6. TERRESTRIAL ECOLOGICAL RISK ASSESSMENT FOR METALS

This section of the Framework provides an overview of how the principles for metals risk assessment apply to ecological risk assessments for terrestrial environments. Receptors typically considered in these assessments include soil invertebrates, plants, and wildlife species. Some assessments also examine effects on microbiota and soil processes. This section of the Framework builds on the information presented in Chapter 2 that lays out issues to be considered during Problem Formulation and that describes metal chemistry associated with soil systems. That information is not repeated here and the reader should refer to Chapter 2 for this information.

6.1. METALS PRINCIPLES

Metals have specific environmental and biotic attributes that should be considered in all risk assessments. These principles for metals risk assessment (see Chapters 1 and 2) apply in various ways to ecological risk assessments depending on the scale of the assessment (site specific, regional, or national). This section describes applications of the principles to terrestrial ecological assessments within the standard risk assessment framework. Specifically, they fall into the risk assessment paradigm as follows:

Background levels	Exposure Assessment
Mixtures	Exposure and Effects Assessment
Essentiality	Effects Assessment
Forms of metals	Exposure and Effects Assessment
Toxicokinetics/toxicodynamics	Exposure Assessment (bioavailability) and Effects Assessment (ADME and toxicity)

6.2. CHARACTERIZATION OF EXPOSURE

Metal exposure assessment includes characterization 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.

6.2.1. Natural Occurrence of Metals

At a national level, metal concentrations vary naturally in soils across the U.S. These variations pose challenges for conducting national assessments of risk to terrestrial ecological receptors. The assessor may decide to use a single toxicity level regardless of background concentrations for a screening type assessment (see text box on ecological soil screening levels or Eco SSLs) (U.S. EPA, 2003c) or may prefer to divide the country into regions of similar metal

background levels (metalloregions). Exposure assessments should consider metal levels inclusive of background.

At the regional and local (site) scales, risk assessors should account for the natural occurrence of metals either at the beginning of an assessment (i.e., during Problem Formulation), during the assessment, or when making risk management decisions about the implications of the predicted or observed levels of metals in soils. Because the national soil survey[4] is over 20 years old, risk assessors should consider the feasibility of generating site-specific concentrations for local risk assessments.

> **EPA's Ecological Soil Screening Levels**
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> EPA's **Ecological Soil Screening Levels** (Eco SSLs) for metals are national-level concentrations of metals in soils that are protective of wildlife, plants, and soil organisms. These values are lower than naturally occurring levels in some parts of the country. Exceedences of such levels does not mean that a risk exists but does mean that a more regional or site-specific assessment may be needed.

More appropriately, risk assessors should avoid single-result assessments for the entire country. 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 water quality criteria (Griffith et al., 1999). The use of metalloregions provides the ability to account for the broad regional parameters affecting metal availability in soils and waters as well as for the differences in organism response to added metal.

The metalloregion concept (McLaughlin and Smolders, 2001), although intuitively appropriate, has not yet been fully developed for the U.S. 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 organic matter [OM]) should be overlaid on the ecoregions, along with soil type (e.g., sandy loam, clay loam) and background concentrations 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. Nevertheless, work is under way to develop metalloregions (e.g., McLaughlin and Smolders, 2001), 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.

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[4] Schacklette, HT; Boerngen, JG. (1984). Element concentrations in soils and surficial materials of the conterminous United States. U.S. Geological Survey Professional Paper 1270. 105 pp.

6.2.2. Forms of Metals

The physical and chemical forms of metals influence exposure and subsequent effects and can be influenced by physical/chemical conditions in the environment. National level assessments involve a broad range of environmental conditions and so the risk assessor should account for different metal species in different locations and soil types. As assessments transition from national, to regional, to local, the assessor should incorporate site-specific soil parameters that influence metal speciation (e.g., pH, CEC, clay content). National values (e.g., geometric mean values) of these parameters should be used, with the same recommendation as discussed in Section 5.1.3 on Natural Occurrence of Metals.

6.2.3. Exposure Routes

The major metal *exposure route* that the risk assessor should consider for wildlife is ingestion, with a minor (and often unknown) inhalation component. For plants, root uptake is the most important with leaf exposures secondary, with the exception of Hg where the majority is accumulated via foliar uptake; Cd and sometimes Pb also may be accumulated through foliage but amounts relative to soil exposure will vary depending upon soil conditions (e.g., pH). Plants may also lose metals through foliar leaching during precipitation events although to a significantly lesser extent than for other micronutrients such as potassium. Soil invertebrates are assumed to be exposed through direct contact. *Pathways* describe transport of the contaminant in the environment and include uptake and bioconcentration (e.g., dietary ingestion of a soil contaminant that has been taken up by plants). Principles of metal transport and fate in soils are applicable to assessments of risk to all terrestrial organisms and will be discussed first. However, because of significant differences in exposure routes and pathways between invertebrates, plants, and wildlife, it is more convenient to discuss exposure assessment methods by receptor group.

6.2.4. Soil Transport and Fate Models

Risk assessors routinely use transport and fate models (i.e., a computational model) to describe and quantify exposure pathways. Models are also useful in situations where risk assessors are trying to estimate exposure levels that are expected to result from the implementation of some permitting action or remediation measures at local, regional, or national scales. Numerous models are available for use; most are based on the same fundamental principles: metals are ubiquitous in the environment and within each media compartment they are present in association with water (freely dissolved metal or as organic and inorganic metal complexes),

> **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).

particles (sorbed, precipitated, or incorporated within a mineral phase), and air. The risk assessor can find a more detailed discussion of these processes in Section 3.2 on Fate and Transport. Currently, there is no single model available that encompasses all the desirable metal-specific features for terrestrial systems. Discussions of the available terrestrial transport and fate models, as well as a number of chemical equilibrium models, may be found in Allen (2002).

6.2.5. Toxicokinetics/Toxicodynamics

Target organ exposure levels and subsequent effects depend on how environmental conditions affect speciation of a metal (e.g., whether an organism actively takes up or excludes metals in soils and how an organism processes metals internally). See Section 3 for details on environmental chemistry and issues relating to bioaccumulation. Risk assessors should specifically address bioavailability and bioaccumulation for each metal of concern in each environment (either a local site for site-specific assessments or some larger estimate for regional and national level assessments).

6.2.5.1. *Bioavailability*

Risk assessors should adjust bulk soil metal concentrations by appropriate bioavailability factors to achieve comparable, actual uptake of metals by soil organisms. This will standardize exposure values across soil types and allow for more accurate comparisons with laboratory toxicity data. Cation exchange capacity (CEC) recently has been shown to be an important factor modifying zinc bioavailability in soils, 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 (OM) and oxyhydroxides present in the soil, and is strongly pH dependent. Surface charge on OM and oxyhydroxides increases with pH, thereby increasing their sorptive capacity for metals (thus decreasing metal bioavailability). Conversely, positive surface charges increase as the pH drops, which increases sorption of anions (e.g., As or Se) 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 for the risk assessor to note that most published values of CEC are measured at pH 7. In general, risk assessors can assume that cationic metals are more bioavailable at lower soil pH (<6) and less bioavailable at higher soil pH (>8). The opposite assumption holds for anionic metals.

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. Preliminary studies suggest that consideration of aging may result in estimates of the bioavailable fraction as low as $0.1 \times$ bulk soil concentrations (McLaughlin et

al., 2002). 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 and the risk assessor should acknowledge this as a significant uncertainty during the risk characterization.

Ideally, exposure should be expressed on the basis of pore water concentration, to account for all factors influencing bioavailabilty; however, there are currently significant limitations to collecting and interpreting metal-related data from soil pore waters and such information generally is not available (even at site-specific assessments and never for regional or national assessments). The risk assessor could estimate metal concentration in soil pore water using EqP theory (as with sediment pore water analyses; see Section 3.1.5). The risk assessor can use published soil binding coefficients (K_ds) to estimate partitioning between soil particles and pore water although these values also are inherently uncertain (published value depends on derivation method, soil type, etc.). Furthermore, toxicity threshold values generally are provided as bulk soil concentrations so the risk assessor would not be able to compare pore water exposure with any effects estimates.

6.2.5.2. *Bioaccumulation*

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 stresses 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 or soil invertebrates, the bioaccumulation factor (BAF; or biota-soil accumulation factor, 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).

Risk assessors should be aware that 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 soils versus laboratory studies using the filter paper substrate protocols. Furthermore, BCFs within earthworms may not include additional feeding of the animals during the study. Field studies are reflective of chronic exposures, whereas BCFs may be calculated from shorter time frames. Ideally, risk assessors should select BCFs reported at equilibrium (i.e., after sufficient exposure time to maximize the BCF). Whole-body BAFs generally are not calculated for birds and mammals, except for small mammals such as rodents (Sample et al., 1998b). Risk assessors should understand the conditions under which metal concentrations were measured and critically examine data to determine whether they are reported

as wet or dry weight (the ratio of tissue to soil concentrations must be done on the same wet/dry weight basis for both).

For soil invertebrates and most plants, metal BAFs are typically less than 1 and usually are based on the total metal in soil and tissue that do not account for bioavailability differences. The risk assessor might consider using 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) for expressing a BAF to allow comparison among different soils, although, in general, data are lacking for using this method.

Furthermore, the risk assessor is reminded that bioaccumulation of metals is not a simple linear relationship. Uptake is nonlinear, increasing at a decreasing rate as medium concentration increases. Models for predicting metal bioaccumulation by soil invertebrates are primarily statistical in nature, describing relationships between metal body burdens in oligochaetes and collembola, soil metal concentrations, and soil physical/chemical characteristics. 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 risk assessors can use as a first approximation for bioaccumulation in soil invertebrates; however, these models are not specific to soil type and, therefore, do not account for bioavailability factors. Furthermore, they do not adequately predict Cr or Ni uptake. An alternative approach that the risk assessor could consider is the use of multivariate statistical models 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) or BAF as a function of soil characteristics (Saxe et al., 2001; Peijnenburg et al., 1999a, b). 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 (Bradham, 2002; Basta et al., 1993).

The absolute level of metal accumulation is not as important as the rate of uptake (Hook and Fisher, 2002; Hook, 2001; Roesijadi, 1992). 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., metallothionein) could be exceeded; cytosolic metal levels then become unacceptably high, and adverse effects can ensue. Because measures of uptake rates are not available, static concentrations are used; the risk assessor should acknowledge this uncertainty during the Risk Characterization.

6.2.6. Soil Invertebrate Exposure

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. However,

the risk assessor should estimate exposure as a function of soil concentration, rather than as a detailed analysis of movement of metals through the food web, to generate data that will be comparable to effects concentrations. This is a reasonable approximation for soft-bodied invertebrates (e.g., earthworms) whose metal exposure is primarily through soil pore water (from both dermal absorption and soil ingestion) (Allen, 2002). There is more uncertainty in correlating soil metal 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, risk assessors should estimate soil invertebrate exposure on the basis of total metal concentration in bulk soils (adjusted for relative bioavailability, where possible) collected in the top 0-12 cm of soil (U.S. EPA, 2003c, 1989b). In detailed, site-specific 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 Coll*embola*) and deeper-soil-dwelling organisms (e.g., various species of earthworms).

6.2.7. Plant Exposure

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).

The risk assessor should consider the default approach to estimating exposure of plants to metal as measuring metal concentrations in bulk soil (top 0-12 cm). However, as with soil invertebrates, this overestimates exposure because it does not account for differential bioavailability and aging. The risk assessor generally can categorize metal bioavailability and uptake based on soil pH and organic matter (see Section 3.1.6.5). 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).

Table 6-1. Qualitative bioavailability of metal cations in natural soils to plants and soil invertebrates

Soil type	Soil pH		
	Low organic matter (<2%)	Medium organic matter (2 <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 6-2. Qualitative bioavailability of metal anions in natural soils to plants and soil invertebrates

Soil type	Soil pH		
	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 (2003c).

Qualitative relationships between soil chemistry and bioavailability are appropriate for national-scale application. However, for site-specific or metals-specific applications, the risk assessor should use quantitative methods. Parker and Pedler (1997) and Lund (1990) have suggested that only uncomplexed, free ionic species of cations can be taken up by roots, and this has been described using a Free Ion Activity Model (FIAM) similar to the Biotic Ligand Model (BLM) used in aquatic systems. However, significant exceptions to the free-ion model have been identified; so until this theory is tested more thoroughly, the risk assessor should continue to estimate exposures using bulk soil values with qualitative estimates of bioavailability based on soil type (pH and OM). Again, the risk assessor should acknowledge these uncertainties during the Risk Characterization.

6.2.8. Wildlife Exposure

The relative importance of exposure pathways and routes varies by animal species and by metal, although, in general, wildlife exposure is primarily through diet and incidental ingestion

of soils or sediments. There are certain chemicals and
exposure situations for which inhalation or dermal pathways
are important, but in most situations the risk assessor can
consider them to be insignificant contributors to total metal
loads (U.S. EPA, 2003c).

Wildlife food chain exposures for metals are
controlled by bioavailability, bioaccessibility, and
bioaccumulation. Bioaccessibility of metals to animals and plants that live on or in the soils can
be influenced by soil parameters, such as pH, CEC, and organic carbon. These soil parameters
tend to be less important for soils that are incidentally ingested by wildlife species.

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. In the absence of site-specific information, the risk assessor can use the following
generalizations to determine the relative importance of incidental soil ingestion versus dietary
metals:

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

2. 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*.)

3. If bioaccumulation is low (<<1), importance of soil ingestion versus diet for metal
    exposure increases.

4. When bioaccumulation is greater (~1 or higher), the food pathway should dominate.

5. 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.

6. The looser the association with the ground (e.g., piscivores, aerial/arboreal
    insectivores, raptors), the lower the importance of soil ingestion.

Figure 6-1 provides a simple scheme for the risk assessor to use for judging the relative
contribution of food and soil before accounting for bioavailability. The assessor should assume
that 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%. However, the risk
assessor should use these generalizations with caution for site-specific assessments. As the risk
assessor acquires more site-specific information, 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. Therefore, when the exposure is being driven by incidental soil ingestion, the risk assessor should consider refinements of exposure estimates through a better understanding of bioavailability, although very little information is available on this for most wildlife species.
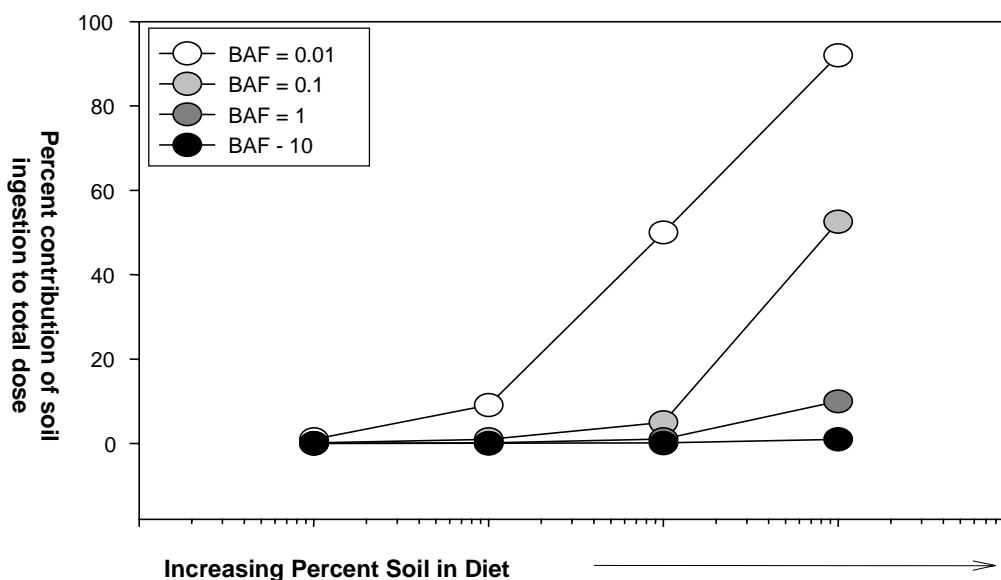

Figure 6-1. Generalized representation of percent contribution of incidental soil ingestion to oral dose for wildlife at different soil ingestion rates and bioaccumulation factors and a bioavailability of 100 percent.

The risk assessor should be cautious about extrapolating bioavailability adjustments for wildlife from models developed for estimation of bioavailability of metals in soils for incidental human exposures. There are significant variations in digestive physiology and anatomy across mammalian and avian species that alter the degree of assimilation and uptake of metals (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.

Food chain modeling can be used to estimate the exposure of wildlife to metals based on the ingestion of soil, food, and water. The risk assessor should use the same dietary uptake

model for metals as is used for organic substances, e.g., Eco SSLs; Ecological Committee on FIFRA Risk Assessment Methods (ECOFRAM) (Sample et al., 1997; U.S. EPA, 1997d). For national or regional risk assessments, the assessor may use trophic transfer rates to model food concentrations but only on the basis of soil measurements (rather than using direct measures of concentration of metals in food items). As with aquatic organisms, trophic transfer values for metals in terrestrial systems are an inverse function of soil concentrations. Therefore, the risk assessor should not use constants for this term but rather should generate regression equations of plant and invertebrate uptake rates as a function of soil concentrations and use which ever value(s) that are consistent with the degree of conservatism or amount of realism appropriate for the assessment. Sample et al. (1998a) developed uptake models to predict concentrations in earthworms from soil concentrations and Efroymson et al. (2001) provides similar information for plants (see Section 6.5.2.3 Bioaccumulation for a more detailed discussion).

With the exception of a few hyperaccumulator species, the risk assessor can reasonably assume that most plant species do not bioconcentrate metals (i.e., BAFs <1). Pb, As, Cr, and Co 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). In contrast, many plants are quite sensitive to some metals (Mn, Zn, Cu, for example); the risk assessor should be aware that plants frequently die before achieving high metal concentration levels that pose a threat to animals via food chain transfer (with the exception of the hyperaccumulator species, as noted above).

6.3. CHARACTERIZATION OF EFFECTS

When assessing metal toxicity to terrestrial organisms, the risk assessor should understand both the natural mechanisms of 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. The risk assessor should also consider interactions between metals in either their uptake or toxicity (such as Cd/Ca/Zn, Hg/Se, Cu/Mo). Risk assessments for metals are further complicated by the need to express the dose-response (or concentration-response) functions in bioavailable units that are functionally equivalent to measures of exposure. This section provides tools and approaches risk assessors can use when addressing issues of essentiality, metal mixtures, and appropriate use of toxicity tests; issues of how acclimation or adaptation to continued exposures may affect toxicity have been addressed in Sections 1.4.1 and 4.2.1 on Natural Occurrence of Metals.

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.

6.3.1. Essentiality

Essentiality, or the requirement for normal organism metabolic function, of some 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 Co, Cu, Fe, Mn, Se, Mo, and Zn, are necessary for the normal development of plants and animals. Other metals, such as As, Cd, Pb, and Hg, have no known functions in plants and animals (Mertz, 1981). Table 6-3 classifies the metals addressed in this Framework by their known essentiality to organisms.

The risk assessor should be sure 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. If TRVs are set too low (i.e., in the range where deficiency can occur), the determination of risk will be erroneous and deficiency effects will be mistaken for toxic responses. For wildlife, the risk assessor can consult the literature on dietary requirements of essential elements for livestock (McDowell, 2003; NAS/NRC, 1994a, 1980). Marschner (1995) summarizes the minimum concentrations required for plant growth.

Because of differences in test conditions among published studies, it may be difficult for the risk assessor to directly compare toxicity threshold values with recommended dietary requirements of essential elements.

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, for the risk assessor 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 can be used by the risk assessor, 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.

Extrapolation of data among species (e.g., from livestock to wildlife species) may also add uncertainty to the effects assessment. Furthermore, addition of safety factors when deriving protective values often results in concentrations significantly below required intake. The risk assessor should address these and similar uncertainties in toxicity threshold derivations as part of the Risk Characterization process. Detailed site-specific assessments, where more accurate estimates of effects thresholds are expected, may require the risk assessor to request additional bioassays to characterize the biphasic dose-response curve and determine both required and excessive threshold levels.

Table 6-3. 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 (Mo)	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.

6.3.2. Toxicity Tests

For assessments conducted for regional or national assessments, criteria development, or ranking purposes, risk assessors should acknowledge that results will be based on organisms and soil types that result in greatest bioavailability and sensitivity. The risk assessor should take great care to ensure that the organism-environment combinations that are assessed are, in fact,

compatible with real-world conditions. 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, risk assessors could use data from standard test species and conditions, but uncertainty factors may be warranted to adjust the final toxicity value.

6.3.3. Metal Mixtures

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

1. Chemical interactions with other constituents in the media,
2. Interactions with the physiological processes of the organism, and
3. Interactions at the site of toxic action.

The joint action of metal mixtures may be expressed in different ways, such as increasing or decreasing the toxicity relative to that predicted for individual components. As a result, the toxicity of metal mixtures has important consequences for metals risk assessments. However, predicting the toxicity of metal mixtures has proven to be a difficult challenge in ecotoxicology.

Much of the difficulty in interpreting the available information on the toxic effects of metal mixtures is due to differences in the bioavailability of metals (and measures used to define the bioavailable fraction) that occur across mixture studies. As discussed in Section 3, the bioavailability of metals depends on a suite of factors affecting their speciation, complexation with ligands, and interaction with biological systems. Nevertheless, the risk assessor needs some measure of the bioavailable metal fraction in the exposure media to accurately predict the effects of metals mixtures (Sauvé et al., 1998; Weltje, 1998; Posthuma et al., 1997). Besides bioavailability issues, the joint action of metal mixtures can depend on the overall mixture concentrations and the relative proportion of the constituent metals, as has been seen in aquatic studies (Norwood et al., 2003; Mowat and Bundy, 2002; Fargašová, 2001; Sharma et al. 1999).

The two most common classes of models used to predict mixture toxicity are the Concentration Addition and Effects Addition models. These models have been used to classify the combined effects of chemical mixtures as being *less than additive* (i.e., when the observed effect is less than the model prediction), *strictly additive* (i.e., matching model predictions), and *more than additive* (i.e., when the observed effect is greater than model predictions; Norwood et al., 2003). Both models use metal concentrations in media to generate concentration-response curves for individual metals, and these data are then used to generate specific critical concentrations for mixture models. 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 converts all metals to one metal concentration). Concentration Addition is used often when the constituents are known or assumed to act through the same or similar MOA. However, the risk assessor should use caution when applying the Concentration Addition model to mixtures containing many metal constituents (particularly those well below toxic levels) because of the potential for an upward bias in predicted mixture toxicity (Newman et al., 2004). In the Effects Addition model, differing potencies are ignored, and the effect of each metal's concentration is combined to predict mixture toxicity. The Effect Addition model is often used when constituents act independently (i.e., different modes of action). Only the Concentration Addition model allows detection of toxicity that is more than additive. Thus, a key issue in applying either the Concentration Addition or Effects Addition model is to define the nature of the metals' joint action (i.e., independent or similar mode of action). The risk assessor can use information on the MOA, capacities to act as analogues for other metals, essentialities and ligand binding tendencies to make this decision.

Risk assessors should keep in mind, however, that toxicities of certain metal elements are associated with deficiencies of others. For example, increased Zn, Cu, and Ni toxicities can be associated with Fe deficiencies (Bingham et al., 1986), and increased Pb and Zn toxicities can also be related to P deficiencies (Brown et al., 2000, 1999; 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). Similar interactions occur in wildlife; for example, Cu toxicity can be a result of Modeficiency and vice versa (McDowell, 2003; NAS/NRC, 1994a, 1980).

It is possible that receptor binding models (e.g., FIAM) 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, to predict metal toxicity using a mechanistic receptor binding approach in an Effects Addition model. Alternatively, if two metals do not compete for the same binding site on the organism, then these models 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. Additionally, 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).

From the preceding discussion, it should be clear that the accurate prediction of joint toxicity of metal mixtures to terrestrial organisms remains a significant challenge.

6.3.4. Critical Body Residues

Critical body residues (CBRs) are internal concentrations of chemicals that are correlated with the onset of a toxic response (Conder et al., 2002; Lanno et al., 1998). CBRs can be based

on whole-body residues (see below for discussion of this approach in soil invertebrates) or concentrations in specific tissues. The risk assessor may choose to use CBRs instead of dietary TRVs to reduce uncertainties because they account for site-specific bioavailability and multipathway issues (Van Straalen, 1996; Van Wensem et al., 1994). Unfortunately, there are major data gaps in available CBRs for many species—metal combinations.

Risk assessors can use tissue-specific critical loads for some metals that have been established for several species of vertebrate wildlife, including Pb in liver, Cd in kidney, Hg in brains, and Se in eggs. See Beyer et al. (1996) for these figures. Only a few CBRs have been developed in soil invertebrates for metals (Conder et al., 2002; Crommentuijn et al., 1997, 1994; and Smit, 1997 for Cd and Zn).

For plants, the use of a tissue residue (CBR) approach is another method that risk assessors might use to address metal toxicity issues, based on the concept that a metal concentration must reach a threshold value in the organism or at the target site before effects begin to occur (McCarty 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 and there is little to no information available. Therefore, this approach, although conceptually sound, requires significant research before risk assessors will find it useful.

6.3.5. Plant and Soil Invertebrate Toxicity

The risk assessor can estimate TRVs (i.e., toxic thresholds) for plants and soil invertebrates from laboratory tests where metals are mixed with standard soils (Fairbrother et al., 2002). 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). Additionally, acclimation and adaptation of test organisms can further complicate test results and aging and other physical/chemical processes that affect metal speciation and uptake are not represented. Because incorporation of sparingly soluble substances, such as many environmental forms of metals, into the soil matrix is difficult, tests generally are conducted using soluble metal salts with the addition of organism to the test matrix immediately after mixing. The risk assessor should be aware of how all these factors influence the test outcome and subsequent TRV derivation.

There is a large body of literature on toxicity of metals to soil organisms (e.g., van Straalen and Løkke, 1997), although often the objectives were to understand processes rather than to develop defensible toxicity thresholds. The challenge for the risk assessor, 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. One approach to addressing variability in soil

toxicity tests is to normalize test results by dividing the LC_{50} (or, more generally, the EC_x) by percent organic matter (Lock and Janssen, 2001). This approach is based on observed correlations between the LC_{50} of Cu to earthworms and soil organic matter content (Lock and Janssen, 2001). More recently, CEC has been shown to be the most important factor modifying Zn bioavailability in soils for both invertebrates and plants. Because CEC is a function, at least in part, of soil pH, normalization using this parameter should be done only among soils of similar pH ranges. However, comparison of field data with laboratory toxicity response information still is best accomplished by measuring metals in soil pore water from field assessments and comparing such data to spiked laboratory soils. Risk assessors can use the guidance document developed for establishing Ecological Soil Screening Levels or Eco SSL to judge the applicability of literature studies to plant or soil invertebrate toxicity threshold determinations. Eco SSLs have been developed for several metals, and the risk assessor should refer to these for national or regional assessments and for screening level, site-specific assessments.

6.3.6. 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 Se (Adams et al., 2003), exposure of waterfowl to Pb-contaminated sediments (Henny et al., 2000; Beyer et al., 1998; Blus et al., 1991), and white-tailed ptarmigan exposure to Cd in vegetation (Larison et al., 2000). Most metals express multiorgan toxicity, resulting in a 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, mice, or domestic livestock species (e.g., cattle and chickens) exposed to soluble metal salts. Risk assessors will need to extrapolate the results of such tests to species of interest because of the paucity of data on the toxicity of metals to wildlife. However, risk assessors should approach this carefully 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 are the selected wildlife species. Toxicological responses vary among species because of many physiological factors that influence the toxicokinetics (absorption, distribution, and elimination) and toxicodynamics (relative potency) of metals after exposure has occurred. For example, differences in digestive tract 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 is a toxicodymamic features leading to interspecific extrapolation uncertainty. Thus, risk assessors should not extrapolate data from mammal studies to birds, and should be aware that 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. These difficulties in interspecific extrapolations are not unique to metals risk assessment except when dealing with essential elements. A review of potential extrapolation methodologies can be found in Kapustka et al. (2004).

> **Risk Characterization**
>
> Have the qualitative assessment, quantitative assessment, and key uncertainties regarding metals been presented in accordance with EPA guidelines?
>
> Do conclusions fully reflect risks in relation to ambient concentrations, essentiality of metals, chemical speciation, and information on variability in species sensitivity?
>
> Have assumptions and uncertainties been documented adequately?
>
> Have available data on mechanisms of action and metal interactions been fully explored in developing the quantitative assessment in accordance with EPA Guidance on Mixtures Risk Assessment?

Currently, the best sources of information for the risk assessor on wildlife metal toxicity thresholds are NAS/NRC (1994a, 1980), McDowell (2003), and the documentation supporting development of Eco SSLs values. The Eco SSL document also includes an approach for screening studies for acceptability for use in derivation of toxicity thresholds for risk assessments, which can then be used for deriving site-specific TRVs for the most applicable endpoints. Risk assessors should apply uncertainty factors for extrapolation of data to species in a different taxonomic category (e.g., genus, family or class) with caution and include a discussion of uncertainty in the risk characterization. Summaries for some metals are available in Beyer et al. (1996) and Fairbrother et al. (1996).

6.4. RISK CHARACTERIZATION

Risk Characterization is the final phase of the risk assessment process, in which information from hazard characterization; dose-response assessment and exposure assessment are jointly considered to determine the actual likelihood of risk to exposed populations (U.S. EPA, 2000c, 1998a). The characterization also should discuss the uncertainties in the exposure and effects assessments, and the level of confidence in the overall determination of risk. At the same time, Risk Characterization is the first phase in the risk management process, in which information from the characterization is integrated into the consequences of rule-making or risk management, such as consideration of cost, alternative solutions, political considerations, community interactions.

Each Risk Characterization should include three components: a qualitative summary of each section of the risk assessment, a numerical risk estimate, and a description of assumptions and uncertainties. These descriptions of variability and uncertainty are particularly important for metals risk assessments given all the components and challenges discussed in this Framework document. These are in addition to the variability and uncertainties that are inherent in all risk

assessments (e.g., species to species toxicity extrapolations). Because information, knowledge, and tools are lacking for many of the metal-specific uncertainties, risk assessors should be particularly diligent in documenting whether these may result in an over- or under-estimation of risk (i.e., result in a conservative risk estimate or not). It is likely that site-specific risk assessments will have fewer uncertainties than regional or national scale assessments because risk assessors have access to local data on key issues such as specific metal species, relative bioavailability, or background metal levels. For national or regional assessments, selection of ranges or specific numbers for these values will depend upon the degree of conservatism desired by the risk assessor and, therefore, should be clearly documented during the Risk Characterization phase.