Fe oxides or phosphates (relative bioavailability 10–15, 30–59%, respectively). Soil pH, organic matter, and cation exchange capacity are the most important variables influencing bioavailability (see Section 4.5, Characterization of Ecological Effects). However, CEC and clay content are not consistently reported in the literature and therefore cannot be used to define relative bioaccessability and toxicity of metals. General categories of uptake based on soil pH and organic matter are shown in Tables 4-17 and 4-18 in Section 4.5 for ranges typically found in soils. These tables are a useful qualitative guide to identify soils with increased (or decreased) metal bioavailability. It is very clear that strongly acidic soils increase plant uptake of Zn, Cd, Ni, Mn, and Co and increase the potential for phytotoxicity from Cu, Zn, and Ni. Alkaline soil pH increases uptake of Mo and Se, while Pb and Cr are not absorbed to any significant extent at any pH (Chaney and Ryan, 1993).

4.4.2.4.2.2. ***Limitations*****.** Qualitative relationships between soil chemistry and bioavailability are appropriate for national-scale application. However, for site-specific or metals-specific applications, quantitative methods are preferred. It has been suggested that only uncomplexed, free ionic species of cations can be taken up by roots, and this has been described using a FIAM similar to the model used in aquatic systems (Parker and Pedler, 1997; Lund, 1990). Studies have been conducted to generate models to predict free copper activity from total metal content, pH, and organic matter content (Sauve et al., 1997a, b, 1995; McBride et al., 1997), and for lead, empirical models were generated using only total metal levels and pH. However, exceptions to the free-ion model have been identified. Ionic or organometallic complexes that increase the total concentration of elements at the root surface have been correlated with increased uptake, either through disassociated ions or through uptake of intact complexes (Parker et al., 2001; McLaughlin et al., 1994). In addition, it is not clear how well plants can distinguish between ions of similar size and charge. Plant uptake of macronutrients is much better understood than is uptake of micronutrients or contaminants, with the primary work on uptake of micronutrients focusing on iron (Welch, 1995). Different mechanisms have been identified that control macronutrient uptake by plants, providing a means through which contaminants can enter root tissue.

4.4.2.4.3. ***Wildlife.*** The relative importance of exposure pathways and routes varies by species of animal as well as by metal, although, in general, wildlife exposure is primarily through diet and incidental ingestion of soils or sediments. The EPA has concluded that there are certain chemicals and exposure situations for which inhalation or dermal pathways are important, but under most situations they can safely be considered to be insignificant contributors to total metal loads (U.S. EPA, 2003c).

Exposure Pathway for Terrestrial Wildlife

Food and the incidental ingestion of soil are the two most important exposure pathways for terrestrial wildlife.

Wildlife food chain exposures for metals are controlled by bioavailability, bioaccessibility, and bioaccumulation. The availability of metals in soils depends on whether exposure occurs via pore water or other pathways external to the organisms. Bioaccessibility of metals to animals and plants that live on or in the soils can be influenced by a number of soil parameters, such as pH, CEC, and organic carbon. These soil factors tend to be less important for soils that are incidentally ingested by wildlife species. For further review of soil bioavailability factors that are important in wildlife exposure, see Section 3.4.6.3, Ecological Effects (Wildlife).

4.4.2.4.3.1. ***Application*.** In the absence of site-specific information, the following generalizations can be used:

- Incidental soil ingestion is a proportionally more important pathway for herbivores than for carnivores or invertivores.

- Uptake into soil invertebrates (e.g., earthworms) is a proportionally more important pathway for animals that feed on these organisms. (Note: This assessment reflects work done with earthworms and may not apply to hard-bodied soil invertebrates such as *Colembolla*.)

The relative importance of exposure pathways (soil vs. food chain) is dictated by the fraction of metal-contaminated soil in the diet and the amount of accumulation of metal in food items. Figure 4-7 provides a simple scheme for judging the relative contribution of food and soil before accounting for bioavailability. Incidental ingestion of soil becomes proportionally more important for exposure to wildlife when (1) the bioaccumulation factor (BAF) from soil to food (e.g., to plants or soil invertebrates) is less than 1 and (2) the fraction of soil in the diet is greater than 1%.

4.4.2.4.3.2. ***Limitations*.** Experience at metals-contaminated sites indicates that the above generalizations should be viewed with caution. As site-specific information is acquired, the relative importance of pathways may change. For example, site-specific data may show that the accumulation of a chemical into plants or soil invertebrates is much lower than indicated by the default assumptions. In such cases, incidental ingestion of soil would become proportionally more important. The bioavailability of metals in incidentally ingested soil is also variable, as discussed later. Therefore, when the exposure is being driven by incidental ingestion, refinements of exposure estimates can benefit from a better understanding of bioavailability. Attention should be paid to the bioavailability of metals for which incidental soil ingestion is the predominant pathway and where ecological risk is indicated, although very little information is available on this for most wildlife species.

Both the bioaccumulation factor and life history components interact to control the relative importance of soil or food as the primary metal transfer medium. In general, the following statements can be made about dietary uptake of metals from soil versus from food:

- If bioaccumulation is low (<<1), importance of soil ingestion versus diet for metal exposure increases.
- When bioaccumulation is greater (~1 or higher), the food pathway should dominate.
- The closer the association an animal has to the ground, the greater the importance of soil ingestion. This association may be due to ground foraging, burrowing habits, etc.
- The looser the association with the ground (e.g., piscivores, aerial/arboreal insectivores, raptors), the lower the importance of soil ingestion.

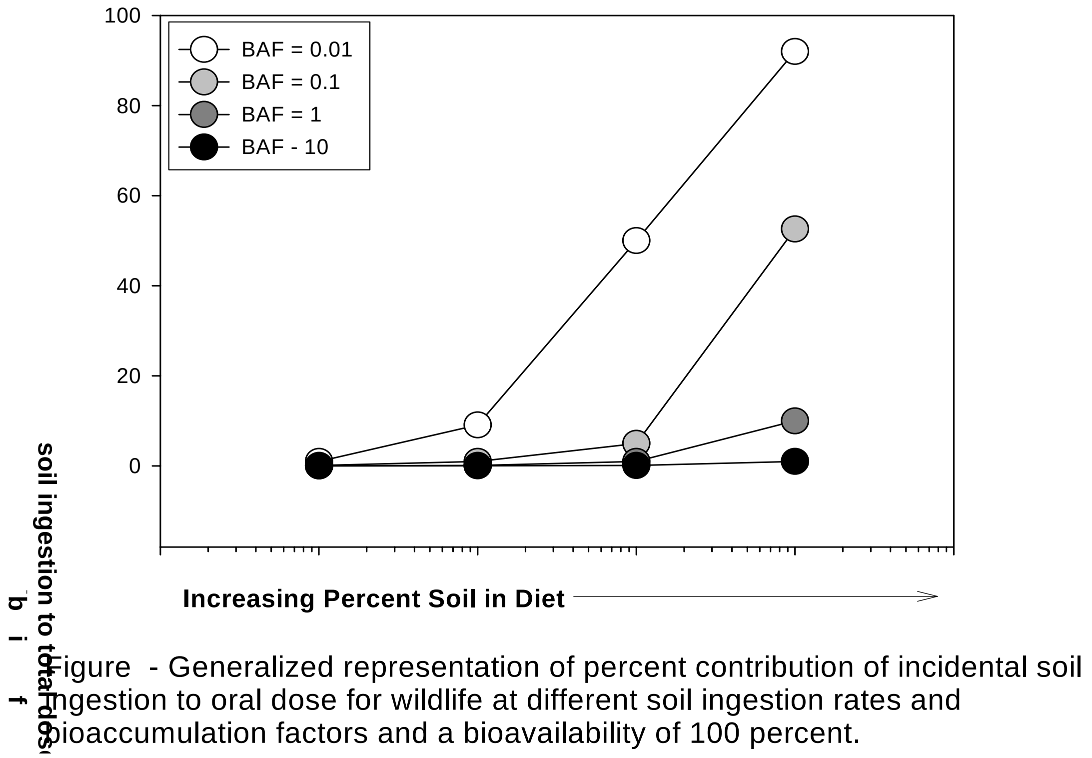

Figure 4-7. Relative contribution of incidental soil ingestion to oral dose for wildlife at different soil ingestion rates and bioaccumulation factors, and a bioavailability of 100%.

Extrapolation from models developed for estimation of bioavailability of metals in soils for incidental human exposures may not be broadly applicable to all wildlife species owing to the influence of differences in digestive physiology and anatomy across the broad and diverse range

of mammalian and avian species (Menzie-Cura and TN&A, 2000). For example, metals present in soils may be more or less bioavailable within the gut of an herbivore that relies on fermentation as compared to the simpler gut of a carnivore that is designed to break down proteins. These gut systems differ in chemistry (including pH) and residence time. For example, ruminants such as deer, antelope, and other hoofed stock initially process food through microbial digestion in the rumen, and their gut pH is general neutral or slightly acidic. Furthermore, end products of microbial digestion in domestic sheep bind to copper and enhance its uptake in the proximal intestine, resulting in a very low tolerance of these animals to dietary copper (NAS/NRC, 1980). Hind gut fermentors such as horses, rabbits, and granivorous birds (e.g., grouse or pheasants) also rely on microbial processes for digestion of lignen and other plant parts but have a more acidic foregut than do ruminants. Most insectivores and carnivores, on the other hand, have relatively acidic digestive systems with a significant amount of protein present. While the low pH may alter the metal speciation (or dissociate ions from attached ligands), most metals require active transport to move through the gut wall and into the circulation. This may be accomplished through binding with transporter proteins present either in the diet or within gastrointestinel cells, which likely differ among the wildlife species (Hill, 1979). Taken together, these physiological differences are significant and make it difficult to accurately extrapolate dietary toxicity thresholds across species. However, thoughtful application of this information will allow appropriate inferences to be made across species with similar physiology and may explain instances where statistical projections of toxic thresholds (e.g., species sensitivity distributions) appear to not be predictive of actual effects.

4.4.3. Food Chain Modeling for Wildlife

4.4.3.1. *Application*

Food chain modeling can be used to estimate the exposure of wildlife to metals based on ingestion of soil, food, and water. The basic format of the model is the same as that for organic substances and is shown in Figure 4-8; detailed explanations are available in several related documents (e.g., U.S. EPA, 2003c [EcoSSLs]; U.S. EPA, 1997d [ECOFRAM]; Sample et al., 1997). Measured, or predicted/estimated, concentrations of metals in soil, surface water, and food items can be used in the model, or concentrations in food can be modeled using trophic transfer factors. TRIM.FaTE (http://www.epa.gov/ttn/fera/human_apex.html can be used for modeling media concentrations of metals as a result of aerial deposition. Information on diet, foraging area, and the like can be found in CH2M HILL (2001) and Sample et al. (1997). The absorbed fraction variable accounts for differences in relative bioavailability (RBA), and is either 1 (default value) or an appropriate estimate. Note that there is no relative absorption factor (RAF) included for food, because the default assumption is that site bioavailability equals that from the toxic form used in the toxicity tests

This approach is the same as that used in risk assessments of organic substances except when trophic transfer rates are used to model food concentrations only on the basis of soil measurements (rather than using direct measures of concentration of metals in food items), in which case metal-specific transfer rates are required. As with aquatic organisms, trophic transfer values for metals in terrestrial systems are an inverse function of soil concentrations. Therefore, it is inappropriate to use constants for this term. Sources, use, and limitations of function of soil where apparent uptake ratios are greater at the lowest and highest concentrations of metals in soils as compared with the middle concentrations of metals in soils and where tissue metal concentrations remain stable over a wide range of soil metal concentrations.

Trophic Transfer Values

Trophic transfer values for metals in terrestrial systems are an inverse function of soil concentrations; therefore, it is inappropriate to use constants for this term.

Sample et al. (1998a) developed uptake models to predict concentrations in earthworms from soil concentrations. These models can be used to estimate the exposure of both the worms themselves and of vermivorous wildlife (e.g., song birds, voles, and shrews). For selected metals (arsenic, cadmium, copper, mercury, manganese, lead, and zinc), the best estimate of tissue concentration in earthworms is a simple ln-ln regression. The addition of soil pH data to the regression model did not markedly improve fit. If soil calcium concentration was incorporated into the regression model, a better fit could be obtained for cadmium and lead but not for other metals. Tissue concentrations were inaccurately estimated for the transition metals nickel and chromium by both simple and multiple regression models. For general estimates, log-linear regression models may be used as bioaccumulation models for arsenic, cadmium, copper, mercury, manganese, lead, and zinc in earthworms. For site-specific assessments, it is recommended that location-relevant bioaccumulation models be developed through direct measurements of local soil and tissue concentrations.

Oral intake (mg/kg/day) =

$$\left[Soil_j \times P_s \times FIR \times AF_{js}\right] + \left[\sum_{I=1}^{N} B_i \times P_i \times FIR \times AF_{ij}\right] \times AUF + \left[Water_j \times WIR\right]$$

where

$Soil_j$ = Concentration for contaminant (j) in soil (mg/kg dw)
P_s = Soil ingestion rate (proportion of FIR)
FIR = Food ingestion rate (kg/kg-body weight/day, dw)
AF = Absorbed fraction of contaminant (j) in biota type (i)
N = Number of different types of biota in the diet
B_i = Contaminant concentration in biota type (i) (mg/kg dw)
Note: ($Soil_j \times T_{ij}$) can be substituted for B_i where T_{ij} = soil-to-biota trophic transfer factor (TTF) [as dry weight to dry weight] for contaminant (j) and biota type (I)
P_i = Proportion of biota type (i) in diet (unitless)
AUF = Area use factor; proportion of available habitat for a wildlife species within the area of concern (%)
$Water_j$ = Concentration for contaminant (j) in water (mg/L)
WIR = Water ingestion rate (mL/kg/day)

Figure 4-8. Wildlife Oral Exposure Model.
Source: U.S. EPA (2003c).

There is no compilation of plant biota/sediment accumulation factors (BSAFs), but U.S. EPA (2003c) (EcoSSLs) provides some data on select metals. For national-level assessments, uptake factors for plants provided by Efroymson et al. (2001) should be used. The highest accumulation of metals in plants occurs in the roots, although other parts of the plant also accumulate metals to varying degrees (Mitrofanov,1993; Greszta, 1982). With the exception of a few hyperaccumulator species, most plant species do not bioconcentrate metals (i.e., BAFs <1). Lead, arsenic, chromium, and cobalt are not taken up by plants in measurable quantities, and the small amount that is taken up is mostly confined to root tissues (Chaney et al., 2000; McGrath, 1995; Chaney and Ryan, 1994; Xu and Thornton, 1985). Exceptions to this exist, notably including, but not limited to, Se and Mo: uptake of these metals into the edible portion of plant tissues can be quite high, generally not sufficient to cause plant toxicities (McGrath, 1995; Bingham et al., 1986; Foy et al., 1978), but may cause significant food chain toxicities .

In contrast, plants are quite sensitive to some metals (manganese, zinc, copper, for example) and may die before achieving high levels that pose a threat to animals via food chain transfer.

4.4.3.2. *Limitations*

Food chain modeling for wildife is similar for both metals and organic substances. All estimates are limited by the information available about the receptor(s) of concern in terms of dietary preferences, relative amounts consumed of various items, relative bioavailability between laboratory and field, and other factors (see Figure 4-8). For metals, the largest uncertainty in the dietary uptake model is in the estimates of trophic transfer factors. As discussed above, these generally are not constants and therefore require the use of uptake equations (i.e., tissue concentrations vary as a function of soil concentration). This necessitates the use of at least quasi-probabilistic modeling, rather than a more simple determination of a single, deterministic oral uptake value.

Use of either measured or modeled tissue metal accumulation levels as an indicator of potential toxicity is limited by the requirement that the accumulated amount be related to a benchmark effect level (i.e., Critical Body Residue, CBR). Very little information is available for metal CBRs in terrestrial wildlife. This is particularly problematic when whole-body tissue levels are reported because what really matters is the effective metal concentration at the site of action of toxicity. If the concentration at the proximate site of action of toxicity is proportional to the whole-body concentration, then this is a lesser concern. However, if the concentration at the site of action is not proportional to the whole-body concentrations, then direct measures of metal concentrations at the site of action is required, especially at higher assessment levels.

> If the concentration at the site of action is not proportional to the whole-body concentration, then direct measures of metal concentrations at the site of action is required, especially when conducting detailed, higher level assessments.

Furthermore, the absolute level of metal accumulation is not as important as the rate of uptake (Hook and Fisher, 2002; Hook, 2001; Roesijadi, 1992). High uptake rates overwhelm the ability of organisms to sequester or excrete the metal, leaving larger proportions of the accumulated metal in a more bioavailable form. Uptake is believed to occur because of the ability of some organisms to sequester metals that enter the cell (e.g., by inducing the synthesis of metallothionein [MT] or granule formation). Adverse effects are avoided as long as the rate of metal uptake does not exceed the rate at which the organism is able to bind the metal, thereby preventing unacceptable increases in cytosolic levels of bioreactive forms of the metal. If the rate of uptake is too great, the complexation capacity of the binding ligand (e.g., MT) could be exceeded; cytosolic metal levels then become unacceptably high, and adverse effects could ensue. Because measures of uptake rates are not available, static concentrations are used instead.

Measurement of the form of the metal that is present in a tissue may be a more predictable indicator of the potential for effects than is total metal concentration (Mason and Jenkins, 1995; Roesijadi, 1992). Metals also may be bound and sequestered by organophosphorus granules, thereby rendering them nonavailable to bind with other intracellular target enzymes (George, 1982; Coombs and George, 1978). Although a wealth of available data exist on measured total tissue levels of metals, there are few data on intracellular speciation or sequestration of the metals. Although models that can be used to perform this sort of evaluation are currently under development, the ability of such models to be used as a tool in exposure assessment remains to be demonstrated. Therefore, measurement of total metal in plant (or animal) tissue remains the accepted default approach.

In sum, the following factors contribute significantly to uncertainty in food chain models:

- Soil ingestion rates are highly uncertain.
- Diet composition can be highly variable, and diet composition has a significant effect on exposure.
- Relative bioavailability from foods is completely unknown.
- Modeling over a number of trophic levels propagates uncertainty.

4.5. CHARACTERIZATION OF ECOLOGICAL EFFECTS

Metals are naturally occurring substances, and organisms have evolved mechanisms for maintaining homeostasis in the presence of expected exposure levels. However, areas of metals enrichment, particularly when generated by anthropogenic activities, can pose challenges to organisms. Toxicity assessment for ecological receptors exposed to metals requires an understanding of both the natural mechanisms for tolerance for (or, in the case of micronutrients, the use of) metals and the toxicological responses that occur when exposure exceeds the capacity of the organism to regulate its body burdens. Interactions between metals in either their uptake or toxicity (such as Cd/Ca/Zn, Hg/Se, Cu/Mo) also should be considered in toxicity assessments. Risk assessments for metals are further complicated by the need to express the dose-response (or concentration-response) functions of bioavailable units that are functionally equivalent to measures of exposure. This section provides tools and approaches for addressing issues of essentiality, appropriate toxicity tests, novel endpoints (e.g., gene expression), and acclimation or adaptation to continued exposures.

4.5.1. Essentiality

Essentiality, or the requirement for normal organism metabolic function, of many metals is one of the primary factors that differentiates risk assessment for metals and metal compounds from that of synthetic organic chemicals (Janssen and Muyssen, 2001). Some trace elements, such as cobalt, copper, iron, manganese, selenium, molybdenum, and zinc, are necessary for the normal development of plants and animals. In many cases, these metals are added to animal feed and pharmaceutical products (SRWG, 2002) or to plant fertilizers. Other metals, such as arsenic, cadmium, lead, and mercury, are not known to be essential to plant and animal growth and development. Trace elements can be divided into three groups:

Essentiality

Essentiality, or the requirement for normal organism metabolic function, of many metals is one of the primary factors that differentiates risk assessment for metals and metal compounds from that of synthetic organic chemicals.

- Those known to be essential.
- Those that have beneficial metabolic effects but have not been shown to be essential.
- Those that occur widely in living organisms but seem to be only incidental contaminants and are not known to be beneficial (Mertz, 1981).

Table 4-14 classifies the metals addressed in this framework by their known essentiality to organisms. The concept that many metals are required for organism health at one range of concentrations and are toxic in quantities outside of that range has been referred to as the "window of essentiality" (Hopkin, 1989) or the "optimal concentration range" for essential elements (Alloway, 1995; Fairbrother and Kapustka, 1997; Van Assche et al., 1997). For essential elements that exhibit biphasic dose-response curves (Figure 4-8, above), adverse effects resulting from deficiency should be considered, as well as those that result from excessive exposure. Recognition of the window of essentiality, as well as consideration of the biochemical and physiological processes that regulate metals within living organisms, are both important components of ecological effects assessment (Abernathy et al., 1993).

4.5.1.1. Application

The optimal concentration range (or safe intake range) for essential elements should ensure that effects thresholds such as Toxicity Reference Values (TRVs) are not lower than the nutritional requirements for the particular plant or animal species being evaluated. Where TRVs or other effects concentrations (or doses) are intended as thresholds for detrimental effects due to excessive intake), care should be taken to ensure that these toxicity thresholds for essential metals are at the upper end of the optimum range or sufficiency range (at the point where toxic effects begin to occur). If set too low (i.e., in the range where deficiency can occur), the determination of risk will be erroneous. For wildlife, the literature on dietary requirements of essential elements for livestock can be consulted. The NAS/NRC has published useful summaries (NAS/NRC, 1994, 1980), and McDowell (2003) updates this information. Minimum concentrations required for plant growth are summarized in Marschner (1995).

4.5.1.2. Limitations

Because of differences in test conditions among published studies, it may be difficult to directly compare toxicity threshold values with recommended dietary requirements of essential elements. Extrapolation of data among species (e.g., from livestock to wildlife species) also may

Threshold Values

For essential elements, it is important to ensure that effects thresholds, such as Toxicity Reference Values (TRVs), are not lower than the nutritional requirements for the plant or animal species being evaluated. It may be difficult, however, to directly compare toxicity threshold values with recommended dietary requirements because of differences in test conditions among published studies.

In screening-level assessments, toxicity threshold values are advised for application, if they are not lower than estimated requirements. Detailed, higher level assessments may require additional bioassays to characterize the biphasic dose-response curve and determination of both required and excessive threshold levels.

add uncertainty to the effects assessment. Furthermore, addition of safety factors when deriving protective values often results in concentrations significantly below required intake. Derived toxicity threshold values should be used in screening-level risk assessments if they are not lower than estimated requirements. Uncertainty in toxicity thresholds or estimated requirements should be addressed as part of the risk management process. Higher level assessments, where more accurate estimates of effects thresholds are expected, may require additional bioassays to characterize the biphasic dose-response curve and determination of both required and excessive threshold levels. See the following sections for considerations of bioavailability factors, mixtures of multiple metals, and interspecific extrapolations.

Table 4-14. Metals classified by their known essentiality

Metal	Essential (known requirement for health and function)		Beneficial (but not known to be essential)		Nonessential (and not known to be beneficial)
	Plants	Animals	Plants	Animals	
Aluminum (Al)					x
Antimony (Sb)					x
Arsenic (As)				x	
Barium (Ba)					x
Beryllium (Be)					x
Cadmium (Cd)					x
Chromium (Cr)		x			
Cobalt (Co)		x	x		
Copper (Cu)	x	x			
Lead (Pb)					x
Manganese (Mn)	x	x			
Mercury (Hg)					x
Molybdenum	x	x			
Nickel (Ni)	x	x			
Selenium (Se)		x	x		
Silver (Ag)					x
Strontium (Sr)					x
Thallium (Tl)					x
Vanadium (V)				x	
Zinc (Zn)	x	x			

Source: Adapted from a table presented in SRWG (2002) and incorporating data from NAS/NRC (1980) and Barak (1999). Fairbrother and Kapustka (1997) discussed the roots of essentiality of naturally occurring elements.

4.5.2. Acclimation and Adaptation

Organisms have developed various mechanisms to cope with variable background metal concentrations, particularly for those metals that are essential elements (see Table 4-14). If the amount is less than required, passive or active uptake mechanisms are used to enhance internal concentrations, whereas during periods of excessive amounts exclusion mechanisms come into play. These various approaches to homeostasis are discussed in detail in Kapustka et al. (2004). Additionally, organisms can acclimate to suboptimal metal levels by changing various physiological functions, or populations can undergo genetic adaptation and develop increased tolerance to different levels (Rusk et al., 2004; Wallace and Srb, 1961).

The genetic makeup of an organism defines its ability to cope with environmental conditions. Genes can be expressed or remain "silent," and shifts in gene expression can occur when the environment changes. Furthermore, organisms use different portions of their total array of genetic information in different life stages. This shifting of tolerance within the genetically defined limit of the organism is known as *acclimation*. Physiological changes induced by acclimation may be reversed if the environment reverts to the original conditions (Posthuma and Van Straalen, 1993; Prosser, 1986). Tolerance acquired through physiological acclimation processes is not always passed on to offspring; however, the same genetic information that allowed acclimation to occur in the parents will be passed on, so the offspring will retain the ability to acclimate in a similar fashion. If the offspring develop in the altered environment, they will express the set of genes most appropriate for tolerance of those conditions.

Tolerance, Acclimation, and Adaption

Tolerance is the ability of an organism to maintain homeostasis under a variety of environmental conditions, such as variable metal concentrations.

Acclimation is how an individual develops tolerance during its lifetime, and it may be gained or lost. Acclimation is also called ***phenotypic plasticity***.

Adaptation is a genetic change over multiple generations as a response to natural selection. Traits are not lost during single life times. Adaptation is also known as ***genotypic plasticity***.

Genetic *adaptation* results from increased survival of tolerant genotypes and subsequent changes in gene frequencies. However, linking these genetic changes to increased tolerance in the field and identifying the specific mechanisms responsible has proven challenging. Laboratory experiments conducted with F_1 generations obtained from metal-contaminated habitats provide the strongest evidence to support a genetic basis of tolerance (Klerks and Levinton, 1993), and new methods in toxicogenomics (e.g., microarrays) are providing additional insights.

Evidence of adaptation (convergent evolution) for metal tolerance in plants comes from the fact that plants of diverse taxonomic relationships grow on soils high in metals. Metal-tolerant flora have been described for soils high in zinc, nickel, chromium, and copper (Antonovics et al., 1971; Brooks, 1972). These reviews indicate that some species are restricted to the high-metal soils, but other species exist across a broad concentration range. These represent differences in niche breadth (i.e., those restricted to high-metal soils vs. those occurring in soils that have either high or low concentrations of metals).

Costs of Metal Tolerance

Although considerable evidence supports the hypothesis that previously exposed populations will be tolerant to metals, both physiological acclimation and adaptation to contaminants may have specific costs. For example, induction of metal-binding proteins increases metals tolerance but also uses energy normally available for other metabolic processes (e.g., growth, reproduction). Similarly, genetic changes associated with metals exposure might harm populations. Reduced genetic diversity has been reported in populations exposed to contaminants and may result in population bottlenecks as well as increased susceptibility to other stressors.

Metal adaptation in plants often is accompanied by metal adaptation in co-located animals, as selection for metal tolerance is expected to improve fitness in exposed conditions (Posthuma and Van Straalen, 1993). Compared with other environmental parameters, metal exposure is regarded as a strong and stable selective force, and it can lead to rapid evolution of tolerance (Posthuma and Janssen, 1995). Metal-tolerant animals tend to grow fast, mature early, and have a high excretion efficiency (Posthuma and Janssen, 1995).

Metal adaptation in natural populations of terrestrial invertebrates has been demonstrated conclusively for several animals: the terrestrial isopod *Porcellio scaber* and the springtails *Orchesella cincta*, *Isotoma notabilis*, and *Onychiurus armatus* (Posthuma and Van Straalen, 1993). Metal tolerance also has been demonstrated in ticks and a fly species in response to the application of a metal-based pesticide (Posthuma and Van Straalen. 1993). There is evidence for increased metal tolerance in other species, but acclimation and adaptation could not be distinguished (Posthuma and Van Straalen, 1993).

Although there is considerable evidence to support the hypothesis that previously exposed populations will be tolerant to metals, both physiological acclimation and adaptation to contaminants may have specific costs. For example, although induction of metal-binding proteins increases tolerance to subsequent metal exposure, it also uses energy normally available for other metabolic processes (e.g., growth, reproduction). Similarly, genetic changes associated with exposure to contaminants might harm populations. Reduced genetic diversity has been reported in populations exposed to contaminants and may result in population bottlenecks. Furthermore, as tolerant genotypes are eliminated from a population, the reduced genetic diversity may increase the susceptibility of this population to other stressors.

4.5.2.1. *Application*

Metal tolerances of plants and livestock are summarized in Table 4-15. It should be noted that the NAS/NRC (1980) committee that identified the maximum levels of metals tolerated by domestic livestock based its conclusions on data from toxicological-type feeding studies in which soluble metal salts had been mixed with practical or purified diets to examine animal response to the dietary metals. If soil is incorporated into diets, metal solubility and bioavailability may be much more limited than in the tests relied on by the NRC. For example, it has been noted that until soil exceeds about 300 mg Pb kg^{-1}, animals show no increased body burden from ingesting the soil (Chaney and Ryan, 1993). Other metals in equilibrium with poorly soluble minerals or strongly adsorbed in soils are often much less bioavailable than they would be if they occurred in more soluble salts.

It has been shown in studies with *Daphnia* and algae that acclimation of test organisms to culture conditions will significantly influence the outcome of toxicity tests (Janssen and Heijerick, 2003; Bossuyt and Janssen, 2002). Presumably, similar effects occur with terrestrial organisms (e.g., earthworms or springtails) (see Fairbrother et al., 2002). Thus, organisms should be acclimated to the same waters or soils that will be used in the toxicity studies for several weeks before study initiation. This will avoid shocking the organisms by placing them in test conditions that have significantly depleted or elevated levels of endogenous metals (e.g., those that are not the subject of the toxicity study) and ensure that any adverse effects are actually due to the metal in question. However, it is not possible to conduct pre-acclimation for soil microbial studies because in situ populations of microorganisms from previously collected soils are used. This calls into question the applicability of results from microbial function studies, and until these issues are resolved, results should not be used for setting soil criteria (U.S. EPA, 2003e).

PICT

For site-specific assessments, the concept of ***pollution-induced community tolerance (PICT)*** has been proposed as a tool to assess community-level effects. PICT is tested by comparing responses of communities collected from polluted and reference sites to contaminant exposures under controlled conditions. The increase in community tolerance at a polluted site that results from the elimination of sensitive species is considered evidence that this restructuring was caused by the pollutant.

Extrapolation of these results to communities of terrestrial plants and animals should be undertaken with caution, since most of the original research on PICT has been conducted using small organisms with relatively fast life cycles (e.g., benthic invertebrates, soil microbial communities).

Table 4-15. Maximum tolerable levels of dietary minerals for domestic livestock compared with levels in forages

Element	Soil-plant barrier[a]	Level in plant foliage[b]		Maximum levels chronically tolerated[c]			
		Normal	Phytotoxic	Cattle	Sheep	Swine	Chicken
		mg/kg dry foliage		mg/kg dry diet			
As (inorg.)	Yes	0.0l–l	3–l0	50	50	50	50
B	Yes	7–75	75	150	~150	~150	~150
Cd[d]	Fails	0.1–1	5–700	0.5	0.5	0.5	0.5
Cr^{3+}	Yes	0.1–1	20	~3000	~3000	~3000	~3000
Co	Fail?	0.01–0.3	25–100	10	10	10	10
Cu	Yes	3–20	25–40	100	25	250	300
F	Yes?	1–5	–	40	60	150	200
Fe	Yes	30–300	–	1000	500	3000	1000
Mn	?	15–150	400–2000	1000	1000	400	2000
Mo	Fails	0.1–3	100	10	10	20	100
Ni	Yes	0.1–5	50–100	50	~50	~100	~300
Pb[d]	Yes	2–5	–	30	30	30	30
Se	Fails	0.1–2	100	~10	~10	10	10
V	Yes?	0.1–1	10	50	50	~10	10
Zn	Yes	15–150	500–1500	500	300	1000	1000

[a] Indicates whether a plant will die at concentrations lower those that which will affect animals.
[b] Based on literature summarized in Chaney et al. (1983).
[C] Based on NAS/NRC (1980). Continuous long-term feeding of minerals at the maximum tolerable levels may cause adverse effects. NRC estimates levels by extrapolating between animal species when data are not available.
[d] NRC based the maximum levels tolerated of Cd or Pb in liver, kidney, and bone in foods for humans rather than simple tolerance by the animals. Because of the simultaneous presence of Zn, Cd in animal tissues is less bioavailable than Cd salts added to diets.
Yes = Plant dies; Fails = Plant does not act as a barrier and concentrates metal to levels for toxic animals; ? = Unknown.

Most research examining tolerance to metals has focused on population-level responses, although consequences at the community level also occur. The most common explanations for increased tolerance of populations within communities include acclimation and selection for resistant genotypes. However, because communities consist of large numbers of interacting species, it is likely that other mechanisms unique to these systems will contribute to increased tolerance. For example, the replacement of sensitive species by tolerant species, termed "interspecific selection" (Blanck et al., 1988), is a common response in polluted systems and one of the most consistent indicators of metal pollution. Pollution-induced community tolerance (PICT) has been proposed as an ecotoxicological tool to assess effects of contaminants on communities (Blanck et al., 1988). PICT is tested by comparing responses of communities collected from polluted and reference sites to contaminant exposures under controlled conditions. The increase in community tolerance at a polluted site that results from the elimination of sensitive species is considered evidence that this restructuring was caused by the pollutant.

Naturally occurring levels of metals play an important role in biogeographic distributions of plants and animals and may, in fact, be limiting factors in species distributions or use of landscapes. Therefore, it is difficult to make generalizations about effects levels that are applicable and consistent to all organisms in all habitats, and it becomes very important to clearly define the geospatial location of the area to which the assessment results will apply. For site-specific assessments, the assessment results will be directly applicable to the entire range of species that may be found on that site (although for assessments conducted over areas larger than several square miles, it is possible that multiple soil types and other local landforms may result in significant differences in metal bioavailability and plant communities.

For assessments conducted for regional or national assessments, criteria development, or ranking purposes, it should be acknowledged that results will be based on organisms and soil types that result in greatest bioavailability and sensitivity. Care should be taken, however, that the organism-environment combinations that are assessed are, in fact, compatible with real-world conditions. For example, benthic organisms generally associated with slow-moving, warm waters would not be expected to tolerate conditions of high metal biovailability such as those occur that in faster-moving, colder waters that have little organic matter. Thus, for site-specific assessments, species tested and water (or sediment) used in the test system should be similar to conditions at the site. In the absence of such information, data from standard test species and conditions could be used, but uncertainty factors may be warranted to adjust the final toxicity value accordingly.

More appropriately, single-result assessments for the entire country should be avoided. Rather, such assessments should be subdivided into metal-related ecoregions known as "metalloregions" (McLaughlin and Smolders 2001) so that protection levels, mitigation goals, and ranking results will be appropriate for the suite of species naturally present within each type of controlling environment. This is directly analogous to the use of ecoregions when establishing WQC (Griffith et al., 1999). The value of metalloregions is that they provide the conceptual framework to account not only for the broad regional parameters affecting metal availability in soils and waters, but also for the differences in organism response to added metal.

4.5.2.2. *Limitations*

Although there is considerable evidence to support the hypothesis that previously exposed populations will be tolerant to metals, both physiological acclimation and adaptation to contaminants may have specific costs (Wilson, 1988). For example, although induction of metal-binding proteins increases tolerance to subsequent metal exposure, it also uses energy normally available for other metabolic processes (e.g., growth, reproduction). Similarly, genetic changes associated with exposure to contaminants might harm populations. Reduced genetic diversity has been reported in populations exposed to contaminants and may result in population bottlenecks. Furthermore, as tolerant genotypes are eliminated from a population, the reduced genetic diversity may increase the susceptibility of this population to other stressors. There is theoretical support for the hypothesis that populations adapted to contaminants have higher metabolic costs or are more susceptible to other stressors (Hoffman and Parsons, 1997; Mulvey and Diamond, 1991); however, few empirical studies have demonstrated increased costs.

Metalloregions

For national-scale assessments, the entire country can be subdivided into metal-related ecoregions (known as “metalloregions”) to help ensure that protection levels, mitigation goals, and ranking results will be appropriate for the suite of species naturally present within each type of controlling environment. However, the metalloregion concept has not yet been applied across

One of the assumptions behind the use of PICT as an ecotoxicological tool is that differences in tolerance among communities can be detected using short-term experiments. This significantly constrains the application of PICT as an assessment tool. Although tolerance at the population level can be assessed using a variety of species, logistical considerations will limit the types of communities where tolerance can be investigated experimentally. Most of the original research on PICT has been conducted using small organisms with relatively fast life cycles (e.g., benthic invertebrates, soil microbial communities). Therefore, extrapolation of these results to communities of terrestrial plants and animals should be done with caution.

The metalloregion concept (McLaughlin and Smolders, 2001), although intuitively appropriate, has not yet been fully developed for the United States. The country has been divided into ecoregions for both aquatic and terrestrial systems (Bailey et al., 1994; Bailey, 1983). These are based on climactic and vegetation factors and form the basis of metalloregions. EPA is still working to complete ecoregion maps at much finer scales for each state (see EPA

Web site at http://www.epa.gov/wed/pages/ecoregions/ecoregions.htm). To complete the metalloregion concept, soil properties that affect bioavailability (e.g., pH, cation exchange capacity (CEC), and OM) should be overlayed on the ecoregions, along with soil type (e.g., sandy loam, clay loam) and background metal concentratons of metals. Similar information is needed for water bodies. Although this type of information is fairly current and available, soil data have not been updated since the mid-1970s, which may limit their usefulness to some extent. Nevertheless, work is under way to develop metalloregions, although it is likely to be several years from the time of this writing before they are available for use in a decision-making capacity.

4.5.3. Metals Mixtures

Mixtures of metals (including metalloids and other contaminants) are commonly encountered in the natural environment as a result of anthropogenic inputs. Metal interactions, according to Calamari and Alabaster (1980), occur at three levels:

- Chemical interactions with other constituents in the media;

- Interactions with the physiological processes of the organism during uptake; and

- Interactions at the site of toxic action.

Much of the difficulty in interpreting the available information on the toxic effects of metal mixtures is due to differing measures and definitions of the bioavailable fraction of metals, whether it is the fraction that is available for uptake from the environment or at the site of toxic action. Some measure of the bioavailable metal fraction in the exposure media is needed to accurately predict the effects of metals and metal mixtures (Di Toro et al., 2001; Sauvé et al., 1998; Weltje, 1998; Posthuma et al., 1997; Ankley et al., 1996). Characterization of effects of metal mixtures has also been reported to be concentration dependent (Mowat and Bundy, 2002; Fargašová, 2001; Herkovits et al., 1999; Spehar and Fiandt, 1986).

4.5.3.1. *Studies of Metal Mixtures*

Few controlled studies exist on the toxicologic interactions of metals found in environmental contamination scenarios. ATSDR has compiled and evaluated interaction studies involving various metals: methyl mercury, arsenic, cadmium, chromium, lead manganese, zinc, copper, cesium, cobalt, strontium, and uranium (draft interaction profiles available online at http://www.atsdr.cdc.gov/iphome.html). Few studies allowed any quantification of interaction magnitude, whether using the authors' definitions of toxicologic interaction or EPA's definitions based on dose and response addition. The summaries below indicate some of the qualitative conclusions available on interaction potential.

A study of a mixture of cadmium, lead, and zinc in rats found slightly more marked adverse hematological effects with the ternary mixture exposure than with the cadmium-lead, cadmium-zinc, or lead-zinc mixtures (Thawley et al., 1977); inconsistencies in dietary levels of calcium and vitamin D in this study, however, may have made comparisons problematic. A well-controlled rat study has reported significant synergism between cadmium and lead regarding testicular atrophy (Saxena et al., 1989). That study also demonstrated protective effects of high dietary levels of zinc, which effectively reduced the testicular effects of the cadmium-lead mixture to control levels. No studies have been located that would allow extrapolation of those high exposure results to more common, lower environmental levels. 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 for binary mixtures compared with ternary mixtures.

The data regarding interactions of environmental metals usually are not adequate for predicting the magnitudes of interactions. Interaction profiles by ATSDR of metal-metal interactions have considered the following combinations: arsenic, cadmium, chromium and lead; lead, manganese, zinc, and copper; and cesium, cobalt, and strontium (see above Web site for draft reports). Experimental efforts to identify and quantify interaction mechanisms among these metals are needed. For some endpoints, the data are not robust enough to show even the direction of interaction (i.e., whether the joint action will be dose additive or greater than or less than additive). The animal studies discussed briefly 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.

4.5.3.2. *Mixtures*

Similar to human health risk assessments, ecological risk assessments commonly involve mixtures of metals. However, Agency guidance is less developed for how to estimate metal mixture effects for wildlife. Binary interactions of dietary metals in livestock are discussed in NAS/NRC (1994, 1980) and McDowell (2003). These references can be used as sources of information from which at least qualitative estimates of additivity, synergy, or antagonism can be developed. Additionally, see section 4.3.6.3. for discussion of human health assessment for mixtures, which provide information useful in consideration of wildlife effects.

4.5.3.3. *Application*

Two key questions should be addressed by effects assessments related to metal mixtures:

- To what extent does each metal contribute to any observed effect?
- Are the effects significantly greater than or lesser than the sum of the individual component effects?

The answers to these questions also have the potential to affect water quality guidelines (WQGs), EcoSSL values, cleanup targets, and other similar management decisions.

Methodologies (graphical and statistical) to predict impacts of metal mixtures and interactions of individual metals within mixtures can be broadly classified as either Concentration Addition models or Effects Addition models. Both models use metal water concentrations to generate concentration-response curves for individual metals, and these data are then used to generate specific critical concentrations for mixture models. Similar models can be developed for soils or sediments.

In the Concentration Addition model, all metals in a mixture are added together to predict toxicity; differing potencies are taken into account by converting chemical concentrations to an equitoxic dose (e.g., Toxic Units (TUs) or Toxicity Equivalence Factors (TEFs), which convert all metals to one metal concentration). In the Effects Addition model, differing potencies are ignored, and the effect of each metal's concentration in a mixture is combined to predict mixture toxicity. Only the Concentration Addition model allows detection of toxicity greater than predicted (more than additive); the Effects Addition model can only predict strict additivity.

The TU approach involves deriving dimensionless units for each metal in a mixture by dividing individual concentrations by their individual toxic concentrations (such as LC_{50} values).

The TUs for all the metals in the test mixture are then summed. A value of 1 ± 0.1 in the case of an LC_{50} would predict 50% mortality (or another effect if TUs are based on that effect rather than on lethality). A value significantly greater than 1 would predict more than 50% mortality. A value significantly less than 1 would predict less than 50% mortality.

The TU approach can be used with any endpoints, for instance LC_{50} or EC_{50} values. However, it assumes the same mode of action for all the chemicals so will only predict additive effects. The toxic concentration can be derived from guideline values (e.g., WQGs), from literature toxicity data, or from specific experiments. This approach can be used when setting WQC. It has, for example, been recommended for use as part of the Australia and New Zealand WQGs (ANZECC and ARMCANZ, 2000). These guidelines employ a concentration addition approach using WQG concentrations of metals as TUs:

$$\mathrm{TTM} = \sum(C_i/\mathrm{WQG}_i)$$

where TTM is the predicted total toxicity of the mixture, C_i is the concentration of the component, and WQG_i is the guideline for that component. If TTM exceeds 1, then the mixture has exceeded the water quality guideline. It is important to note that this has been developed for water, and significant limitations may be associated with applying it to soil systems.

Norwood et al. (2003) conducted a literature review on the effects of metal mixtures. Mixtures varied from 2 to 11 metals. The investigators determined that the TU approach is presently the most appropriate model for predicting effects of metal mixtures, based on currently available data (e.g., effect concentrations, ECx values). Effects addition models, especially if based on body or tissue concentrations, might be more accurate in the future, but they require reliable dose-response and bioaccumulation curves for all single metals (not just ECx values) and then careful testing of the models (research on tissue concentration effect levels is ongoing).

Application of HSAB. The QICAR approach (described in Section 4.1, Environmental Chemistry) might also be applicable to predict the potential for interactions of metals in mixture. Unsatisfied with the qualitative conclusions of Newman and McCloskey (1996), Ownby and Newman (2003) fit binary metal mixture data derived from the Microtox assay to the following model of joint independent action (Finney, 1947):

$$\text{Predicted } P_{A+B} = P_A + P_B - P_A P_B$$

where:

P_{A+B} = The biological response to the metal mixture, expressed as a proportion
P_A and P_B = The biological responses to A or B when present singly

This relationship would hold if the actions of the paired metals were independent. However, if the metals were not independent, the P_AP_B term will deviate from its expected value of zP_AP_B where $z = 1$. Assuming that paired metals with very similar binding tendencies are more likely to interact with the same biological ligands than are metals with very dissimilar tendencies, deviations of the z coefficient from 1 would reflect departure from complete independence of metal action. HSAB theory allowed prediction of metal interactions in this model system. Although Microtox is considered to be a useful tool for organic contaminants, it is very sensitive for metals (Willemson et al., 1995).

4.5.3.4. *Limitations*

At present, the most appropriate approach for determining the toxicity of metal mixtures and addressing the two key questions listed above is to use the TU approach as a screening-level assessment. This approach cannot, however, be used beyond screening because it does not provide enough certainty. Norwood et al. (2003) found that of 191 case studies examined, 70% were additive or less than additive. Thus, this approach was primarily either appropriate or overprotective, but 30% of the case studies indicated that this approach would be underprotective. For aquatic organisms acutely exposed to cationic metals, the assumption of additivity is sufficient, particularly if bioavailability adjustments are made using the BLM (see Section 3.4.8).

Currently, there are no realistic means of ranking mixtures of metals or individual metals within mixtures. National criteria for mixtures also are not possible at this time. Furthermore, the concentration addition (TU) approach is recommended only for application to mixtures with fewer than six components. This issue remains site specific because interaction responses are dependent on both the actual metal mixture combinations (metals and ratios) and the exposed organisms.

Toxic Unit Approach

Currently, the most appropriate approach for determining the toxicity of metal mixtures is to use the ***Toxic Unit (TU) approach*** as a screening-level assessment tool. This approach cannot, however, presently be used beyond screening because it does not provide adequate certainty since combined effects depend on the relative amounts of each metal. Furthermore, the TU approach is recommended for applications with mixtures containing less than six components.

Researchers are presently attempting to predict the effects of metal mixtures on the basis of critical body concentrations of metals. Ongoing research is attempting to integrate the effect accumulation functions of each metal into a metal mixture model in which an effect addition formula will be compared with a concentration addition formula, both based on body concentration. Current work is focused on aquatic invertebrates, and similar research is required for other organisms.

It is possible that the BLM may be expanded in the future to include mixtures. In theory, if two metals compete for binding to the same site of toxic action on an organism, it should be possible to model the total metal bound to that site and hence predict metal toxicity using a mechanistic BLM approach in an Effects Addition model. Alternatively, if two metals do not compete for the same binding site on the organism, then the BLM may provide more reliable estimates of individual metal bioavailability, and these estimates can then be combined in more accurate Effects Addition models. However, at present, these possibilities remain theoretical and need testing. However, this possibility, while improving the ability to assess the effects of metal mixtures, does not include temporal aspects (i.e., “time-to-response” versus concentration).

4.5.4. Background

Background is defined as the amount of metals occurring in soils, water, or air as a result of anthropogenic and natural processes. Anthropogenic contributions are limited to those that are not influenced by current, direct releases (i.e., emissions, discharges, or disposal) from a source or site of concern. This includes metals that may arise from manmade substances (particularly metalloids) or from natural substances (metallic ores) present in the environment as a result of human activity that are not specifically related to the release in question (U.S. EPA, 2003c). Background should be defined in a specific spatial and temporal aspect that is related to the scope of the particular hazard or risk assessment. Background concentrations can vary by as much as five orders of magnitude, depending on soil type, geography, and other factors (Chapman and Wang, 2000).

Background

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Background may exacerbate toxicological effects and accumulations of metals from direct emissions or other regulated sources or, conversely, it may result in adaptation of organisms to higher metal concentrations and result in increased tolerance to emissions (see above section). Furthermore, because metals occur naturally, and some are essential macro- or micronutrients, they are at least partially responsible for how plants and animals are distributed within various ecoregions. The distribution of plants and animals, local species diversity, species survival, and the vitality of individuals can be profoundly affected by background levels of metals in an area. Humans, on the other hand, are distributed throughout the world, irrespective of naturally occurring levels of metals, so knowledge of background levels is not as significant.

4.5.4.1. *Application*

Concentrations of metals in soils and waters of the United States vary tremendously. Thus, use of a single number to represent all areas within the United States is to be discouraged. Statewide averages (U.S. EPA, 2003c) provide somewhat better resolution, but even these are constrained by political boundaries, not by geochemical characteristics. Additional information on concentration of metals in soils at smaller spatial resolutions is provided in Shacklette and Boerngen (1984). Some metals (e.g., Fe, Cu, and Zn) are included in the State Soil Geographic Database (STATSGO) available at www.nrcs.usda.gov/technial/techtools/stat_browser.html. These data can be grouped at whatever spatial scale is required, but they are not screened for whether they represent true background concentrations. Similarly, data on water concentrations can be retrieved from EPA's STORET database (www.epa.gov/storet/index.html). These data should be used with caution, however, as this is a voluntary-entry database, and there is no consistent method for measurement or for quality assurance/quality control (QA/QC) of the data. Like the STATSGO information, STORET data do not necessarily represent true background levels; additionally, there is incomplete coverage across the United States.

Similar data for sediments are available from the EPA's National Sediment Quality Survey database, which is available in the form of an MS Access 1997 database and can be obtained on a CD from the EPA's Office of Water, Office of Science and Technology (EPA, OW, OST). It contains survey data from 1980 to 1999, including sediment chemistry data, tissue residue data from selected organisms, and toxicity data (lethal and sublethal effects on various test organisms). Overall, there are data from more than 50,000 stations and 4.6 million analytical observations. The data were complied from a variety of sources, but mostly from state and federal monitoring programs, and sampling and analysis strategies varied among sources. All data have "data qualifiers" associated with them in the database. However, this was not a statistically designed survey, and it is heavily biased toward contaminated sites, so inferences to areas that were not sampled should be made with caution.

A more appropriate approach is to define average (and ranges) of background concentrations for various ecoregions (Hargrove and Hoffman, 1999; Bailey, 1998; Omernick, 1986). Work is under way in this regard, but such values currently are not available. Therefore, the recommended default is to use state averages where possible and always to define the range that might be encountered within the spatial scale being considered. It is also important to recognize that background concentrations in water are highly variable through time because flooding or drought conditions substantially change the relative concentrations of metals in a water body. Again, it is suggested that ranges rather than single number averages be used.

Several practical issues should be considered when evaluating the contribution of background to hazard or risk and its implications for various risk management options when conducting site-specific assessments. First, a physical and/or temporal boundary should be defined for the analysis. Next, background should be described, estimated, or measured. U.S. EPA (2002b, c) provides detailed guidance on how to estimate local background concentrations and notes that locations of background samples should be areas that could not have received contamination from the site but that have the same characteristics as the medium of concern (i.e., water, soil, or air).

Another reason to include background in the assessment is to evaluate the effect of the remedial options. Although some areas of the site may have elevated concentrations for certain metals, other metals may not be elevated. Remedial actions could cause these naturally occurring metals to become more bioavailable, thereby resulting in unintended toxicity. The HSAB theories and QICAR models (see Section 4.1.2) can be applied to derive useful estimates of biological activity for metals to aid in making decisions regarding remedial actions.

Incorporating background. How the information on background concentrations is used in the final risk assessment depends on how data on toxic responses were generated and the relationship of the bioavailability characteristic of the naturally occurring material to the source-related additional metal. For aquatic organisms, toxicity tests generally are conducted in waters that are relatively low in background metals and are of moderate hardness. Therefore, the toxicity thresholds described (e.g., in WQC) represent the total amount of metal in the water, not the amount that can be added to the natural background levels. If, however, the tests are run on site-specific waters where metal background and bioavailability may differ significantly from standard waters and where organisms have been acclimated to such conditions, then the toxicity threshold reported should include both the background and the amount that is added. When using this approach, caution should be exercised, because the background levels of metals in the aquatic system may be highly variable over short time periods. The primary consideration is that the *bioavailable fraction* of the natural background concentrations should be added to the *bioavailable fraction* of the added metal.

In terrestrial systems, toxicity bioassays for soil organisms (plants and invertebrates) are conducted either in artificial soils or in actual soils, both of which contain background concentrations of metals (particularly of essential elements). Therefore, the toxic levels reported are the amount added during the study. Unfortunately, the background amounts frequently are not reported, so it may not be feasible to take a true "added risk" approach. Furthermore, the amount of metal added to the test system generally is in a much more bioavailable form (e.g., a metal salt) than is the background material. Therefore, for a site-specific release of a highly bioavailable form of the metal, the background concentration may not contribute significantly to total metal uptake. Thus, it becomes extremely important to measure exposure in terms of the *bioavailable fraction*, so field exposures can be expressed in a manner comparable to the highly bioavailable material used in toxicity tests for threshold setting.

For human health and wildlife assessments, the amount of metal in food material should be taken into consideration in a manner similar to that discussed above for soil organisms. Again, differences in bioavailability of food-incorporated metals and top-dressed metal salts should be considered. Additionally, natural uptake and the amount of metals in forage and other food items will vary, depending on the amount of metal in soil and the particular species of plant/animal present in the area. Site-specific assessments can take this into consideration. For national or large-scale assessments, a default assumption can be made that food items contain sufficient amounts of micronutrients to meet dietary needs and that the toxic threshold value represents the *bioavailable fraction* that is added above these values.

4.5.4.2. *Limitations*

National databases of metal concentrations in soil or water do not currently differentiate between naturally occurring levels and levels that are elevated owing to anthropogenic sources. However, the databases can be screened to ascertain whether specific areas are affected by point source emissions and so can be used as reasonable estimates for large-scale background levels. More important, however, is that background concentrations are most frequently reported as total amount of metal, without specifying the bioavailable fraction or chemical speciation or the data (e.g., pH or redox potential) needed to estimate bioavailability. Information on probable mid- to long-term changes in soil or water properties that might enhance bioavailability of background metals also should be provided for accurate assessments of future risk.

4.5.5. Indirect Effects of Metals

Metals can initiate ecological changes by directly affecting individual organisms (i.e., through toxic responses). Organisms can die, fail to reproduce, or have altered behavioral patterns. As a consequence of such actions on individuals, other organisms within the community will be indirectly affected through reduced number of prey items or predators or changes in competition for resources. Additionally, indirect interactions between organisms can and do occur independently of initiating effects from toxicity due to metals exposures (described by Dill et al., 2003). Although "initiators" may be biotic, physical, or chemical, in all cases there is an effect to a species (the "transmitter") that has an effect on another species (the "receiver").

Indirect Effects

Indirect effects to organisms initiated by metals toxicity can be negative (density dependent or independent) or positive (density dependent) and can occur between species or within the same species. Table 4-16 summarizes some of these relationships.

Indirect effects to organisms initiated by metals toxicity can be negative (density dependent or independent) or positive (density dependent) and can occur between species or within the same species. Some examples are summarized in Table 4-16; additional examples are provided by Chapman et al. (2003). Interactive effects can also occur due to the combined effects of environmental stressors and metals toxicity. For instance, Liess et al. (2001) found that the toxicity of copper to an Antarctic amphipod was increased by food shortage and excessive ultraviolet-B radiation. Some authors have considered such interactive effects to be indirect; however, they can be addressed as direct effects in risk assessments and hence are not considered further.

Table 4-16. Examples of Indirect Effects of Metal Toxicity

Transmitter organism	Receiver organism	Comment
Prey populations reduced	Predator populations reduced	Negative indirect effects due to reduced prey (food)
Predator populations reduced	Prey populations increased	Positive indirect effects due to reduced predation
Competitor populations reduced	Competitor populations increased	Positive indirect effects due to reduced competition
Toxicity to some larvae of a species	Increased adult-to-larval survival, increased growth and biomass of adults of the same species	Positive indirect effects due to density-dependent compensation

4.5.5.1. *Application*

Functional redundancy (species having similar roles in ecosystem processes) is a well-known but arguably not well-understood phenomenon in ecosystems. It is often assumed that if an organism's primary prey item is reduced or eliminated, then the organism can switch to another prey item due to functional redundancy. However, in the case of food web-mediated effects, the following sequence can and does apply: chronic metal exposure reduces the food abundance of certain dietary components; as a result there are increased energetic costs of feeding and an associated reduced growth efficiency. It is important that all components of the food web are exposed to metals; thus, the determination of the final effect should include the concurrent changes to all elements.

A determination of whether indirect effects such as loss of preferred prey can occur requires three components. First, appropriate conceptual diagrams should be developed in the problem formulation phase of the risk assessment and subsequently refined. Such diagrams should incorporate sufficient detail regarding key biotic interactions (e.g., competition and predation) and the ecological context in which the species exist and pollution occurs. As noted by Chapman et al. (2003), seasonal and life-stage changes in feeding patterns can occur and will "require iterative temporal diagrams showing the various reasonable possibilities." If the factor is limiting, then the organism will exhibit a response; however, if it is not a limiting factor, the likelihood that it will lead to organismal changes decreases.

Second, risk assessors should focus proactively on the first three possibilities in Table 4-16 because it is extremely unlikely that indirect effects due to density-dependent compensation will be detectable without extraordinary effort. Increased numbers of receiver organisms due to reduced competition or predation are more likely to be detected and more likely to have overall adverse implications for communities and/or ecosystems, despite their apparent positive aspects (to the particular receiver species).

Third, risk assessors should focus on any cases of reduced growth of individuals, particularly where the individuals affected are relatively tolerant to metals toxicity *and* their normal feeding patterns are disrupted. These are clear indications (although not conclusive proof) of indirect effects of metals toxicity. An example of negative indirect effects due to reduced prey is provided by Campbell et al. (2003). These authors summarize extensive field research into yellow perch in metal-impacted lakes in eastern Canada. Yellow perch in lakes shift from feeding on zooplankton to feeding on littoral macrobenthos during their second year of growth and then begin to include a significant amount of fish in their diet during their third to fifth year of growth. Fish in the most metal-impacted lakes did not undergo this normal sequence of diet shifts. Instead, they continued to utilize smaller prey throughout their lives owing to the loss of their primary prey species to direct metal toxicity. A bioenergetic bottleneck developed because the perch's growth efficiency was reduced by the need to catch and eat smaller prey. The perch were more tolerant to metals toxicity, and thus there were no major direct effects on their survival (though multiple physiological effects were recorded). However, the loss of their primary prey species resulted in smaller or stunted perch, a major indirect effect of metals toxicity. Many methods exist in the ecological literature to gather population, community, or ecosystem function data that may be achieved without significant levels of effort. Some elegant analyses of population demographics can be accomplished within a single sampling effort (e.g., Wilson et al., 1996; Heyer et al., 1994; Davis, 1982).

4.5.5.2. *Limitations*

> Presently there is no realistic means of ranking metals on the basis of indirect effects, nor are national criteria possible. This issue remains a site-specific one because interactions between the initiator (metal toxicity), the transmitter, and receptor organisms are dependent on both the level and type of toxicity and the sensitivities of individual organisms within structurally and functionally unique populations, communities, and food chains.

It is difficult to predict natural (e.g., behaviorally mediated), indirect interactions in nature (Dill et al., 2003). It is also difficult to predict indirect interactions due to abiotic factors such as metals toxicity. Accurate predictions require good understanding of the functional interactions within and between populations, particularly along food chains, as well as density-dependent and density-independent processes between and within species.

Presently there is no realistic means of ranking metals on the basis of indirect effects, nor are national criteria possible. This remains a site-specific issue because interactions between the initiator (metal toxicity), the transmitter, and receptor organisms are dependent on both the level and type of toxicity and the sensitivities of individual organisms within structurally and functionally unique populations, communities, and food chains.

As noted by Campbell et al. (2003) and Dill et al. (2003), indirect effects of stressors on organisms probably are not uncommon occurrences. However, there are few documented cases of this occurring, and it is likely that most cases go unrecognized. It would be useful to review previous detailed-level ecological risk assessments involving metals toxicity to determine and document previous cases.

Indirect effects from metals toxicity are primarily associated with ecosystem function, not structure. However, ecological risk assessments typically are focused on determining risk to structure, not function. The assumption is made that measuring structure protects function because of functional redundancy. Clearly, given the reality of indirect effects, this is at best a questionable assumption that needs to be tested.

4.5.6. Bioavailability in Terrestrial Systems

Bioavailability of soil metals to terrestrial organisms is closely linked to dynamic soil physical and chemical parameters, and biotic processes. As discussed in Section 4.1, there are qualitative and quantitative methods and models for considering soil chemistry issues and aging of metals in soil that modify metal bioavailability and its toxicity in soil. Unless specific bioavailability data exist, even with measures of soil physical and chemical parameters such as soil loading capacity, aging of metals, and speciation, accurate estimates of exposure to the terrestrial biota cannot be made. In situations where information is not available, bulk soil chemistry is typically used with a default of 100% relative bioavailability.

> Unless specific bioavailability data exist, even with measures of soil physical and chemical parameters such as soil loading capacity, aging of metals, and speciation, accurate estimates of exposure to terrestrial biota are not possible. In situations where information is unavailable, bulk soil chemistry is typically used with a default of 100% relative bioavailability (i.e., the bioavailability of the chemical in soil is presumed to be the same as the bioavailability in the tests used to develop the toxicity data).

4.5.6.1. *Soil Organisms: Invertebrates and Microorganisms*

Metal speciation is the primary consideration in assessing the bioavailability of metals to soil invertebrates and microbes. Major assumptions regarding metal exposure in aquatic systems, such as a the relatively homogeneus dissolution of metals in the exposure water, may not be applicable or may apply at different scales in soil systems. Although soil microbes may be immersed in soil solution films surrounding soil particles, few invertebrates are exposed to metals in this manner. Exposure usually consists of partial contact of soil solution films with the surfaces of the invertebrates that are capable of absorbing metals (e.g., earthworm dermal surfaces). Direct contact with membranes across which metal uptake can occur does not take place for many hard-bodied soil invertebrates (e.g., arthropods), and metal uptake is almost entirely through the ingestion of metal associated with particle matter or soil solution. For these reasons, exposure and relative bioavailability cannot be expressed similarly for each organism in the soil ecosystem, and an understanding of primary routes and mechanisms of metal exposure should be established for species or groups of similar organisms.

Often, direct toxicity testing of the soil of concern is the best method for assessing bioavailability and toxicity-to-soil biota (Fairbrother et al., 2002). Issues such as spiking of metals solutions onto soils, aging, and laboratory-to-field extrapolation should be considered. In the ecological soil screening document (U.S. EPA, 2003c), published literature was evaluated using primary soil parameters affecting lability of metals in soils in a matrix to qualitatively indicate metal bioavailability. Further information on this topic and factors relating soil chemistry to soil biota toxicity are discussed in Section 4.1.6. A terrestrial BLM method currently under development (Allen, 2002) may provide a useful tool to link bioavailability, soil chemistry, and toxicity to soil biota.

> Exposure and relative bioavailability cannot be expressed similarly for each organism in the soil ecosystem, and an understanding of primary routes and mechanisms of metal exposure should be established for species or groups of similar organisms.

4.5.6.2. *Plants*

The most common route of metal exposure in plants is through the roots. Ions and organic molecules contact roots via the transpiration stream, diffusive transport, and microbe-facilitated transport. At the root surface, soluble contaminants have the potential to enter the root tissue through the transpiration stream or through a range of mechanisms designed to facilitate nutrient uptake. In general, it is thought that only uncomplexed, free ionic species of cations and ions can be taken up by roots. This has been described using a FIAM (Parker and Pedler, 1997; Lund, 1990). Sauvé et al. (1998) put forward

> Ionic or organometallic complexes that increase the total concentration of elements at the root surface have been correlated with increased uptake, either through disassociated ions or through uptake of intact complexes. Thus, research into how to quantify metal complexes in soils relative to their lability is important.

a method to calculate free metal activity levels for copper and lead to derive a pH-dependent soil criterion for soil biota. The soil criteria paper was based in part on previous studies (Sauvé et al., 1997a, b, 1995; McBride et al., 1997) to generate models to predict free copper activity from total metal content, pH, and organic matter content or, in the case of lead, using only total metal levels and pH. The equations were generated from more than 60 soils using ion selective electrodes and standard methods for determining pH, organic matter content, and total metal levels. However, many exceptions to the FIAM have been identified, such that it has been abandoned in favor of its close cousin, the BLM. Ionic or organometallic complexes that increase the total concentration of elements at the root surface have been correlated with increased uptake, either through disassociated ions or through uptake of intact complexes (Parker et al., 2001; McLaughlin et al., 1994). In addition, it is clear that plants do not distinguish well between many pairs of ions of similar charge and size (e.g., As and P or Cd and Zn).

Plant bioassays can be used to measure the relative bioavailability of metals in various soil types. Results can be used to determine either the direct or the indirect value of bioavailability of contaminants in plants and to extrapolate an indirect estimate of relative bioavailability to organisms that consume the plants (assuming a correlation between plant and animal uptake). This type of testing has been routinely done in agriculture for decades and has been used to validate extraction tests. Tests have most often focused on identifying plant deficiencies of particular elements, but they are easily adapted to evaluate toxicities (Gettier et al., 1985).

In the absence of test data, relative bioavailability can be estimated qualitatively based on relative pH and organic matter content (Tables 4-17 and 4-18), although other soil factors can be significant, most notably CEC. However, combinations of these two soil parameters and their ranges are sufficient as a qualitative guide for assessors to identify soils where metals may have increased (or decreased) availability to plants. This is particularly noted when soil pH exceeds 7 and is a mechanism to obtain essential elements under conditions of low availability.

Table 4-17. Qualitative bioavailability of metal cations in natural soils to plants and soil invertebrates

	Soil pH		
Soil type	**Low organic matter (<2%)**	**Medium organic matter (2 to <6%)**	**High organic matter (6 to 10%)**
4 ≤ Soil pH ≤ 5.5	Very high	High	Medium
5.5 < Soil pH ≤ 7	High	Medium	Low
7 ≤ Soil pH ≤ 8.5	Medium	Low	Very low

Table 4-18. Qualitative bioavailability of metal anions in natural soils to plants and soil invertebrates

	Soil pH		
Soil type	**Low organic matter (< 2%)**	**Medium organic matter (2 to <6%)**	**High organic matter (6 to 10%)**
4 ≤ Soil pH ≤5.5	Medium	Low	Very Low
5.5 < Soil pH < 7	High	Medium	Low
7 ≤ Soil pH ≤ 8.5	Very High	High	Medium

Source: U.S. EPA (2003e).

4.5.6.2.1. ***Critical Tissue Residues.*** When used appropriately, plant tissue analysis can provide an indirect, semiquantitative assessment of bioavailability processes (NAS/NRC, 2002). For phytotoxic metal concentrations to be effectively used as an indirect measure of bioavailability, it is important that the threshold values of the plant tested be well understood. In addition, toxicities of certain metal elements are associated with deficiencies of others. For example, increased zinc, copper, and nickel toxicities can be associated with iron deficiencies (Bingham et al., 1986), and increased lead and zinc toxicities can also be related to phosphorus deficiencies

(Brown et al., 2000, 1999a; Laperche et al., 1997). The behavior of plant species in response to nutrient deficiencies varies, and this behavior can affect the uptake of metal elements (Marschner, 1998). Because of these multiple confounding factors, the bioavailability of metals in plants (as well as to consumers) is more accurately and reliably measured directly as the edible plant tissue concentrations of the metal in association with soil metal concentrations in the root zone (NAS/NRC, 2002).

4.5.6.3. *Wildlife*

For most metals, the dietary intake pathway is the main route of exposure for wildlife (NAS/NRC, 2002; see also Section 4.3). However, the incidental ingestion of soil can often contribute a large portion to the majority of the exposure to a wildlife consumer. Because many inorganic metals do not readily accumulate in food (organometalic compounds are excluded from discussion here), highly contaminated soil may result in higher exposures to metals through activities such as grooming fur, preening feathers, consuming soiled prey or forage, burrowing, and taking dust baths. However, canopy feeders would be anticipated to have less incidental soil ingestion and therefore less exposure to inorganic metals than wildlife that consume food that is in more intimate contact with the ground.

The relative importance of the dietary and incidental soil ingestion pathways is dictated by (1) the fraction of total metal available in soil versus that in food and (2) the relative bioavailability of the metal in the soil as compared to metal in food items. Figure 4-7 in Section 4.2.3. shows the relative contribution of food and soil to total metal exposure before accounting for bioavailability. Understanding of the bioavailability of metals in incidentally ingested soils becomes necessary when there is a high amount of metal in the soil that is not taken up by soil organisms (plants or invertebrates). However, the same variables that restrict uptake by plants or other soil organisms act to reduce bioavailability to wildlife that ingest soil directly. Therefore, a qualitative estimate of low relative bioavailability could be made for these soils. Furthermore, data that are generated for human health studies could be used to estimate soil bioavailability for wildlife, acknowledging the uncertainty inherent in such interspecies extrapolations.

Dietary bioavailability. Very little information is available on dietary bioavailability for most wildlife species (see Menzie-Cura and TN&A, 2000), for a review, cited in NAS/NRC, 2002). One of the most significant challenges is that the bioavailability of metals may be influenced by differences in digestive physiology and anatomy across the broad and diverse range of mammalian and avian species. For example, metals present in soils or food may be more or less bioavailable within the gut of an herbivore that relies on fermentation as compared to the comparatively simpler gut of a carnivore that is designed to break down proteins. These gut systems differ in chemistry (including pH) and residence time. The potential differences between species have not yet been explored rigorously. In general, ruminants absorb lower amounts of metals than do monogastric animals such as rats (NAS/NRC, 1980). Some general guidelines are provided for some metals (e.g., lead in NAS/NRC (1980)), and human-derived values can be used as default values in the absence of species-specific data.

Critical body residues (CBRs). CBRs are internal concentrations of chemicals that are correlated with the onset of a toxic response (Conder et al., 2002; Lanno et al., 1998). The use of CBRs reduces uncertainties in ecological risk assessment procedures because they account for site-specific bioavailability and multipathway issues (Van Straalen, 1996; Van Wensem et al., 1994). CBRs can be based on whole-body residues (see below for discussion of this approach in soil invertebates) or concentrations in specific tissues. Tissue-specific critical loads have been established for several species of vertebrate wildlife for lead in liver, cadmium in kidney, and selenium in eggs. See Beyer et al., 1996, for these figures.

4.5.7. Bioavailability in Aquatic Systems

Many factors influence the bioavailability of inorganic metals in aquatic systems. Abiotic (e.g., organic carbon, pH, cations) and biotic (e.g., uptake and metabolism) modifying factors determine the amount of metal that interacts at biological surfaces (e.g., at the gill) and subsequently is taken up. In the dissolved phase, metals can exist as free ions as well as in a variety of complexed forms. These forms, or species, are of key importance in understanding potential impacts because they have differing bioavailabilities, and therefore water quality can dramatically influence the proportion of bioreactive forms of a metal. For many metals in aquatic systems, it is the free ionic form which is believed to be responsible for toxicity. For example, Cu^{2+} has been directly linked to toxicity in fish and invertebrates, while Cu complexed by dissolved organic matter does not induce toxicity to the same degree (Ma et al., 1999; Erickson et al., 1996) because bioavailability for uptake is reduced.

The relationship between speciation and bioavailability is expressed through the FIAM (Campbell, 1995). However, the FIAM is not without limitations, as links between metal speciation and toxicity are complicated and the free metal ion is not always the only bioreactive form. For example, complexed metal, including Cu bound to DOC can be taken up and contribute to toxic impacts and effects (McGeer et al., 2002; Erickson et al., 1996). Although the link between bioavailability of metals and factors influencing speciation (such as pH, temperature, and organic and inorganic anionic complexation) are of prime importance, other abiotic factors, particularly cations, influence metal bioaccumulation and toxicity. Dissolved cations such as Na^+, K^+, Ca^{2+}, and Mg^{2+} can competitively inhibit metal uptake. BLMs, the recently developed integrated toxicity prediction models, have successfully combined abiotic speciation, cationic competition, and bioaccumulation at the presumed site of toxic action (reviewed by Paquin et al., 2002a). The influence of exposure conditions on metal bioavailability is beginning to be understood for some metals, but speciation of metals within the organism is generally less well understood. However, speciation within the organism is likely important for storage, metabolism, elimination, and delivery to and bioavailability at the site of toxic action (Mason and Jenkins, 1995).

It is important to account for bioavailaiblity in relation to exposure conditions when comparing toxicity studies and relating these to natural environments. This includes knowing the species of metal that is likely to be present in the environment. A number of other variables also can influence the outcome of a laboratory toxicity assay, including the acclimation of test animals to the culture conditions (see Section 4.5.2, Acclimation, for further discussion), the natural background concentrations of the metals (including, but not limited to, the metal of interest) in either the test water or the site of concern (see Section 4.5.4, Background), potential interactions of the various metals (see Section 4.5.3, Mixtures), and the potential of the metal to transform to a bioreactive species (see Section 4.1, Environmental Chemistry). The relative compositions of the pretest culture medium and the underlying test medium also should be examined because sudden changes in ionic composition have been shown to cause sufficient stress to sensitize organisms and enhance toxicity (Taylor et al., 1990). This is a necessary prerequisite for successful comparison of effect levels derived from laboratory tests to predicted exposures.

The bioavailability of metals in aquatic systems can vary significantly among taxonomic groups. For example, although most mayflies (*Ephemeroptera*) are generally sensitive to metals, caddisflies (*Trichoptera*) and many stoneflies (*Plecoptera*) are relatively tolerant (Clements et al., 1992). In fact, these species-specific differences in sensitivity to contaminants have motivated the development of numerous indices of water quality that are based on composition of benthic communities (Barbour et al., 1992; U.S. EPA 1989a; Hilsenhoff 1987). Because responses of aquatic organisms to chemical disturbances are usually contaminant specific

(Slooff, 1983), field assessments of metal effects may be difficult when more than one contaminant is present, and caution is required when using biotic indices to assess effects of complex effluents. In some situations, it may be necessary to either develop chemical-specific indices on the basis of sensitivity to specific classes of contaminants or calibrate metrics used in individual field assessments. For example, metals discharged into a system dominated by highly sensitive taxa (e.g., certain heptageniid mayflies) will have greater effects than the same effluent discharged into a system dominated by tolerant organisms (e.g., certain chironomids). Kiffney and Clements (1996) showed that benthic communities from headwater streams were more sensitive to metals than were communities from mid-elevation streams.

4.5.7.1. *Application*

A variety of methodologies (e.g., hardness adjustments, FIAM, Water Effect Ratio (WER), and aquatic BLMs) can be used to account for differences in concentrations of bioavailable metal species when assessing the effects of metals in aquatic systems (U.S. EPA, 1999b). These bioavailability considerations are important in understanding the exposure conditions of interest and how these relate to toxicity study results. WER determinations can account for site-specific (i.e., dependent on the water quality at a specific location) bioavailability (U.S. EPA, 1994c), although they require the development of animal test data. Hardness adjustments were among the first computational methods to account for bioavailability when applying WQC. FIAM approaches that produce speciation profiles of a metal in an aquatic system provide insight into the relative bioavailabilities of the different forms of metal and the importance of complexation. Several models are available for the calculation of metal speciation in natural waters, including MINEQL (Schecher and McAvoy, 1994; Westall et al., 1976), MINTEQA2 (Brown and Allison, 1987), CHESS (Santore and Driscoll, 1995), WHAM (Tipping, 1994) and PHREEQ (Parkhurst, et al., 1980). The BLM approach further extends bioavialability considerations because it applies the latest information on the chemistry and physiological effects of metals in aquatic environments (Di Toro et al., 2001; Santore et al., 2001; Paquin et al., 1999).

Quantitative Ion Character Activity Relationships (QICARs) can be used to extrapolate from availability and/or toxic data for a metal for which there are data to a metal for which data are limited. The approach is similar to the Quantitative Structure-Activity Relationships (QSARs) that have been used for organic compounds. The QICAR approach was recently integrated into the general context of modern QSAR practices by McKinney et al. (2000), and Weltje (2002) successfully applied it to the lanthanides. The QICAR approach is best applied with a full understanding of the system under study, and the approaches should be validated before application. This approach is but one example of the types of tools that are available but that still need further development to support metals risks assessments for aquatic systems.

In the BLM, chemical speciation is simulated as an equilibrium system that includes complexation of inorganic ions and NOM using the CHemical Equilibria in Soils and Solutions (CHESS) model (Santore and Driscoll, 1995). It includes a description of metal interactions with NOM based on the WHAM (Tipping, 1994). The simulation of biological interactions is based on the binding affinity characteristics measured in gill-loading experiments, originally described by Playle et al. (1993a, b). The biotic ligand (i.e. the gill) is represented as having a characteristic binding site density and conditional stability constants for each of the dissolved chemical species with which it reacts. Predictions of metal toxicity are made by assuming that the dissolved metal LC50, which varies with water chemistry, is always associated with a fixed critical level of metal accumulation at the biotic ligand. This fixed level of accumulation at 50% mortality is referred to as the LA50. The LA50 is assumed to be constant, regardless of the chemical characteristics of the water (Meyer et al., 1999, 2002).

The FIAM and BLM approaches both consider:

- Chemical speciation of the metal and the relative amounts of complexation to inorganic and organic ligands.
- The relative bioavailability of metal species (for example but not limited to the free metal ion) and interactions with other cations that may compete for binding sites of uptake or toxicity.
- Accumulation of metal by the organisms at the site of toxic action.

The BLM approach has been applied successfully to combine the influences of speciation and cationic competition on metal toxicity. (Di Toro et al., 2001; Santore et al., 2001; McGeer et al., 2000; de Schamphelaere and Janssen, 2002, 2004; de Schamphelaere et al., 2002, 2003, 2004; Heijerick et al., 2002a, 2002b, also see example application for Cu below)., The model can distinguish, at least conceptually, metal that will bioaccumulate and cause toxicity from the total metal pools in an organism and the bioavailable metal pool in the exposure media. Themodeling approach has been extended to species such as algae (Heijerick et al., 2002; de Schamphelaere et al., 2003) and *Daphnia* (de Schamphelaere and Janssen, 2002, 2004; de Schamphelaere et al., 2002, 2004), which are toxicologically relevant but more difficult to characterize in terms of accumulation at the site of toxic action.

While the focus of currently existing BLMs is acute toxicity, the Ion Balance Model (Paquin et al., 2002a, b,c) can be used to extend the applicability of the BLM to evaluate chronic toxicity The Ion Balance model uses the BLM to predict accumulation levels at the biotic ligand and then explicitly represent the degree of the physiological response of the organism to metal exposure over time (i.e., disruption of ionoregulation and gradual loss of plasma sodium). Although the approach, which was initially applied to silver, may ultimately provide a way to predict effects due to metals over varying exposure durations, further development and testing is required. Chronic toxicity models have recently been developed for the impacts of Cu on *Daphnia magna* (de Schamphelaere and Janssen, 2004) and of Zn on fish, *Daphnia* and algae in European Union risk assessments on zinc.

Overall, the BLM approach has wide application in terms of understanding bioavailability in relation to toxicity. It incorporates the influence of speciation in the exposure medium, bioaccumulation and toxic impacts in a robust approach, and has been applied in a variety of contexts. For example, the BLM has recently been incorporated into draft revisions to EPA's water quality criteria for Cu, it has been used in risk assessment and it is being applied as an alternative to the Water Effect Ratio approaches for setting site specific discharge objectives. When considering the application of this approach, as with all models, care should be taken to understand and explicitly account for the assumptions and potential sources of uncertainty.

4.5.7.2. *Limitations*

Novel applications such as the FIAM and BLM have been shown to offer dramatic improvements over traditional approaches such as the hardness equations which depend on the empirical relationship between water hardness and toxicity and the correlation of water quality variables with hardness. While these new approaches offer improvements, there are still many unknowns and uncertainties in relation to bioavailability and the biotic and abiotic influences on metal toxicity. For example, the role of natural dissolved organic matter in bioavailablity and moderating toxicity is not well understood. There are considerable research efforts ongoing currently, and it is likely that our understanding of metal bioavailability and the method for integrating this knowledge into prediction models will improve quickly in the coming years.

EPA has developed a draft criteria document for copper that incorporates the BLM and will undergo peer review. The draft criterion document is available on the EPA/OST Web page at www.epa.gov/waterscience.

The BLMs that have been and are being developed for a subset of metals (e.g. Cu, Ni, Cd, Ag, Pb, Zn) are based on a limited number of species. BLMs are generally focused on predicting acute toxicity, although there are a few examples of BLMs that predict chronic toxicity, as discussed previously. The development of BLMs to predict chronic toxicity will likely require new knowledge about the combined effects of dietary and waterborne exposure, the mode of toxicity, and the accumulation of metal or dose delivered to the biological site of toxic action. Similarly, acclimation responses, which can influence bioaccumulation at the site of toxicity, are not well understood, and plant and animal species can differ considerably with regard to the forms of metal taken up as well as their relative toxicities. Although these and other issues add complexity to the evaluation, the BLM approach considers metal biogeochemistry; and therefore represents a viable avenue toward understanding and predicting the toxicity of metals.

4.5.7.3. *Example: BLM Application to Development of Copper Aquatic Life Criteria*

The BLM's ability to incorporate metal speciation reactions and organism interactions allows for the prediction of metal toxicity to a variety of organisms over a wide range of water quality conditions. Accordingly, the BLM is an attractive tool for deriving WQC in EPA's water program. Application of the BLM may eliminate the need for site-specific criteria modifications, such as water effect ratios, which are currently used to account for site-specific chemistry influences on metal toxicity. EPA currently is using the BLM to develop a freshwater aquatic life criteria criterion maximum concentration (CMC) for copper. The BLM accounts for inorganic and organic ligand interactions of copper and also considers competitive interactions that influence binding of copper at the site of toxicity. Although a new model is being used, the criterion derivation is still based on the principles set forth in the 1985 guidelines (Stephen et al. 1985). To develop a BLM-based criterion, model predictions of critical accumulations on the biotic ligand (LA50 values) and either LC50 or EC50 values are needed to calculate species mean acute values (SMAVs) and genus mean acute values (GMAVs) as well as to derive a species sensitivity distribution.

4.5.7.3.1. ***Model Input Parameters.*** Much of the aquatic toxicity literature reviewed for the derivation of the copper criterion neither measured nor reported many of the key BLM input parameters. In these cases, the input parameters were estimated. A detailed description of the methods used to obtain or estimate these input parameters is included in U.S. EPA (2003a). Briefly, when critical water chemistry parameters were not available, a variety of strategies were employed to find the additional or surrogate data (e.g., authors or lab personnel were contacted, and alternative sources such as studies with similar water quality U.S. Geological Survey's National Stream Quality Accounting Network (NASQAN; http://water.usgs.gov/nasqan/) and the EPA STOrage and RETrieval (STORET; http://www.epa.gov/STORET/) were used). Where sources could not be used to develop geochemical input parameters for the BLM, data were generated using the reported water hardness and regression relationships constructed from NASQAN data.

4.5.7.3.2. ***Quality Ranking of Water Chemistry Input Parameters.*** A ranking system of 1 to 6 was devised to evaluate the quality of the chemical characterization of the test water (but not the overall quality of the study). Studies that included all of the necessary BLM input parameters, based on measurements from either the test chambers or the source water, were assigned a ranking of 1. Rankings of 2 to 4 were assigned to studies that did not measure all parameters but provided reliable estimates of ion concentrations. Studies were assigned a ranking of 5 to 6 when one of the key parameters (DOC, Ca, pH, or alkalinity) was not measured and could not be reliably estimated or if two or more key parameters (DOC, Ca, pH, or alkalinity) were not measured. Only those studies with a rank of 1 to 4 were used to derive the criterion.

As with any modeling effort, the reliability of model output depends on the reliability of the input. Although the input data have been extensively scrutinized and filtered, the reliability of the BLM-derived values developed for copper in this project are subject to the limitations of the input measurements/estimation procedures described above.

4.5.7.3.3. ***Criteria Generation.*** To calculate an acute criterion or CMC, reported acute toxicity values (e.g., LC50s) and individual test water chemistry parameters were used to calculate LA50 values by running the model in the speciation mode. These LA50 values were then "normalized" to a standard water condition by running the model in the toxicity mode and specifying user-defined LA50s. These normalized LC50s were used to calculate SMAVs, GMAVs, and a final acute value (FAV) pursuant to the 1985 guidelines procedure. The FAV represents a hypothetical genus more sensitive than 95% of the tested genera. The FAV was derived from the four GMAVs that have cumulative probabilities closest to the 5th percentile toxicity value for all the tested genera. Inputting this FAV as an LC50 concentration and running the model in speciation mode determines the lethal accumulation associated with the FAV in the standard test water.

The Cu BLM assumes that most of the parameters are invariant for different organisms. Despite the complexity of the modeling framework, the thermodynamic constants used to simulate the inorganic and organic chemical equilibrium reactions are determined by the characteristics of the metal and available ligands. As such, the constants do not change for simulations involving different organisms. Although most BLM parameter values (including the biotic ligand binding constants and site densities) are consistent across organism types, differences in sensitivity across organisms types should still be accounted for. This is accomplished by adjusting the critical biotic ligand concentration (e.g., LA50) values for each species.

This criterion LA50 is programmed into the model as a constant. To derive a criterion for a specific site, the site water chemistry data are input to the model. The model then uses an iterative approach to determine the dissolved copper concentration needed to achieve a Cu-biotic ligand concentration equal to the criterion LA50. This dissolved Cu concentration is, in effect, the FAV based on site water chemistry. The site-specific CMC is this predicted dissolved metal concentration (or criterion FAV) divided by two. The site-specific CCC is the CMC divided by the final acute-chronic ratio.

4.5.7.3.4. ***Next Steps.*** EPA has developed a draft criteria document that will undergo peer review. When EPA solicits scientific views from the public on the draft criterion document, the model will be made available on the Office of Water web page at www.epa.gov/waterscience. Until the peer review is completed and a final copper criteria document is published by EPA, the procedures described here are draft and subject to change, and the criteria are not considered to be available for use. After completing the copper criteria update, EPA will consider incorporating the BLM into derivation procedures for other metal criteria, such as for silver, cadmium, and zinc. Although the BLM is currently appropriate for use in deriving an updated freshwater copper CMC, further development is required before using it to evaluate a saltwater copper CMC, a CCC, or a chronic value.

Alternative approaches may be considered to establish the database of input parameters for the acute toxicity studies, because the approach described in this document is labor intensive.

Alternatives may include either developing a few high-quality data sets that satisfy the minimum data requirements of the guidelines for a limited set of organisms or developing data sets for known sensitive species. Estimating missing input parameters by relying on statistical techniques or Monte Carlo approaches may also be explored.

4.5.8. Bioaccumulation and Bioconcentration in Aquatic Organisms

Bioaccumulation can be defined as the net accumulation of a metal in a tissue of interest or a whole organism that results from exposure from all relevant sources (e.g. water, food, and sediment). Metal bioaccumulation can apply to the entire organism, including both metal adsorbed to surfaces or absorbed by the organism or to specific tissue. It is usually expressed on a weight (dry or wet)- adjusted basis. Bioaccumulation that occurs under steady-state conditions (i.e., where accumulation remains relatively constant due to uptake being offset by elimination) is often of primary concern in risk assessments. Bioaccumulation of metals is a concern with respect to the accumulating organism when it occurs in the toxicological form(s) and concentrations at the site(s) of toxic action. It also can be indicative of dietary exposure to aquatic organisms at higher trophic levels. While inorganic metals can bioaccumulate in animal and plant tissues, biomagnification across three or more trophic levels is rare.

The simplest tools for estimating bioaccumulation include direct measures of tissue levels and the derivation of simple relationships between tissue levels and environmental concentrations. These simple empirical relationships have often been expressed as bioaccumulation factors (BAF) or bioconcentration factors (BCF) and various data bases summarize values reported in the literature. It is well recognized, however, that there can be considerable uncertainty associated with the application of literature-derived, bioaccumulation or bioconcentration factors to specific risk assessment situations. In other cases, efforts have been made to establish more detailed mathematical relationships between exposure concentrations and tissue levels. For example, empirical approaches that extend beyond simple factors include the use of regression equations that describe the relationship between exposure and accumulation. Significant advances are also being made on the application of kinetic or steady-state uptake models for describing and predicting bioaccumulation (Reinfelder et al., 1998; Chang and Reinfelder, 2002; Kahle and Zauke, 2003; Wang and Zauke, 2004;). These offer promise for understanding the contribution of differing routes of exposure (e.g., water vs. diet). There is considerable experience in using mechanistic bioaccumulation models for estimating

Biomagnification is defined as an increase in the concentration in an organism from a lower trophic level to a higher trophic level within the same food web due to bioaccumulation from the diet (also see trophic transfer). Biomagnification is expressed as the ratio of the concentration in the organisms of the higher trophic level to the concentration in the organisms of the lower trophic level (i.e., biomagnification factor). Note, however, that biomagnification that is quantified using field data makes an explicit assumption that bioaccumulation results from the diet only, when actually multiple sources may be involved (e.g., water or sediment). Biomagnification factors greater than one indicate biomagnification, while factors less than one indicate no biomagnification or biodilution (see below). Inorganic forms of metals rarely biomagnify across three or more trophic levels.

Biodilution, a decrease in organism concentration with increasing trophic level has generally been more commonly observed when concentrations are evaluated across three or more trophic levels.

Trophic transfer. The transfer of a bioaccumulated substance in a prey species to a predator species via dietary exposure. When the concentration in the predator species is increased relative to the prey, trophic transfer is a form of biomagnification. Trophic transfer is important due to its relationship to dietary toxicity (also called secondary poisoning) in which toxicity is manifest through accumulation in prey species and subsequent dietary exposure to predatory species. Importantly, however, dietary toxicity cannot be directly inferred from an evaluation of trophic transfer alone, but rather the combination of trophic transfer and toxicity information for predator/prey species within an ecologically-linked food web.

risks of organic chemicals in aquatic systems and it is anticipated that such models will have an increasing role for risk assessments of metals.

Because the simpler empirical approaches such as the BCF and BAF have received much use and attention in the past, they are discussed in further below with respect to applications and limitations. However, the kinetic and steady-state uptake and bioaccumulation models will likely emerge as important approaches.

4.5.8.1. *Scientific Issues with BCF/BAF*

The BCF is the ratio of contaminant concentration in an organism to its concentration in water at steady state conditions and water-only exposures. Metal concentrations are usually expressed on a weight-adjusted, whole-organism basis, and waterborne metals may be expressed as total or dissolved metals. BCFs have been developed primarily with hydrophobic organic chemicals in aquatic systems, but similar such accumulation factors have been applied to other matrices (e.g., sediment and soils) for both organic chemicals and metals. Strictly speaking, metal bioconcentration in sediment and soil systems is the net accumulation of a metal in or on an organism from pore water only. Hence, in sediment and soil, the denominator for the BCF ratio should comprise the pore water concentration of metal, not the total metal concentration in the sediment or soil. In the broadest context, the BAF is the ratio of a contaminant concentration in an organism to that in a specified medium at steady state in situations where the organism is exposed to multiple environmental media.

Although BAFs and BCFs are calculated in a similar manner, the interpretation is slightly different with metal accumulation in organisms arising from water only for BCFs and from both water and dietary sources for BAFs. For aquatic organisms, BAFs are derived from measurements in natural environments, and BCFs are nearly always measured under laboratory conditions where exposure can be effectively limited to the water column. Unless metal concentrations in pore water serve as the denominator for the ratio, soil and sediment BAFs are usually termed BSAFs. Concentrations are usually measured on a total-metal and weight-adjusted whole-organism (or tissue) basis.

Toxicological bioaccumulation is the fraction of the metal that bioaccumulates and is distributed to receptors at sites of toxic action (Figure 2-2). For metals, this would include reactions with target proteins or other receptors that result in toxicity but not interactions with metallothionein and other metal-binding ligands or incorporation into granules that make metals unavailable for interactions with target molecules. This fraction is conceptual in nature and difficult to measure in practice; it is akin to the minimal effective dose measured in blood that is often used in medicine for assessing therapeutic effects. It could be conceptually defined as a toxicological bioaccumulation fraction or the ratio of total metal concentration in an organism to the metal concentration at the site(s) of toxic action.

As discussed in more detail in McGeer et al 2004, there are fundamental differences in the physical, chemical and toxicological properties of inorganic metal substances and organic substances, such that the BAF/BCF model would not apply to the former. The success of the BAF/BCF model as a valid indicator of the environmental and toxicological behavior of neutral organic substances is due to their hydrophobic/lipophilic chemical properties, and this has important consequences for application to inorganic metals. Many of the assumptions and characteristics of the BAF/BCF model openly conflict with the physical, chemical, biological, and toxicological realities associated with inorganic metal substances. For example, the BAF/BCF model works well for neutral organic substances because uptake of lipophilic substances into biota occurs via simple passive diffusion. However, uptake of the vast majority of inorganic metals is a physiological process, which occurs via a number of specific routes, most of which involve saturable transport kinetics. The degree of uptake and ultimate internal fate of inorganic metals is strongly influenced by ligand binding and receptor site competitive interactions which control metal availability and/or transfer processes, and cannot be reliably represented by a simple partitioning between water and organism. As a result of the differences explained below, simple application of these models is meaningless in assessing acute and chronic toxicity (adapted from McGeer et al., 2004):

- The principal theoretical features of the BAF/BCF model that make it applicable to neutral organic substances also make it inapplicable to inorganic metal substances. These factors produce an inverse relationship between BAF/BCF and exposure concentration; this has been observed for both essential metals and nonessential metals.

- The approach of using one simplified bioaccumulation model (BCF and BAF) and applying it to inorganic metals ignores the basic physical and chemical differences between organic and inorganic substances and is not supported by theoretical and empirical weight of evidence.

- Based on the inherent assumptions of the BCF and BAF model and on the environmental and toxicological behavior of the organic substances from which they were developed and validated, for the vast majority of inorganic metals evaluated, the scientific basis for broad application of the BAF/BCF model is lacking in the context of hazard assessment.

- The complexity of assessing and predicting the bioavailability and bioaccumulation of metals in aquatic systems arises from many factors including:
    - Essentiality of some metals resulting in a "U"-shaped dose response curve.
    - Variation in assimilation efficiency for different species of metal, for different biota and at different sites of uptake.
    - Ability to modulate uptake at the various sites of uptake.
    - Contributions of different routes of entry to the metal body burden and effects.
    - Ability to sequester, store, detoxify and eliminate bioaccumulated metals.
    - All metals will bioaccumulate to some degree without impacts as a result of exposure to natural background concentrations

- Based on reviews (e.g., McGeer et al., 2003), it would appear that for the vast majority of the metal/taxonomic group combinations assessed, the assumptions regarding the independence of BCF/BAF with exposure concentration and proportionality of hazard with increasing BCF/BAF do not hold true.

- The latest scientific data on bioaccumulation do not currently support the use of BCF and BAF data when applied as generic threshold criteria for the hazard potential of inorganic metals (e.g., for classification as a "PBT" chemical).

- In cases where the use of BCF/BAF data are being considered, a careful evaluation of existing data for metals that formally documents the extent to which values represent bioaccumulation linked to toxic impact (both direct and indirect) and the resulting uncertainty introduced by concentration dependency and other aspects of the BCF/BAF data should be done.

- Moving from the current situation to a revised, improved, and validated set of criteria and/or methodologies for assessing metal hazards and risks presents a number of options and challenges, as currently there would appear to be no clear alternatives ready for application.

- BCFs for metals will not be absolute values, but will vary widely with both the specific exposure circumstances/conditions as well as the status/age/condition of the particular organism measured. This applies to all substances, organic and inorganic, although the relative scale of the uncertainty added is greater for inorganic substances.

The Organization for Economic Co-operation and Development (OECD) has published guidance for the hazard classification of chemical substances (OECD, 2001). The hazard classification schemes presented in the OECD guidance incorporates, among other parameters, evidence of bioaccumulation as a basis for hazard ranking. The OECD guidance recognizes the shortcomings associated with the use of BAF/BCF data as a surrogate for the hazard potential of metals, and therefore cautions that metals should be assessed on a case-by-case basis rather than recommending the application of the simple bioaccumulation model (BAF/BCF). The principle features of the BAF/BCF model that make it applicable as a surrogate for acute and chronic toxicity to neutral organic substances also make it inapplicable to inorganic metal substances as discussed above and in McGeer et al. (2004).

The phenomena described above regarding the limitations of the BCF/BAF model also have a bearing on the use of BCFs and BAFs in national-level assessments (e.g., water quality criteria and national-scale risk assessments) in addition to site-specific assessments. For example, variability in BCFs and BAFs caused by an inverse relationship of BCF with exposure concentration can confound the application of BCFs in risk assessments, the extent to which depends in part on the magnitude of this relationship (i.e., its slope) and the extent of extrapolations made (i.e., extrapolation of BCFs across differing exposure concentrations and species). For organic chemicals, generic normalizing factors have been shown to reduce variance in BAFs due to bioavailability differences (e.g., normalizing to organic carbon and lipid; see Burkhard et al., 2003). However, analogous normalizing factors have yet to be developed and widely applied in the context of BAFs or BCFs for metals, although some progress has been made with relating accumulation parameters to body weight (Hendriks and Heikens, 2001) or permeable surface area in the case of the amphipod, *Gammarus zaddachi* (Wang and Zauke, 2004). Simple normalizing factors can be confounded by the fact that animals and plants have evolved physiological and anatomical means for regulating and or storing internal concentrations of metals and the species-dependent specificity of these mechanisms. Regulation of metals by biota can actually result in decreased apparent BAF/BCF factors with increasing exposure concentrations. Thus, a fundamental understanding of such mechanisms is important for reducing the uncertainties associated with interpreting tissue residues. The mere presence of a metal in a plant or animal does not mean it is at a site of toxic action. In summary, caution should be exercised when using published BAF and BCF values to make judgements about resultant tissue levels of metals in animals and plants. Where this is found to be necessary as an initial, screening level of assessment, the associated uncertainties should be identified and discussed with respect to their potential impact on the risk estimates and resultant decisions.

In situations where a decision has been made to employ a BAF or BCF factor, there may ways to reduce some of the uncertainty. One idea involved subtracting “normal” accumulation from the calculation of the BCF. This involves separating the portion of metal that bioaccumulates from exposure under “normal” or background conditions from that portion that occurs as a result of exposure to elevated levels of metals (McGeer et al., 2003). The ACF value also accounts for the accumulation of essential metals required for physiological function. These ACF values were dramatically lower than BCF values (illustrating the importance of normal bioaccumulation) for some metals, particularly essential metals. However, for most metals ACFs still varied with exposure concentration and were not viewed as a sufficient replacement to the BCF (McGeer et al., 2003).

Another approach that may reduce uncertainty caused by concentration dependency of BCFs and BAFs involves limiting the selection and application of BCF and BAF values to concentration ranges that more closely correspond to toxicological thresholds. For example, limiting the calculation of BAF and BCF values to concentrations that approximate the applicable water quality criterion has also been suggested as a method for reducing the uncertainty around BCF and BAF values in situations where concentration dependency is evident. This would account for bioaccumulation at an exposure level where concern over bioaccumulation might be expected. This approach has limitations (McGeer et al., 2004) and does not appear to reduce the overall variability associated with BCF and BAF measurements when evaluated across broad classes of organisms. An additional issue for this approach is that WQC reflect some of the more sensitive organisms, whereas the BCF and BAF measurements are not necessarily from these same organisms and include data from biota that may not be as sensitive to chronic effects. Therefore, as a modifier for broad-based application, this variation of the BCF/BAF methodology does not appear to explain variability. However, on a site-specific basis where toxicity thresholds and species are better characterized, this approach may have value in reducing uncertainty. As already noted, caution should be exercised with any of the BAF or BCF applications.

4.5.8.2. ***Bioaccumulation in Relation to Dietary Toxicity***

Discriminating between metals that have the potential to cause effects via trophic transfer and metals that do not is another approach that might be useful in distinguishing between metals, based on bioaccumulation and impacts. Metals taken up and stored within an organism may not cause direct effect to that organism but may be bioavailable to organisms in the next trophic level that feed on it. Bioaccumulation of metals in prey organisms may be quite high, especially in organisms such as high volume filter feeders and those that accumulate elevated levels of metals, for example, those that store detoxified forms. Metals that bioaccumulate to levels in prey organisms that cause impacts in predatory organisms are clearly important issues to address however, there is a general lack of understanding of the potential for toxicity.

A prey organism with a high concentration of a particular metal represents a potential opportunity for the trophic transfer of the metal from an enriched source to a predator at the next trophic level. The form of detoxified storage of that accumulated metal in the prey species has a significant effect on the potential assimilation of that metal by the predator (Wang and Fisher, 1999). For example, Nott and Nicolaidou (1990) have shown that the bioavailability to neogastropod mollusc predators of metals present in detoxified metalliferous granules in prey varies among metals and with type of granule; thus the zinc-rich pyrophosphate granules accumulated in barnacles are not digested in the digestive tract of the predator *Nucella lapillus* and are therefore not bioavailable to that predator. Similarly the physico-chemical form of accumulated cadmium in the oligochaete *Limnodrilus hoffmeisteri* is critical in the assimilation of cadmium by a predator, in this case the decapod *Palaemonetes pugio* (Wallace et al., 1998; Wallace and Lopez, 1997). Other studies have indicated that lead bioaccumulated in mussels and stored in a detoxified form within the soft tissues of the mussels is bioavailable and may cause impacts (Regoli and Orlando, 1994). The potential effects of food preparation (cooking) on metal bioavailability should be considered when assessing risks to human consumers.

Currently there are no standardized tools for incorporating the potential impact of dietary exposure into assessments. Research efforts are focusing on the relative importance of: dietary pathways in relation to waterborne exposures, transfer from sediments into food chains, the potential of bioaccumulated to cause impacts in consumer organisms. One example of an approach that illustrates how exposure, bioaccumulation and trophic transfer can be linked is illustrated below.

A general approach to account for potential impacts arising from trophic transfer would be to link bioaccumulation in prey items to exposure in the water column as well as potential impacts in consumers. One methodology to achieve this is to integrate tissue burden to toxicity relationships (prey: predator interactions) with exposure to bioaccumulation relationships (e.g., BCF and BAF values). A theoretical example of this approach, developed by Brix et al. (www.epa.gov/ncea/raf/pdfs/metals/sumryrprt_metals.pdf) is shown in Figure 4-9.

Within the context of this approach, it would be necessary for relevance to ensure that the species being considered were linked within trophic food webs (i.e., the exposure to bioaccumulation relationship was for prey items being consumed by the predators that are sensitive to trophic transfer). Some data already exists to begin these evaluations. For example, it is possible to derive the water concentrations necessary to produce impacts via dietary exposure (see Brix et al. reference above) and site-specific case studies would be valuable in illustrating, testing, and validating these relationships. The potential benefits from this approach would be reducing the uncertainty from extrapolations across exposure concentrations currently being made with metals BCF/BAF data. Also, there would be an ability to link impacts through to waterborne metal concentrations. However, particularly if BCF and BAF data is used, this approach would bring with it the inherent uncertainty associated with predicting tissue metals burdens (e.g., the high variability) and the inability to account for geochemical influences on uptake and accumulation. Ensuring that the local site specific factors that influence the bioaccumulation and the expression of impacts are understood and recognized would be important, to ensure for example that criteria and guideline values were not set at existing background levels. Two regression relationships, one for exposure and bioaccumulation (green line) and the other for bioaccumulation to dietary toxicity thresholds (blue line) are used to line exposure and dietary impacts.

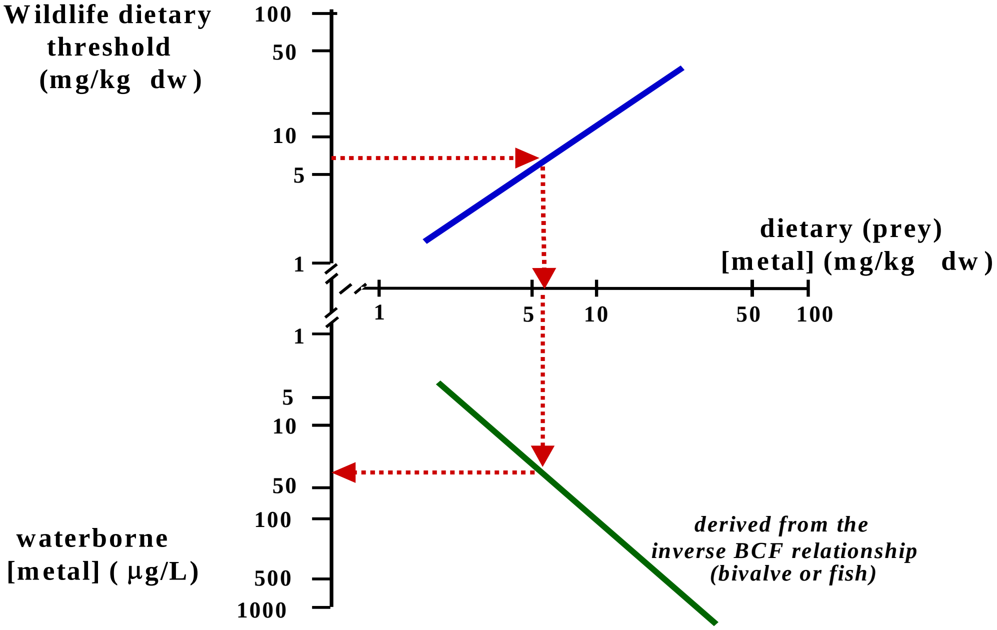

Figure 4-9. Linkages between dietary toxicity threshold, bioaccumulation in prey organisms and waterborne exposure.

4.5.8.3. *Alternatives to Tissue Burdens and Bioaccumulation*

A key parameter that a bioaccumulation measure should be validated against is chronic toxicity. Because bioaccumulation criteria within the context of persistent, bioaccumulative, toxic substances are used as indicators of chronic toxicity (Franke et al., 1994; OECD, 2001), validation of linkages to chronic metal toxicity would provide confidence in their use and application. A number of key issues should be addressed when considering bioaccumulation of metals in relation to the potential for chronic impacts, and these add uncertainty to the interpretation of data. However, unlike the substances that the PBT concept was originally developed for, there is often substantial information on the chronic toxicity of metals.

In some regards, our ability to understand and interpret chronic metal toxicity is as advanced, or possibly more advanced, than metal bioaccumulation. Therefore, rather than trying to derive and validate a surrogate for the chronic impacts of metals, it might, in some cases, be feasible to eliminate bioaccumulation and only consider chronic toxicity data. The development of a criterion based on chronic toxicity could be based on a variety of approaches, all of which will require a modified framework for consideration as BCFs, BAFs, and bioaccumulation would be replaced. Despite the challenges associated with this degree of change, it is worthy of consideration.

4.5.9. Bioaccumulation in Terrestrial Organisms

For terrestrial ecosystems, the concept of bioaccumulation is intended to capture the potential for two ecologically important outcomes: (1) direct toxicity to plants and wildlife and (2) secondary toxicity to animals feeding on contaminated plants and animals. This approach captures the potential for trophic transfer of metals through the food web, so total exposure can be calculated, including dietary intake as well as intake from contaminated environmental media (soil and water). For vegetation, the BAF (or BSAF) is defined as field measurements of metal concentration in plant tissues divided by metal concentration in soil (or soil solution); the BCF is defined as the same measurement carried out in the laboratory (Smolders et al., 2003).

Data applicability is directly related to which tissue is sampled and how it is processed. BAFs for plants include metals aerially deposited on leaves as well as those in soil particles adhering to roots. Such metals will not be part of BCFs, which frequently are determined in hydroponic culture. Similar differences between BCFs and BAFs apply for earthworms exposed in laboratory studies using the filter paper substrate protocols. Furthermore, BCFs with earthworms may not include additional feeding of the animals during the study. Additionally, field studies are reflective of chronic exposures, whereas BCFs may be calculated from shorter time frames. For birds and mammals, whole-body BAFs generally are not calculated, except for small mammals such as rodents (Sample et al., 1998b). Rather, concentrations in target tissues are measured for comparison with critically toxic levels (Beyer et al., 1996).

The BAFs for metals by soil invertebrates and most plants are typically less than 1, although they usually are based on the total metal in soil and tissue. BSAFs expressed in this manner may be suitable for comparisons of metal uptake within the same soil type, but they would be misleading if soil bioavailability factors (e.g., pH and organic carbon) differ. Unfortunately, the literature database is populated almost entirely with BAFs derived from measurements of total metal. Furthermore, bioaccumulation is not a simple linear relationship. Uptake is nonlinear, increasing at a decreasing rate, as medium concentration increases. Reliance on incorrect relationships of the bioavailable portion will be trivial compared to the error associated with BAFs at high concentrations. In the future, a ratio of total metal in the organism to some measure of the bioavailable fraction of metal in the soil (e.g., free ion concentration or weak salt extractable) should be used for expressing a BSAF that allows comparison among different soils. An alternative approach currently under study is to use a multivariate statistical model to look for patterns of uptake of multiple metals to predict the potential bioconcentration of one metal of particular interest (Scott-Fordsmand and Odegard, 2002).

4.5.9.1. *Models for Bioaccumulation in Soil Inverterbrates*

4.5.9.1.1. *Application*

4.5.9.1.1.1. *__Univariate models__*. The bioaccumulation of metals in soil organisms cannot reasonably be modeled from information based solely on soil concentrations. Therefore, models for the prediction of metal bioaccumulation by soil invertebrates are primarily empirical in nature, describing relationships between metal body burdens in oligochaetes and collembola, soil metal concentrations, and soil physical/chemical characteristics. Statistical relationships have been established using univariate and multiple regression

> The bioaccumulation of metals in soil organisms cannot be modeled from information based solely on soil concentrations. Two types of models are available to help describe the relationships between metals body burden and soil parameters: (1) ***univariate models*** that describe statistical relationships between body burdens, soil metal concentrations, and soil physical and chemical properties; and (2) ***multivariate models*** that explain BAFs as a function of soil properties.

approaches. Sample et al. (1998a) and Peijnenburg et al. (1999b) have each developed univariate uptake models for earthworms that are based on empirical data (metal concentrations in worms vs. the natural log of amount of metal in soils) that are widely used as a first approximation. However, these models are not specific to soil type and therefore do not account for bioavailability factors such as pH, clay content, or cation exchange. Furthermore, they do not adequately predict Cr or Ni uptake.

4.5.9.1.1.2. ***Multivariate models.*** Multivariate models also are available (Peijnenburg et al., 1999a, b) for *Eisenia andrei* and *Enchytraeus crypticus* that explain BAF as a function of soil characteristics. The soil parameters that generally contributed the most to explaining the variance between uptake rate constants and BAFs were pH (for Cd, Zn) and also CEC (for Pb) and clay content (for Cd). Similar studies are needed for describing relationships between soil physical/chemical characteristics and metal bioaccumulation in other groups of soil invertebrates such as collembola and isopoda. Until these are available, the models for earthworms and enchytrids can be applied to other groups, although the added uncertainty should be acknowledged.

Path analysis has been suggested as an alternative for multiple regression in describing these relationships. It partitions simple correlations into direct and indirect effects, providing a numerical value for each direct and indirect effect and indicates the relative strength of that correlation or causal influence (Basta et al., 1993). Bradham (2002) used path analysis and backwards stepwise regression analysis to derive statistical models capable of predicting uptake and effects of As, Cd, Pb, and Zn in earthworms as a function of soil properties.

Saxe et al. (2001) described a model for predicting whole-body concentrations of Cd, Cu, Pb, and Zn in *Eisenia andrei* as a function of pH, soluble metals in the soil at gut and environmental pH, and soluble organic carbon in soil extracts. The model also includes parameters that characterize the ability of worms to regulate the metal body burden, whether metal uptake is via the epidermal or gut surface and whether the metal is essential. The model has been validated against a series of Dutch soils and is very good at correctly predicting metal accumulation.

4.5.9.1.2. ***Limitations*****.** Bioaccumulation of organic substances is typically modeled using a One-Compartment, First-Order Kinetics (1CFOK) model. However, most of the assumptions of the model are violated when applied to bioaccumulation of metals by soil invertebrates. Soil invertebrates are not exposed to a constant concentration of metals in the soil over space and time, making it difficult to accurately define exposure. Sufficient data exist on the metabolism of metals to show that all pools of metal taken up by the soil invertebrates are not equally available for depuration (some are actually never depurated and are released only when the organism dies), making a 1CFOK model an inaccurate approximation.

Metal concentrations do not reach a steady state that is proportional to external (i.e., soil) concentrations. This is similar to the situation in aquatic systems where metal bioaccumulation is a function of water concentration. Internal essential metal concentrations are regulated and remain relatively constant over a wide range of soil metal concentrations. Only when normal regulatory mechanisms are overwhelmed do internal levels of essential metals increase. Accumulation of nonessential metals also violates the assumption of steady state, as organisms have evolved mechanisms for the detoxification of nonessential metals that involve the internal accumulation of the metal in forms that are not toxic to the organism (e.g., incorporation into inorganic granules or binding to organic molecules to form metal ligands such as metallothioneins).

4.5.9.2. *Critical Body Residues*

CBRs are an extension of the concept of bioaccumulation to internal concentrations of metals that are correlated with some toxic response and hence represent toxicological bioavailability (Conder et al., 2002; Lanno et al., 1998). Use of CBRs in appropriate species may reduce uncertainties in ecological risk assessment procedures (Van Straalen, 1996; Van Wensem et al., 1994). However, only a few CBRs have been developed in soil invertebrates for metals. Crommentuijn et al. (1997, 1994) and Smit (1997) established CBRs for sublethal effects for Cd and Zn, respectively, in the springtail (*Folsomia candida*). Conder et al. (2002) demonstrated that effects of Cd in earthworms (*Eisenia fetida*) are

Knowing the species of metal that is likely to be present in the environment is important for extrapolating results from laboratory tests to natural settings. Laboratory studies are often done under simplified environmental conditions (compared to the natural environment) that may enhance aquatic bioavailability. Conversely, these studies frequently do not include potential effects of dietary metal exposure.

In addition to abiotic factors that can influence metal bioavaiability, other variables may potentially affect the outcome of laboratory toxicity assays and should be considered in interpretation of data. These include acclimation of test animals to culture conditions, natural background concentrations of the metal (including, but not limited to the metal of interest), potential interactions of the various metals, and potential transformations to bioreactive species. The similarity of the pretest culture medium to the underlying testing medium also should be considered.

correlated with concentrations of the metal in the solid phase of the worms (i.e., the pellet fraction, following homogination and centrifugation). If future research can isolate the fraction of an invertebrate that represents toxicological bioavailability, then it may be possible to estimate a toxicological BSAF representing a relationship between a specific fraction of metal that accumulates in the organism and a measure of chemical bioavailability in the soil. Until then, CBRs based on whole-organism analyses are a reasonable approximation for use in ecological risk assessments.

4.5.10. Sediment Toxicity - Equilibrium Partitioning Approach for Metals

4.5.10.1. *Rationale for Use of EqP Benchmarks*

Toxic pollutants in bottom sediments of the nation's lakes, rivers, wetlands, estuaries, and marine coastal waters create potential for continued environmental degradation even where water column concentrations comply with established human health and aquatic life WQC. In addition, contaminated sediments can be a significant pollutant source that may cause water quality degradation to persist even when other pollutant sources are stopped (U.S. EPA 1997d,e,f; Larsson, 1985, Salomons et al., 1987; Burgess and Scott, 1992).

Because of their widespread use and associated environmental releases, metals such as cadmium, copper, lead, nickel, silver, and zinc are commonly elevated in aquatic sediments. Various types of sediment guidelines have been proposed for assessing the potential effects of these metals on benthic invertebrate communities. Many of these involve empirical correlations of metal concentrations in sediment with associated biological effects (e.g., sediment toxicity); these include ER-M, ER-L, TEL and PEL, SLC, AET and others (Sullivan et al., 1985; Persaud et al., 1989; Long and Morgan, 1990; Ingersoll et al., 1996; MacDonald et al., 1996). Most of these approaches use measures of total metal in sediment, and do not account for differences in bioavailability of metals among sediments. Nevertheless, when examined across large data sets, these empirical guidelines do show relationships between concentrations of total metal and biological effects. However, a limitation to these empirical approaches is that the causal linkage between the measured concentration of metals and the observed toxicity cannot be established, in part because of the procedures used to derive correlative values and because values derived are based on total rather than bioavailable metal concentrations. That is, for any given total metal concentration, adverse toxicological effects may or may not occur, depending on the physicochemical characteristics of the sediment of concern (Tessier and Campbell, 1987; Luoma, 1989; Di Toro et al., 1990).

An alternative to empirical approaches is equilibrium partitioning (EqP) theory. The EqP approach is intended to predict concentrations that will or will not cause adverse effects based on an understanding of the factors that control metal bioavailability in sediments and the relationship between that bioavailability and biological effects. For cationic metals (e.g., cadmium, copper, lead, nickel, silver, and zinc), these factors include the presence of acid volatile sulfides (AVS), which form insoluble metal sulfides that are believed to have low biological availability. Beyond AVS, particulate organic carbon (POC) and iron and manganese hydroxides in sediment, and dissolved organic matter (DOM) in interstitial water are also believed to influence metal bioavailability in sediment. By recognizing differences in bioavailability among sediments, the intent of the EqP approach is to produce guideline values that are applicable across a wide variety of sediments and represent causal relationships between specific chemicals and sediment toxicity.

In 1987, EPA reviewed proposed approaches to developing numerical sediment quality guidelines (Chapman, 1987). All of the approaches reviewed had strengths and weaknesses, and no single approach was found to be applicable for the derivation of sediment benchmarks in all situations (U.S. EPA, 1989d). The EqP approach was selected for further development because it presented the greatest promise for generating defensible national chemical-specific sediment benchmarks applicable across a broad range of sediment types. While the Agency has never adopted formal sediment quality criteria, the technical development of sediment quality guidelines was pursued and has resulted in the publication of several numerical guidelines (See http://www.epa.gov/nheerl/publications/). These guidelines are called “EqP sediment benchmarks” (ESBs) and are numerical concentrations for individual chemicals or mixtures of chemicals derived using the EqP approach. As described below, these are intended to be concentrations below which there should not be direct toxicity to most benthic organisms as a result of the chemical or chemicals addressed in the guideline.

4.5.10.2. *Application of the EqP Approach*

As originally proposed, the EqP approach for copper, cadmium, lead, zinc, nickel, and silver focused on AVS as the principal partitioning phase. Because these metals form sulfides that are highly insoluble, toxicity from these metals is not expected if there is sufficient sulfide available to bind all available metal. Because some metals in sediment are present in mineral forms that are not highly reactive, the metal concentrations to which the sulfide concentration is compared is not the total metal in sediment, but the metal extracted simultaneously with the sulfide, and is therefore referred to as simultaneously extracted metal (SEM). Because cadmium, copper, lead, nickel, and zinc are divalent metals, 1 mol of each metal can bind with 1 mol of AVS. The molar concentrations of these metals are compared with AVS on a one-to-one basis. Silver, however, exists predominantly as a monovalent metal, so that silver monosulfide (Ag_2S) binds 2 mols of silver for each mol of AVS. Therefore, SEM Ag will represent the molar concentration of silver divided by two, [Ag]/2, which is compared with the molar AVS concentration. Thus, the solid-phase AVS ESB is defined as:

$$\Sigma_i\,[SEM_i] \le [AVS]$$

where:

$$\sum_i [SEM_i] = [SEM_{Cd}] + [SEM_{Cu}] + [SEM_{Pb}] + [SEM_{Ni}] + [SEM_{Zn}] + 1/2[SEM_{Ag}]$$

When this sum is less than the molar concentration of AVS, no toxicity is expected because there is sufficient sulfide to bind all SEM. Results of calculations using chemical equilibrium models indicate that metals act in a competitive manner when binding to AVS. That is, the six metals will bind with AVS to form their respective sulfides in the order of their increasing solubility: silver, copper, lead, cadmium, zinc, and nickel. Therefore, they should be considered together. There cannot be a guideline just for nickel, for example, because all the other metals may be present as metal sulfides and therefore, to some extent, as AVSs. If these other metals are not measured as a mixture, then the $\sum$SEM will be misleadingly small, and it might appear that $\sum[SEM]<[AVS]$ when in fact this would not be true if all the metals were considered together. It should be noted that EPA currently restricts this discussion to the six metals listed above; however, in situations where other sulfide-forming metals (e.g., mercury) are present at high concentrations, they also should be considered.

While the SEM-AVS comparison has been found effective for predicting when metals in sediment will not cause toxicity, the presence of excess SEM above AVS does not always portend toxicity. This is, at least in part, because other components of sediments can also reduce the bioavailability and toxicity of metals. To address these additional factors, a second component of the metal mixture ESB was developed: the interstitial water benchmark. The concept behind the interstitial water component is that even if there is excess SEM above AVS in a sediment, if there is sufficient additional binding available to maintain non-toxic concentrations of metals in the intersititial water, then the sediment should not be toxic, despite the excess SEM. The aggregate toxicity of metals in the interstitial water is assessed by dividing the concentration of dissolved metal in interstitial water by the final chronic value from the EPA water quality criterion for the protection of aquatic life. This ratio is called the Interstitial Water Benchmark Unit (IWBU), and these are summed across all of the metals (using an assumption of addivity, as described in Section 4.5.3); if the sum is less than 1, then the interstitial water-phase ESB is met, and toxicity is not expected. Mathematically, this equates to:

$$\Sigma[M_{i,d}]/ [FCV_{i,d}] \le 1$$

where:

$$\textstyle\sum_i([M_{i,d}]/FCV_{i,d}) = \sum_i([M_{Cd,d}]/FCV_{Cd,d}) + \sum_i([M_{Cu,d}]/FCV_{Cu,d}) + \sum_i([M_{Pb,d}]/FCV_{Pb,d}) + \sum_i([M_{Ni,d}]/FCV_{Ni,d}) + \sum_i([M_{Zn,d}]/FCV_{Zn,d})$$

For freshwater sediments, the FCVs are hardness dependent for all of the divalent metals under consideration, and thus should be adjusted to the hardness of the interstitial water of the sediment being considered. Because there are no FCVs for silver in freshwater or saltwater, this approach is not applicable to sediments containing significant concentrations of silver (i.e., ΣSEM>AVS). Because silver has the smallest solubility product and the greatest affinity for AVS, it would be the last metal to be released from the AVS or the first metal to bind with AVS. Therefore, it is unlikely that silver would occur in the interstitial water of any sediment with measurable AVS (Berry et al., 1996).

When used together, the overall metal mixture ESB is met if either the SEM-AVS or interstitial water ESBs are met. Further, both guidelines are no-effect guidelines, in that they predict the absence of toxicity if they are met. Though the probability of toxicity is higher if both components of the ESB are exceeded, and this probability increases as the magnitude of exceedance increases, failing to meet the ESB does not imply that toxicity will necessarily occur. This is because neither the SEM-AVS or interstitial water components address all factors that may reduce the bioavailability and toxicity of metals in sediment. Site-specific toxicity testing may be advisable to refine assessments for specific sites.

4.5.10.3. ***Enhancements to the Metals ESB***

The AVS guideline is a “no effects” guideline; that is to say that if the guideline is not exceeded, the sediments should not be toxic due to the metals included in the guideline, but an exceedence of the guideline does not necessarily mean that the sediments will be toxic due to the presence of those metals. One way to reduce the uncertainty of a prediction of toxicity is to normalize for the fraction of organic carbon in the sediment and use $\sum$SEM-AVS)/f_{OC} for the solid-phase guideline. The use of this guideline is described in the draft metals ESB (EPA 2002f).

A refinement of this organic carbon correction has been proposed by Di Toro et al. (2004, submitted). In this analysis, organic carbon partition coefficients are calculated for each metal and used to estimate combined AVS-organic carbon partitioning. Although this approach has not been widely applied as yet, it has a strong theoretical basis, and initial applications suggest that it can strengthen the ability of EqP to predict metal toxicity (rather than just non-toxicity) in sediments.

The metals ESB currently applies to only six metals: cadmium, copper, lead, nickel, silver, and zinc. However, AVS can also be used to predict the lack of toxicity in sediments due to chromium because the presence of AVS in sediments is indicative of reduced sediments. Chromium is present primarily in its reduced form (CrIII) in reduced sediments, and in this form it is much less toxic and bioavailable than the oxidized form of chromium (CrVI) (Berry et al., in prep.). The use of AVS in the prediction of the lack of chromium toxicity in sediments will be described in an addendum to the metals ESB.

4.5.10.4. ***Endpoints of Concern***

The metals ESB should be viewed as an approach, as opposed to a specific number. The ESB calculates a concentration in sediment that will correspond to a given toxicological endpoint using partitioning theory. However, the correspondence of the benchmark is variable, depending on which toxicological endpoints are selected. The FCVs from the metals WQC are used in the ESB as examples because they are familiar and provide a widely accepted level of protection. Other toxicological endpoints could be chosen, however, if a different level of protection was desired. For example, the water-only, chronic no-effect concentration for a threatened or endangered species might be used as the toxicological endpoint. EqP theory could then be used to calculate a sediment concentration that would be protective of that species.

4.5.10.5. *Limitations*

It should be emphasized that the metals ESBs are intended to protect benthic organisms from the direct effects of these *six metals* in sediments that are permanently inundated with water, intertidal, or inundated periodically for durations sufficient to permit development of benthic assemblages. They do not apply to occasionally inundated soils containing terrestrial organisms. These benchmarks do not address the possibility of bioaccumulation and transfer to upper-trophic-level organisms or the synergistic, additive, or antagonistic effects of other substances.

The EqP approach to metal assessment has been questioned on several technical issues, such as whether it applies to organisms that live in oxygenated burrows, whether it adequately accounts for ingestion of sediment, and whether it is appropriate to use bulk sediment chemistry to represent responses to microenvironments that exist in bedded sediment. Many of these issues are difficult to address in a comprehensive manner, and more study will be required to fully evaluate them. In the interim, it should be noted that there is a considerable body of experimental data supporting the EqP approach to metals (Berry et al. 1996; Hansen et al. 1996; Di Toro et al. 2004; EPA 2002f), and these data include tests with organisms with varying life histories, that use irrigated burrows, and that ingest sediment. While this does not prove that the criticisms above are not legitimate, it provides support for continuing to apply the EqP approach as a reasonable representation of metal toxicity in sediment, at least until a superior approach is developed.

Because AVS is a product of microbial activity, AVS can vary seasonally with changes in microbial activity. The degree to which such cycling can affect the potential risk from metals in sediments is unclear but should be considered in sampling programs designed to assess SEM-AVS.

Because metals bound to sulfide do not appear to be sufficiently bioavailable to cause toxicity in sediment toxicity tests, one would not expect bioaccumulation of metals in sediments with more AVS than SEM. However, several studies have found bioaccumulation of metals even when excess AVS is present (Ankley et al., 1996). This has caused considerable debate about the appropriateness of SEM-AVS for assessing metal toxicity in sediments (e.g., Lee et al., 2000), because it suggests that metal bioavailability may not be effectively represented by SEM-AVS analysis. However, there is a large number of studies indicating that toxic effects of metals are absent in sediments when SEM is less than AVSs, even when bioaccumulation is observed. This suggests that the bioaccumulated metals may not be toxicologically available or of sufficient concentration in the organism to cause effects. In addition, these metals do not biomagnify to higher trophic levels in aquatic ecosystems (Suedel et al., 1994). Therefore, an ESB based on the difference between the concentrations of SEM and AVS still appears appropriate for protecting benthic organisms from the direct effects of sediment-associated metals, but not for protecting against metal bioaccumulation.

4.5.10.6. ***Use and Implementation***

In practice, the sediment benchmarks for these six metals are not exceeded, and benthic organisms are sufficiently protected (defined in this case as the level of protection afforded by the WQC), if the sediment meets either one of the following benchmarks:

- The solid phase benchmark: $\sum_i[\mathrm{SEM}_i] < [\mathrm{AVS}]$; in other words, if metal, measured as SEM, does not exceed AVS; or

- The interstitial water benchmark: $\sum_i([M_{i,d}]/\mathrm{FCV}_{i,d}) < 1$; in other words, if the sum of the contributions of all six metals in the interstitial water would not be expected to cause chronic effects at the level of protection afforded by the WQC.

If the AVS or interstitial water ESBs is exceeded, there is reason to believe that the sediment might be unacceptably contaminated by these metals. Further evaluation and testing would, therefore, be necessary to assess actual toxicity and its causal relationship to the metals of concern. If data on the sediment-specific SEM, AVS, and organic carbon concentrations are available, the uncertainty bounds for $(\sum\mathrm{SEM\text{-}AVS})/f_{OC}$ described in the metals ESB could be used to further classify sediments as those in which metals are not likely to cause toxicity, metal toxicity predictions are uncertain, or metal toxicity is likely. For sediments in which toxicity is likely or uncertain, acute and chronic tests with species that are sensitive to the metals suspected to be of concern, acute and chronic sediment toxicity identification evaluations (TIEs), in situ community assessments, and seasonal and spatial characterizations of the SEM, AVS, and interstitial water concentrations would be appropriate (Ankley et al., 1994).

4.5.11. Soil Toxicity

Variability among soil toxicity test results is due in part to the influence of soil properties on bioavailablity of metals (e.g., pH, organic matter and CEC). See Sections 3.1, 3.4.6, and 3.2, covering environmental chemistry bioavailability, terrestrial bioavailability, terrestrial exposure issues. Additionally, incorporation of sparingly soluble substances, such as many environmental forms of metals, into the soil matrix is difficult, and acclimation/adaptation of test organisms can further complicate test results. Use of soluble metal salts with the addition of organism to the test matrix immediately after mixing is not representative of most environmental situations, where aging and other physical/chemical processes affect metal speciation and uptake. Furthermore, testing of soil microbial function is particularly problematic because the test substance is added to soils with the microbial population already in place and that contain background amounts of metals.

4.5.11.1. *Application*

Modifications to standard toxicity bioassays for plants and soil organisms to account for properties of metals were discussed in an expert workshop and subsequently provided in Fairbrother et al. (2002). These modifications include directions on type of soil matrices to use, mixing and aging of metals into the soil, and cautions about acclimation of test organisms. Future studies conducted specifically for development of toxicity endpoint values for metals can follow these suggested protocols and circumvent many of the past problems.

There is, however, a large body of literature on toxicity of metals to soil organisms that has already been developed, although often the objectives were to understand processes rather than to develop defensible toxicity thresholds. The challenge, therefore, lies in how to use these data, taking into account the test-to-test variability in soil chemistry parameters, and how to develop a technically defensible means of extrapolating toxicity responses across soil type—in other words, how to adjust the toxicity threshold values for bioavailability differences in test conditions. Ideally, an aquatic BLM could be extended to terrestrial setting to account for differences in bioavailability due to environmental chemistry, particularly for plants and soft-bodied soil invertebrates. Studies have been initiated to develop what has been referred to as a *terrestrial BLM*, or tBLM. The conceptual approach to development of a tBLM is very much the same as for the variations of the BLMs that have been developed for aquatic settings (Allen, 2002). Although not currently developed to the point of being of practical use, experimental testing and model development programs are fully underway in the hope of providing a tool that will be of great practical utility in the relatively near future.

Another approach to addressing soil variability in soil toxicity tests is to normalize test results by dividing the LC50 by percent organic matter (van Gestal, 1992). This approach is based on observed correlations between the LC50 of copper to earthworms and soil organic matter content. Most recently, CEC has been shown to be the most important factor modifying zinc bioavailability in soils for both invertebrates and plants, and it will presumably show a similar relationship with other cationic metals (Smolders, 2003 presentation in DC; based on work by C. Janssen, Univ. Ghent). Therefore, effect responses in different soil types can be normalized on the basis of relative CEC. It should be remembered that CEC is a function, at least in part, of soil pH. Therefore, normalization can be done only among soils of similar pH ranges. However, comparison of field data with laboratory toxicity response information is best done through measuring metals in soil pore water from field assessments and comparing such data to spiked laboratory soils.

Several studies of plants and invertebrates have suggested that consideration of aging may result in significant overestimates of field effects using data laboratory studies (e.g., Smit and Van Gestel, 1998). Estimates of effects of aging (see Section 4.1.7.2., Environmental Chemistry for an aging discussion) range from 3 to 8-fold differences between laboratory and field results.

Because most soil properties are correlated to some degree, isolating individual soil parameters and relating them to soil biota toxicity is difficult. Treating the soil like a black box and using sequential extraction techniques has been useful in determining what fraction of the metals are in a labile form (Kabata-Pendias and Pendis, 2000). However, some scientists have questioned the usefulness of this process (Morgan and Morgan, 1988).

4.5.11.2. *Limitations*

Only two methods have been proposed in the literature for normalizing toxicity data across soils to account for differences in bioavailability: adjusting of endpoint values by percent organic matter in the soil or as a function of organic matter plus clay content. Both methods fail to incorporate either pH or cationic exchange capacity, both of which are of critical importance in determining bioavailability. Aging of metals in soils also is not included in these approaches. Furthermore, the data sets used to generate the relationships were not sufficiently robust to make generalizations possible across all soils and all organisms. The development of a tBLM shows promise as a method that will overcome these limitations; however, it likely will not be completed for at least 2 years after the publication of this Framework.

The use of a tissue residue approach has been suggested as another method to address soil chemistry and metal toxicity issues, suggesting that a metal concentration must reach a threshold value in the organism or at the target site before effects begin to occur (McCarthy and Mackay, 1993; Lanno and McCarty, 1997). For essential elements in plants, deficiency/sufficiency concentrations in foliage have been developed. However, the relationship between toxicity and tissue residues is complex and varies depending on tissue type (roots vs. shoots), plant species, and metal. Little information is available for soil invertebrates, so relationships between tissue concentration and toxic response cannot yet be developed. Therefore, this approach, although conceptually sound, requires significant research before critical tissue levels can be established.

For plants, a large proportion of the toxicity literature was developed in support of understanding potential toxicity and metal uptake from biosolids (e.g., sewage sludge). It is difficult to determine single-species, single-metal thresholds from this database for several reasons. First, biosolids tend to contain a mixture of metals, so any response observed cannot be attributed to a single metal and should account for potential antagonism or synergies that might occur. Second, biosolids are, by their nature, high in organic matter, which significantly affects bioavailability of the metals. Until a robust method is developed to adjust toxicity endpoints for the influence of organic matter (and other bioavailability factors), it will remain difficult to apply such results to unamended soils. The additional organic matter also provides excess nutrients to the plants, which further confounds possible metal effects. The guidance for development of Ecological Soil Screening Levels or EcoSSL (U.S. EPA, 2003c) can be used to judge the applicability of literature studies to plant or soil invertebrate toxicity threshold determinations.

4.5.12. Food Chain (Wildlife) Toxicity

Toxicity in wildlife from metals exposures is generally poorly understood and is rarely quantified in field settings. A few notable exceptions are those mechanisms described in avian waterfowl exposure to selenium (Adams et al., 2003), exposure of waterfowl to lead-contaminated sediments (Beyer et al., 1998; Blus et al., 1991; Henny et al., 2000), and white-tailed ptarmigan exposure to cadmium in vegetation (Larison et.al., 2000). Most metals express multiorgan toxicity, resulting in a general decrease in overall vigor, as opposed to well-defined mechanisms of action documented from organic xenobiotics such as pesticides. Typically, toxicological data used to assess the risk of many metals to wildlife are derived from laboratory species such as rats or mice or domestic livestock species (e.g., cattle and chickens) exposed to soluble metal salts. Extrapolating the results of such tests to evaluate toxicity to wildlife is necessary because of the paucity of data on the toxicity of metals to these receptors. However, extrapolation of results should be approached with caution due to the large amount of uncertainty that could be introduced into the risk assessment process (Suter, 1993).

Laboratory and domestic species may be more or less sensitive to chemicals than is the selected receptor. Toxicological responses among species vary because of many physiological factors that influence the toxicokinetics (absorption, distribution, and elimination) and toxicodynamics (relative potency) of metals after exposure has occured. For example, differences in gut physiology, renal excretion rates, and egg production influence the toxicokinetics of metals. The ability of some species to more rapidly produce protective proteins such as metallothionein after exposure to metals are toxicodymamic features leading to interspecific extrapolation uncertainty. For example, mammal studies should not be extrapolated to birds, and extrapolation of data from rats (simple, monogastric digestive physiology) to ruminants introduces more uncertainty than does extrapolation from rats to canids, and so on. In the case of metals, which some species are able to regulate or store in their tissues without experiencing toxic effects (i.e., biota-specific detoxification), extrapolations between species used to assess bioaccumulation and toxicity can be especially problematic.

4.5.12.1. *Application*

Methods for extrapolating metal effects data among species are not unique to metals risk assessment, with the exception of understanding different requirements for essential elements. Some of the methods for extrapolating effects data among species include body weight normalization (Sample and Arenal.,1999), distribution-based approaches (Van Straalen, 2001), or the use of uncertainty factors (e.g., Calabrese and Baldwin, 1994). All of these approaches suffer from a lack of an underlying physiological basis quantifying toxicokinetic and toxicodynamic responses among species. A review of potential extrapolation methodologies can be found in Kapustka et al. (2004).

Currently, the best sources of information on metal toxicity thresholds are NAS/NRC (1980; 1994), McDowell (2003), and the documentation supporting development of EcoSSLs values (U.S. EPA, 2003c). The EcoSSL document also includes a general approach for screening studies for acceptability for use in derivation of toxicity thresholds for risk assessments that can be used for deriving site-specific TRVs for the most applicable endpoints. These endpoints should then be extrapolated to species with similar physiology, particularly of the digestive system, due to the predominance of the dietary exposure pathway (e.g., cow data can be applied to wild bovids such as bison and possibly to other ruminants such as deer or elk). Uncertainty factors can be carefully applied if there is concern for extrapolation of data to species in a different taxonomic category (e.g., genus, family or class). General summaries for some metals are available in Beyer et al. (1996) and Fairbrother et al. (1996).

4.5.12.2. *Limitations*

Information on toxicity of metals to wildlife under field conditions is severely limited, focusing on only a few species and a few metals. Deriving TRVs for metals in wildlife is problematic because the administered form of metal is typically not found in most applied settings. For example, lead has one of the largest databases for laboratory exposures but has been limited by the form of the metal studied. Until recently, almost all field studies were conducted in support of toxic effects of lead shot (i.e., pure elemental lead), whereas almost all laboratory studies have administered lead to test subjects as lead acetate. These extreme forms of lead relative bioavailability make extrapolations to dietary exposures difficult for this substance. The best approach in this case is the use of critical tissue residues, because liver lead levels indicative of lead poisoning are well established (Beyer et al., 1996). Selenium is another example where tissue-based toxicity thresholds (in this case, in the avian egg) may be most appropriate (e.g., Adams et al., 2003).

Cross-species extrapolations should be conducted with some knowledge of animal physiology and specific responses to metals. Digestive physiology is the most important distinction, because most metal exposures in wildlife are by the dietary route. However, other specific organismal responses should be understood as well. For example, sheep are much more sensitive to copper than are other ruminants because of the particular nature of their gut flora (NAS/NRC, 1980). Therefore, extrapolation of sheep data to other ruminants would be highly overconservative. On the other hand, pigs are extremely tolerant to copper, possibly due to low gut uptake rates (NAS/NRC, 1980), so extrapolations of such data to other monogastric animals would not be protective.

Interactions of metals (see Section 3.3. Human Health) also should be taken into account when analyzing metal toxicity data for wildlife. As noted above, values that may be protective for a particular metal within a certain animal may not be so if other metals are present or deficient (or vice versa). Therefore, dietary studies should be examined to understand the presence of other metals and to ascertain the sufficiency of essential elements. Application of single-metal thresholds to field situations, whether in a site-specific context or on a national scale, should make provision for adjustment to account for interactive effects.