5. AQUATIC ECOLOGICAL RISK ASSESSMENT FOR METALS

This chapter describes how to incorporate the metals risk assessment principles described in Chapters 1 and 2 into ecological risk assessments involving aquatic-based receptors. Specifically, the following discussion focuses on the relationship between each metal principle and components of the EPA's Framework for Ecological Risk Assessment (U.S. EPA, 1992a) and subsequent guidelines (U.S. EPA, 1998a). These components include Problem Formulation, Characterization of Exposure, Characterization of Effects, and Risk Characterization. The Problem Formulation phase consists of defining assessment endpoints, risk hypotheses, and a conceptual model to produce an analysis plan for the risk assessment. Chapter 2 discussed the consideration of the metals principles in the Problem Formulation phase. In this chapter consideration of the metals principles in the characterization of exposure, effects, and risk to aquatic organisms is discussed.

Consistent with the previously stated scope and purpose of this Framework, not all aspects of the ecological risk assessment process are discussed. Only those aspects of the aquatic ecological risk assessment process and associated technical issues with the greatest relevancy to the metals principles are included. Also emphasized is how the geographic scale (e.g., site specific, regional, national) and analytical scope (e.g., screening vs. definitive analysis) of aquatic risk assessments affect the extent to which the metals principles can be incorporated. Although these principles apply equally to risk assessments involving terrestrial-based ecological receptors, many of the methods and tools that can be used to implement these principles differ between the aquatic and terrestrial environments. Thus, a separate discussion of how the metals principles apply to ecological risk assessment in the terrestrial environment is provided in Chapter 6.

5.1. METALS PRINCIPLES

Metals have specific environmental and biotic attributes that should be considered in all risk assessments. Specifically, these attributes 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 (absorption, distribution, metabolism, and excretion [ADME] and toxicity)

5.2. CHARACTERIZATION OF EXPOSURE

The Exposure Characterization phase describes the potential or actual contact or co-occurrence of stressors with receptors. It includes analysis of stressor sources, their distribution in the environment, and the extent and pattern of contact or co-occurrence to produce an exposure profile for the ecological receptor(s) of concern. Further guidance on characterizing exposure in ecological risk assessments is found in U.S. EPA (1998a, 1992a).

Exposure Profile

The exposure profile should describe the exposure pathways from stressor source to the receptor, the exposure intensity, its spatial and temporal distribution of co-occurrence, and the impact of variability and uncertainty on the exposure estimates.

5.2.1. Background Levels

Background levels refers to those concentrations of metals that derive from natural as well as anthropogenic sources that are not the focus of the risk assessment. In aquatic ecosystems, metal concentrations vary widely over space and time owing to differences in watershed geology, hydrology, anthropogenic and natural loads from "nontarget" sources, and other factors. Depending on the magnitude of the exposure associated with these factors, background metal concentrations can account for a significant portion of total metal exposure. Furthermore, certain essential metals can bioaccumulate to high levels in some aquatic organisms (e.g., Zn in barnacles, Cu in crayfish) due to species-specific physiological requirements, regardless of source. Even some nonessential metals can naturally bioaccumulate to significant levels as a result of mimicry of essential metals or sequestration and storage. Thus, the risk assessor needs knowledge of background concentrations in order to characterize exposure and to differentiate risk associated with metal sources already in the environment from risk associated with the metal sources of concern in the assessment.

Environmental Background in Exposure Assessment

What are the environmental background concentrations at the site(s) of interest? How do they vary over relevant spatial and temporal scales?

What is the relationship between environmental background and toxicologically relevant metal concentrations?

Can natural and anthropogenic metal be distinguished?

To what extent are background concentrations being extrapolated over space and time?

What level of confidence (uncertainty) exists in the estimate of environmental background concentrations?

Depending on the design and context for the assessment, the risk assessor needs to address several questions and issues pertaining to background concentrations of metals. Obtaining reliable estimates of background concentrations can be challenging, particularly at larger spatial scales. Risk assessors are cautioned against using background metals in soils as surrogates for sediments due to differences in the biogeochemical processes between these two environments. Metal concentrations in

sediments can be impacted by sediment physicochemical composition and localized sediment transport processes. Fractionation of sediment cores, in combination with careful estimates of sedimentation rates, has been used to distinguish pre- vs. post-industrialized contributions of metals via atmospheric transport (e.g., mercury) (U.S. EPA, 1997b). Risk assessors may find this type of sediment core analysis useful for differentiating natural levels versus levels associated with anthropogenic sources.

Concentrations of metals in the water column vary over time and are highly responsive to hydrological changes. In site-specific risk assessments, the risk assessor may quantify background levels by measuring metal concentrations at sites upstream from the area of concern. National databases of metal concentrations in various aquatic media (sediments, water, biota) include the following:

- The EPA STOrage and RETrieval (STORET) database (accessible at http://www.epa.gov/storet/);
- The National Sediment Quality Survey (NSQS) (accessible at http://www.epa.gov/waterscience/cs/nsidbase.html);
- Environmental Monitoring and Assessment Program (EMAP) (accessible at http://www.epa.gov/emap/);
- The National Stream Quality Accounting Network (NASQAN) (accessible at http://water.usgs.gov/nasqan/), and
- The Hydraulic Benchmark Network (HBN) (accessible at http://ny.cf.er.usgs.gov/hbn/).

The risk assessor should keep in mind that with the exception of the HBN, these databases have not been established to distinguish background concentrations from local, anthropogenic sources of metal loadings (e.g., industrial discharges, stormwater runoff) and, thus, may reflect significant anthropogenic loadings of metals to environmental media. The HBN was established to provide long-term measurements of streamflow and water quality in areas that are minimally affected by human activities. While the HBN contains long-term measurements of a number of parameters that are known to affect the bioavailability of metals (e.g., dissolved organic carbon [DOC], inorganic ions such as Ca and Mg, pH, conductivity), it does not contain information on metals of typical regulatory concern, with the exception of Al.

5.2.2. Forms of Metals

The physical and chemical forms of metals affect exposure, bioavailablity, and subsequent effects and are influenced by physicochemical environmental conditions. National-level assessments involve a broad range of environmental conditions; therefore, the risk assessor

should account for different metal species in different locations and water body types. As assessments transition from national, to regional, and to local, the assessor should incorporate site-specific sediment and water quality parameters that influence metal speciation, complexation, and sorption onto biological surfaces (e.g., pH, organic carbon, inorganic ligands, Ca, Mg, sulfide). Speciation models (e.g., MINEQL) combined with biotic ligand models offer a framework for addressing the differential occurrence and toxicity of various metal forms. Risk assessors should be aware of the difficulty in applying this approach to assessments involving large regional or national scales because of the variability in model parameter values (including covariance among parameters) that occurs across locations. Information about the range of the input parameters can be derived from available databases (see Section 5.2.1), and the risk assessor can decide what value to use (e.g., minimum, maximum, mean) depending on the degree of conservatism desired in the assessment. The risk assessor should include this information in the Risk Characterization as part of the overall discussion of assumptions and uncertainties in the assessment. Risk assessors can directly assess the metal forms for site-specific assessments or estimate what these would be based on sediment/water parameters (see Section 3.1.3, Environmental Chemistry).

5.2.3. Exposure Pathway Analysis

Exposure Routes

For aquatic organisms, pathways of exposure to metals include movement from water to sediments (and vice versa to a lesser extent) and through the food web; air deposition directly into aquatic systems or through run-off; and sedimentation from soils.

For aquatic organisms, potential routes of exposure to metals include absorption across (or in some cases adsorption to) respiratory organs, dermal absorption, sediment ingestion, and food ingestion. Quantifying exposure and uptake by the respitory route is a particular challenge to aquatic risk assessors because of the differing types of respiratory organs among aquatic species, the dynamic nature of the respiratory process in water, and the intimate contact between the receptor and metals dissolved in waters. Further complicating the issue, some respiratory organs can also be involved in locomotion, excretion, ion regulation and the capture, sorting, and ingestion of food. Similarly, risk assessors may find the ingestion route 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 use of stable isotope techniques has contributed greatly to evaluating the role of diet in contaminant accumulation (including metals) by precisely defining trophic interactions (e.g., Kidd et al., 1995; Jarman et al., 1996). The absorption route can involve uptake across a phytoplankton cell membrane, amphibian skin, arthropod exoskeleton, the egg membrane, or the integument of an infaunal clam or annelid.

Despite the complexities associated with quantifying exposure of aquatic animals to metals from multiple routes of uptake, risk assessors can find a significant amount of information

on the relative importance of the different uptake pathways (Wang, 2002; Hook and Fisher, 2001b; Fisher et al., 1996; Bjerregaard et al., 1985). Applications of one-compartment biokinetic models using laboratory-based measurements of key model parameters (assimilation efficiency, metal uptake rates from water and food elimination rates) have been extended to field situations for populations of a diverse array of aquatic species, including freshwater and marine bivalves, various crustaceans such as copepods, amphipods, and crab, aquatic insects, and fish (e.g., Luoma and Rainbow, 2005; Stewart and Fisher, 2003; Griscom et al., 2002; Baines et al., 2002; Fisher et al., 2000, 1996; Roditi et al., 2000; Wang et al., 1996; Luoma et al., 1992). 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 for risk assessors to account for the major processes governing contaminant concentrations in aquatic animals and that laboratory-derived kinetic parameters are applicable to natural conditions (Luoma and Rainbow, 2005). Thus, these models provide tools for risk assessors to use when addressing metal exposure and uptake, and they can be used to determine the relative importance of different routes of exposure (Landrum et al., 1992; Wang et al., 1996).

Risk assessors should always consider temporal aspects of exposure, particularly in aquatic systems that respond to frequent shifts in hydrology. 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 typical or average concentrations but last for only a few hours. These episodic exposure scenarios have been poorly characterized for metals (Butcher et al., 2006). Any risk assessment for metals should clearly state all assumptions about duration of exposure and what uncertainties are added to the risk model as a consequence.

5.2.4. Fate and Transport of Metals

Risk assessors routinely use transport and fate computational models to describe and quantify exposure pathways. Models also are useful in situations where risk assessors need an estimate of future 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 air, water (freely dissolved metal or as organic and inorganic metal complexes), and particles (sorbed, precipitated, or incorporated within a mineral phase). The risk assessor can find a detailed discussion of the fate and transport of metals in Section 3.2. No single,

currently available model includes all the desirable metal-specific features for aquatic systems. Discussions of the family of available aquatic transport and fate models, as well as a number of chemical equilibrium models, may be found in Paquin et al. (2003).

5.2.5. Toxicokinetics and Toxicodynamics (Bioavailability and Bioaccumulation Issues)

5.2.5.1. *Aqueous Phase*

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 bioavailability, and the hazard and risk assessments of waterborne metals are complicated by the fact that metal species differ in their toxicological properties. For many metals in aquatic systems, it is the free ionic form that is most 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 of its reduced availability for uptake by the organism. However, the risk assessor should be aware that although toxicity of metals bound to DOC is reduced, it is not eliminated entirely and can contribute to the total metal loading to an ecosystem and subsequent toxic effects (McGeer et al., 2002; Erickson et al., 1996). On the other hand, there are cases where nontoxic metal species are bioavailable and taken up by the organism but cause no adverse response (e.g., Ag-Cl complexes in rainbow trout) (McGeer and Wood, 1998). Risk assessors should recognize that the presence of metal within an organism cannot always be used as a surrogate for toxic response.

Risk assessors can choose among a variety of methods to account for relative bioavailability of metals in aquatic systems, including hardness adjustments, water-effect ratio (WER), Free Ion Activity Model (FIAM), and aquatic Biotic Ligand Models (BLMs) (Paquin et al., 2002a). Each method contains strengths and limitations and may not be amenable to all types of assessments (e.g., ranking/classification, national, and site-specific assessments). For example, adjustment of aqueous metal concentrations for differences in water hardness was among the first computational methods to account for bioavailability differences between the laboratory and the field when applying EPA water quality criteria. Although these adjustments are relatively easy to apply, they require empirical data to define the toxicity-water hardness relationship. Thus, they are more amenable to site-specific risk assessments, although even in those cases they do not account for other water quality factors that affect bioavailability (e.g., DOC, pH). The water hardness approach has been applied at the national level through a statement of water quality criteria as hardness-based equations rather than as single values. Risk assessors can choose to use ranges, means, or median values when conducting large-scale (e.g., regional) assessments, but they will need to acknowledge this uncertainty during the risk characterization phase.

Bioavailability adjustments using WERs incorporate the combined effects of all water quality parameters present in site water on bioavailability of metals of interest relative to what was measured in laboratory tests (U.S. EPA, 1994c). Thus, compared to hardness adjustments, WERs encompass a broader array of water quality factors that can impact bioavailability. However, WERs are relatively resource intensive (requiring toxicity testing), are applicable only on a site-specific basis, and are not easily adjusted to account for temporal or spatial variability.

This relationship between speciation and bioavailability also has been explained through the free ion activity model (FIAM, Campbell, 1995). This model produces speciation profiles of a metal in an aquatic system and provides insight into the relative bioavailabilities of the different forms of metal as well as the importance of complexation. Models available for the calculation of metal speciation in natural waters are reviewed in Section 3.1.5, Sediment Chemistry, and include 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 risk assessor should review Paquin et al. (2003) for a more in-depth understanding of these models and how to select among them for particular places and types of assessments.

The BLM approach successfully combines the influences of speciation (e.g., free metal ion, DOC complexation) and cationic competition (e.g., K^{+}, Na^{+}, Ca^{+2}, Mg^{+2}) on metal toxicity in fish (De Schamphelaere and Janssen, 2004, 2002; De Schamphelaere et al., 2004, 2003, 2002; Heijerick et al., 2002a, b; Di Toro et al., 2001; Santore et al., 2001; McGeer et al., 2000). The model can be used to distinguish, at least conceptually, metals that will bioconcentrate at the site of toxicity (e.g., gill or other biotic ligand) from the total metal pools in an organism and the bioavailable metal pool in the exposure media. The model also can be applied to algae (De Schamphelaere et al., 2003; Heijerick et al., 2002) and *Daphnia* (De Schamphelaere and Janssen, 2004, 2002; De Schamphelaere et al., 2004, 2002). The BLM recently has been incorporated into draft revisions to EPA's national water quality criteria for Cu (it has been used in risk assessments at a range of geographic scales), and it is being applied as an alternative to the WER approaches for setting site-specific discharge objectives. The BLM has the potential to address spatial and temporal factors that affect bioavailability, provided that the variability in water quality parameters used as inputs to the model can be quantified or predicted (e.g., pH, DOC, K^{+}, Na^{+}, Ca^{+2}, Mg^{+2}). However, the risk assessor should be aware of the many limitations in applying the BLM (or any Free Ion model). For example, the development of the BLM has focused primarily on bioavailability and acute exposures. Work has begun to extend the BLM to chronic toxicity for some organism/metal combinations (e.g., De Schamphelaere and Janssen, 2004; Paquin et al., 2002a, b), and further development is expected. Also, the BLM is currently based on metal uptake through the dissolved phase; thus, additional research is needed to address metal uptake and toxicity via the diet.

5.2.5.2. *Sediment Phase*

Sediment-Associated Metals

When the molar concentrations of acid-volatile sulfide in sediment exceed the amount of simultaneously extracted metal, the metals are expected to be associated with the solid phase and not to be toxic.

Risk assessors have several approaches for estimating exposures to sediment-associated metals that account for bioavailability differences. The equilibrium partitioning approach (EqP) 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 cationic metals should be present as insoluble sulfides and relatively nontoxic. The amount of reactive sulfide is quantified by measuring the amount of sulfide freed when sediment is extracted with 1 N HCl. This procedurally defined quantity is known as *acid volatile sulfide* (AVS). The amount of reactive metal is determined from the same extraction by measuring the metal concentration in the acid extract. This quantity is known as *simultaneously extracted metal* (SEM). The risk assessor then can determine the potential bioavailability of a metal by comparing the relative molar concentrations of the SEM and AVS. 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 many metals (Ankley et al., 1996, 1991; Berry et al., 1996; Hansen et al., 1996; Carlson et al., 1991; Di Toro et al., 1990).

However, risk assessors should be aware that although the correspondence of SEM-AVS to toxicity was found, some questions remain about 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 (Ankley et al., 1996). However, the lack of toxicity observed when AVS exceeds SEM suggests that this bioaccumulated metal may not be biologically available. 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, risk assessors can use the SEM-AVS model in exposure estimations as long as its shortcomings are acknowledged appropriately and uncertainties are recorded in the Risk Characterization phase of the assessment.

Other tools risk assessors can use to determine the bioavailable concentrations of sediment-bound metals include metal concentrations in the chemical extracts (Fan and Wang,

2001; Babukutty and Chacko, 1995; Tessier et al., 1984), acid extracts (Langston, 1980; Luoma and Bryan, 1978), or biomimetic extracts (Weston and Maruya, 2002; Mayer et al., 2001; Chen and Mayer, 1998). However, no consensus yet exists on their best use for different types of metals or metalloids. Several other methods have been proposed. Based on the premise that iron oxides in oxic sediments lower metal bioavailability, Fe 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 conducted 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 have been used to estimate bioavailable metal.

5.2.5.3. *Dietary Phase*

Dietborne Metal Exposure

Risk assessors should consider dietborne metal exposure 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.

As discussed in Section 5.2.3 (Exposure Pathway Analysis) and illustrated by the conceptual model for bioavailability (Figure 2-2), it is well established that dietary exposure to metals can result in accumulation of metals in aquatic organisms. What is less well established is the best way to express dietary exposure in a way that can be linked to potentially toxic effects (either directly to the aquatic organism or its predator). The main reason for this ambiguity is that the bioavailability of dietary metals varies widely across organisms and exposure conditions and standardized approaches are not available for predicting toxicity. The subsequent discussion elaborates on this point and provides some suggestions for how risk assessors might address dietary metals in different assessment contexts (screening vs. definitive).

After ingestion, some of the dietary metal can be released from the ingested particle into the gastrointestinal fluids of the animal (Chen and Mayer, 1999; Mayer et al., 1997; Gagnon and Fisher, 1997) and become available for assimilation into the tissues of the animal and the tissues of its consumer (i.e., trophic transfer). Assimilation efficiency (i.e., the net amount of metal retained in tissues relative to the amount ingested from food) is a common measure of the bioavailability of a chemical from food, and the risk assessor may find this to be a useful parameter for comparing the potential for toxicity among different types of organisms. Assimilation efficiency is also an important input parameter for estimating metal bioaccumulation using kinetic-based bioaccumulation models (e.g., Luoma and Rainbow, 2005). Assimilation efficiencies can vary widely depending on the metal, its form and distribution in prey, species digestive physiology (e.g., gut residence time), environmental conditions (e.g., temperature), food quality, food ingestion rate, and metal concentration in the diet. Thus, risk

assessors should consider likely ranges of assimilation efficiencies for a particular metal-animal combination when evaluating metal bioavailability from the diet. A number of reviews have summarized current knowledge of assimilation efficiencies of ingested metals among different aquatic animal species (Wang and Fisher, 1999; Fisher and Reinfelder, 1995).

The distribution and form of metals in dietary organisms is of critical importance for understanding the bioavailability of dietary metals and trophic transfer potential. For example, metals in the cytosolic fraction of phytoplankton and "soft parts" of zooplankton have been shown to correlate well with bioaccumulated metal in their predators (e.g., herbivores and plantivorous fish, respectively) (Wang and Fisher, 1996; Reinfelder and Fisher, 1994a, b). Metals sorbed to the cell wall of phytoplankton and the exoskeleton of zooplankton were poorly assimilated by consumers. The bioavailability of metal-enriched granules in prey (a detoxification and storage mechanism exhibited by some organisms) has been shown to be negligible or substantially reduced when consumed by certain predators (e.g., Wallace et al., 2003, 1998; Wang, 2002; Wang and Fisher, 1999; Nott and Nicolaidou, 1990). However, the risk assessor should be aware that the bioavailability of metal-enriched granules in prey can vary among metals and with type of granule (Wang, 2002; Mason and Jenkins, 1995; Nott and Nicolaidou, 1990) and may also depend somewhat on digestive physiology of the predator (e.g., gut pH, retention time). As a result of these findings, fractionating body burdens of metals (e.g., cytosolic metal vs. metal granules) has been suggested as a better means of identifying the bioavailable fraction of dietborne metals (Seebaugh and Wallace, 2004; Wallace et al., 2003; Fisher and Reinfelder, 1995; Reinfelder and Fisher, 1994). Although such techniques show promise for operationally defining the extent to which dietary metals may be bioavailable for trophic transfer in aquatic food webs, risk assessors should understand that broad-scale application of these techniques to metals risk assessments is presently limited by the relatively small number of metals and predator-prey relationships evaluated.

Despite the uncertainties associated with bioavailability and trophic transfer of dietary metals, the use of whole-body inorganic metal concentrations in prey species may have some utility to risk assessors for conservatively screening for exposure and potential risks to consumers (i.e., in cases where whole-body residues are below dietary toxic thresholds). For more definitive assessments, further research is needed to quantify the bioavailability and effects of inorganic dietary metals, with the exception of certain organometallics (e.g., methyl mercury) and metalloids (e.g., Se) where dietary toxicity has been well established.

Trophic Transfer

Trophic transfer is the transfer of a chemical from prey species to a predator species via dietary exposure.

Biomagnification is a type of trophic transfer where chemical concentrations increase in organisms from a lower trophic level to a higher trophic level within the same food web.

Biodilution represents a decrease in organism concentration with increasing trophic level.

5.2.5.4. *Bioaccumulation and Trophic Transfer*

Assessing and predicting the bioaccumulation of metals in aquatic ecosystems is a component of many Agency regulatory and nonregulatory activities (e.g., chemical ranking/classification, derivation of national water quality criteria, Superfund site risk assessments; see Section 2.3.1 for definitions and a conceptual model related to bioaccumulation). Interest in metals bioaccumulation originates from concerns regarding the direct impact of metals on organisms accumulating the metal and indirect impacts on their consumers (i.e., trophic transfer). Unlike certain persistent and bioaccumulative organic compounds, which tend to biomagnify in aquatic food webs (e.g., DDT/DDE, PCBs, PCDD/PCDF), inorganic metal compounds rarely biomagnify across three or more trophic levels (McGeer et al., 2004; Suedel et al., 1994); however, certain organometallics can biomagnify in aquatic food chains. Some metals (e.g., lead) tend to biodilute in aquatic food webs. Risk assessors should not interpret lack of biomagnification as lack of exposure or concern via trophic transfer (see text box). Even in the absence of biomagnification, aquatic organisms can bioaccumulate relatively large amounts of metals and become a significant source of dietary metal to their predators (Reinfelder et al., 1998).

For many nonionic organic chemicals, risk assessors can derive first-order approximations of bioaccumulation potential from information on chemical properties and organism attributes (e.g., K_{ow} and lipid content) and their use as inputs to simplified, fugacity-based models (e.g., Gobas et al., 1993). For metals, analogous methods to predict bioaccumulation based on simple chemical properties are not available or are not widely validated. The lack of analogous models for metals is likely due, at least in part, to the high degree of specificity exhibited by the mechanisms and processes underlying metals bioaccumulation (e.g., speciation, exposure conditions, and organism physiology) (see McGeer et al., 2004; Rainbow, 2002; Mason and Jenkins, 1995). As a result, risk assessors currently are limited to using an empirical approach for assessing and predicting metals bioaccumulation. Typically, this requires direct measurement of metal concentrations in the organism or experimentally-determined parameters for use as input to bioaccumulation models (e.g., gill uptake rate, elimination rate, assimilation efficiency).

Aquatic ecological risk assessors commonly use bioconcentration factors (BCFs) and bioaccumulation factors (BAFs) to quantify chemical accumulation in tissue relative to concentration in water. BCFs and BAFs are determined as the ratio of the chemical concentration in tissue to its concentration in water (using the steady-state method) or as a ratio of uptake rate (k_u) and elimination rate (k_e) constants (using the kinetic method). Measurement of BCFs or BAFs usually is conducted for conditions that approximate steady-state (i.e., where accumulation remains relatively constant due to chemical uptake being offset by its elimination by the organism). It is assumed that the greater the BAF or BCF, the greater a chemical's bioaccumulation or bioconcentration potential.

Risk assessors should recognize that considerable uncertainty can be associated with the application of literature-derived BCFs and BAFs for assessing the risks of metals, as variability in BCFs and BAFs for metals is known to be high (e.g., 50-fold or higher within a metal). Most of this uncertainty results from bioavailability differences between the studies from which the BCF or BAF is measured and the site(s) to which it is being applied (e.g., water quality characteristics, metal speciation, exposure pathways).

Other sources of uncertainty that risk assessors should consider in the broad application of BCF/BAF data are rooted in the complex mechanisms of metal toxicokinetics (uptake, metabolism, distribution, elimination). For example, unlike hydrophobic, nonionic organic chemicals where uptake across biological membranes generally occurs via passive diffusion, the uptake of metals is believed to involve a number of specific transport mechanisms. Some of these transport mechanisms involve binding with membrane carrier proteins, transport through hydrophilic membrane channels, and endocytosis. Passive diffusion is thought to be reserved for certain lipid soluble forms of metals, such as alky-metal compounds and neutral, inorganically complexed metal species (e.g., $HgCl_2^0$). The implication of these specific transport mechanisms is that metal bioaccumulation can involve saturable uptake kinetics, such that BCFs and BAFs depend on exposure concentration. The existence of saturable uptake mechanisms, the presence of significant amounts of stored metal in organisms, and the ability of some organisms to regulate bioaccumulated metal within certain ranges are all thought to be responsible for the inverse relationship that has been frequently reported between BCFs/BAFs and metal exposure concentrations (McGeer et al., 2003; Borgman et al., 2004). In these cases, higher BCFs or BAFs are associated with lower exposure concentrations and also can be associated with lower tissue concentrations within a given BCF or BAF study. This is counter to the implicit assumption that higher BCFs or BAFs indicate higher metal hazard.

As a result of the aforementioned uncertainties, risk assessors must be careful in broad-scale application of BCF and BAF data for metals. Specifically, the current science does not support the use of a single, generic threshold BCF or BAF value as an indicator of metal hazard. Similarly for national risk assessments, use of a single BCF or BAF value holds little utility due to high uncertainty that results from differences in bioavailability, exposure conditions, and species-specific factors that influence metal bioaccumulation by aquatic organisms. When extrapolation across sites is necessary and limited data prevent application of alternative approaches (discussed below), uncertainty in the use of BCFs and BAFs can be reduced by expressing them as a function of media chemistry (i.e., to address bioavailable metal), exposure concentrations (i.e., to address concentration dependency issues), and limiting extrapolations to within a particular species or closely related species. The use of BCFs and BAFs for metals assessments appears to have most value for site-specific applications, when appropriate measurements are taken from the site(s) of interest and extrapolation of BCF/BAF values across differing exposure conditions and species is minimized.

Risk assessors should be aware of several alternatives for assessing metals bioaccumulation that address some of the concerns listed above. One of these is to develop regression relationships between tissue and exposure concentrations. Such regression relationships have been used to characterize bioaccumulation of metals by soil organisms (U.S. EPA, 2003c; Sample et al., 1999), but they have not yet been compiled for aquatic systems. The advantage of this technique is that it addresses the dependency of BCF or BAF on exposure concentration. However, it does not explicitly adjust for bioavailability differences that occur across sites. Another alternative is to use a kinetic-based model for describing bioaccumulation (Luoma and Rainbow, 2005; Wang and Zauke, 2004; Kahle and Zauke, 2003; Chang and Reinfelder, 2002; Reinfelder et al., 1998). These models can improve predictions of metal bioaccumulation in aquatic organisms because they incorporate different exposure routes (e.g., water vs. diet) and the dynamic nature of metal bioaccumulation processes. For example, Luoma and Rainbow (2005) reviewed the DYNBAM model (a single-compartment, kinetic-based bioaccumulation model) and found it to accurately predict metal bioaccumulation for a wide range of metals, organisms, and habitats based on data derived from 15 separate studies. Importantly, DYNBAM and similar such models require experimental data measured under environmentally-realistic conditions in order to derive model parameters for each metal-species combination (e.g., uptake and elimination rates, assimilation efficiency, food ingestion rates). Compilations of such data on model input parameters are available for some species and metals (e.g., Wang and Fisher, 1999, for aquatic invertebrates). Clason et al. (2004) and Kahle and Zauke (2003) have developed two-compartment bioaccumulation models for amphipod crustaceans that incorporate background metal and saturation of uptake kinetics. Currently, however, these models include only the dissolved phase and do not account for uptake from the diet.

The bioaccumulation models described above offer strong promise for improving bioaccumulation predictions in aquatic risk assessments for metals and should be considered by risk assessors. However, risk assessors should be aware of their limitations. For example, they currently do not account for differential partitioning and bioavailability of metal in organisms (see Figure 2-2). Empirical methods are being developed to predict metal compartmentalization in tissues of aquatic organisms (e.g., Wallace and Luoma, 2003; Wallace et al., 2003), but these have not been incorporated into bioaccumulation models. These models also do not explicitly address the impact of metal speciation on bioaccumulation or link bioaccumulated metal to toxic effects, although such models are under development (Paquin et al., 2002b). Regardless of the type of bioaccumulation model used, reductions in uncertainty in metals bioaccumulation assessments should be directed at achieving robust connections between the bioaccessible/bioavailable form(s) of metals in various exposure media, their accumulation, metabolism, and distribution in tissues, and the form(s) of metals that exert their toxicity directly to the organism or indirectly to its consumers.

5.3. CHARACTERIZATION OF EFFECTS

5.3.1. Essentiality

Essentiality refers to the nutritional requirements of an organism for normal metabolic function. A key difference between metals and organic chemical contaminants is that some metals are required either as macronutrients (e.g., Fe, Ca, Mg) or micronutrients (e.g., Cu, Zn, Ni) to maintain a healthy organism. Table 6-3 in Section 6.2.1 classifies the metals addressed in this Framework by their known essentiality to plants and animals.

Consideration of essentiality by the risk assessor is important for several reasons. First, the risk assessor should ensure that toxic effects thresholds calculated from an assessment are not lower than the nutritional requirements for the particular plant or animal species being evaluated. As discussed in Sections 4 and 6, the risk assessor should be aware that such elements exhibit classic bell-shaped or biphasic dose-response (or exposure effect) relationships, with adverse effects occurring at both high and low concentrations and an optimal mid-range dose or exposure (see Abernathy et al., 1993; Chapman and Wang, 1998). For aquatic organisms, information about nutritional requirements is available for many commonly tested or cultured species. Essentiality issues also impact the bioaccumulation and toxicity of metals since organisms have evolved various mechanisms to maintain homeostasis of essential metals. Such mechanisms may also impact the bioaccumulation and toxicity of nonessential metals, particularly those that share similar binding and uptake mechanisms. In these cases, accumulation is nonlinear with respect to exposure concentration, whereby greater uptake and retention of metals occurs at low concentrations and uptake rates decrease as exposure media concentrations increase. The impact of homeostatic and other mechanisms on bioaccumulation has been discussed in Section 5.2.

5.3.2. Toxicokinetics/Toxicodynamics (Toxicity Issues)

For organometallic compounds such as organo-selenium and methyl mercury, toxicity from dietary exposure has been shown to contribute substantially to ecological risk at environmentally-realistic concentrations and thus should be considered by the risk assessor when characterizing the effects of these compounds. Beyond those two organo-metal compounds, however, the importance of exposure to dietary metals is much less clear. Toxicity to aquatic organisms from dietary exposure to metals has been demonstrated where exposure is sufficiently high, although, in some cases, these concentrations are extreme (e.g., 10,000 g/g Cu) (Handy, 1993). In such cases, 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 first via other pathways (e.g., direct toxicity of waterborne metal).

In other studies, however, effects from dietary exposure have been demonstrated at relatively low exposure concentrations (e.g., zooplankton studies by Hook and Fisher, 2002,

2001a, b). This raises additional concern for metals assessment because it increases the potential for toxicologically significant exposures to occur in cases where risk via a waterborne pathway is low. However, other studies with the same organisms and metals, but somewhat different test methods, reached different conclusions regarding the significance of dietary exposure (e.g., De Schamphelaere and Janssen, 2004; Meyer et al. [in press]). Dietary exposure of aquatic organisms to metals is an active area of research, and it is likely that new data and insights will result in a more comprehensive understanding of dietary effects. Until that time, risk assessors should make decisions regarding potential risks of dietary metal exposure on a case-by-case basis.

5.3.3. Metal Mixtures

Mixtures of metals (including metalloids and organic substances) 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:

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

The joint action of metal mixtures may be expressed in different ways, including 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. For example, toxicity has been observed for mixtures of metals present individually at nontoxic levels (e.g., at levels corresponding to water quality guidelines) (Enserink et al., 1991; Spehar and Fiandt, 1986). Despite the importance of considering the effects and mixtures of metals to aquatic organisms, risk assessors will find that predicting the toxicity of metal mixtures has proven to be a difficult challenge in aquatic toxicology.

Much of the difficulty in predicting the toxicity of metal mixtures to aquatic organisms results from differences in the bioavailability and/or methods used to define the bioavailable fraction among toxicological studies and subsequent ambiguity in interpreting mixture toxicity data. As discussed in Chapter 2, the bioavailability of metals depends on a suite of factors that can affect their speciation, complexation with ligands, and interaction with biological systems (e.g., pH, DOC, inorganic anions, and cations). Apart from bioavailability differences, the joint action of metal mixtures to aquatic organisms (i.e., antagonism, additivity, synergism) has been reported to depend on other aspects of toxicity test design, including the degree of toxicity associated with the overall mixture concentration (Mowat and Bundy, 2002; Fargašová, 2001; Herkovits et al., 1999; Spehar and Fiandt, 1986), the relative proportion of constituent

concentrations (Norwood et al., 2003; Sharma et al., 1999), the duration of the exposure (Marr et al., 1998), and several other factors related to experimental design (Norwood et al., 2003). In a similar review, the European Centre for Ecotoxicology and Toxicology of Chemicals concluded that the acute and chronic toxicity to aquatic organisms of mixtures of metals could not be reliably predicted or generalized although they recommended that, in the interim, assuming additive effects is likely "a balanced approach" for acute toxicity of metal mixtures (ECTOC, 2001).

Given these difficulties in evaluating mixtures effects, risk assessors commonly use two simplifying models: concentration addition and effects (response) addition. These models are used to classify the combined effects of chemical mixtures as being antagonistic, additive, and synergistic (also referred to as "less than additive," "strictly additive," and "more than additive," respectively). 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, such as toxic units (TUs) or toxicity equivalence factors (TEFs), which converts all metals to one metal concentration. Concentration addition is often used when the constituents are known or assumed to act through the same or similar MOA. However, risk assessors should be aware that applying the concentration addition model to mixtures containing many metal constituents (particularly those well below toxic levels) can result in 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 in a mixture is combined to predict mixture toxicity. The effects addition model is used when constituents act or are assumed to act independently (i.e., different MOAs). Thus, the risk assessor defines the nature of the metals' joint action (i.e., independent or similar MOA) to decide when to apply either the concentration addition or effects addition model. The risk assessor should consult information on the MOA, capacities to act as analogues for other metals, essentialities, and ligand binding tendencies to choose among these types of models.

The assumption of additivity has some regulatory precedence for use when addressing the toxicity of mixtures although not necessarily for metals. For example, the concentration addition approach is recommended for use by Australia and New Zealand for evaluating whether a mixture of less than six constituents exceeds their water quality guidelines (ANZECC and ARMGANZ, 2000). Similarly, additivity is assumed by EPA when evaluating the combined *acute* toxicity of multiple toxic effluents on the basis of whole-effluent toxicity data (U.S. EPA, 1991). Additivity is not assumed for *chronic* exposures due to lack of supporting data. For predicting the direct toxicity of mixtures of cationic metals in sediments to benthic organisms, EPA uses the ∑SEM-AVS approach described previously (see Section 5.2.5 Toxicokinetics and Toxicodynamics (Bioavailability and Bioaccumulation). Note, however, that this method is

limited to six cationic metals (Cd, Cu, Pb, Ni, Ag, and Zn). Furthermore, this method is considered a "no effect guideline," whereby the absence of toxicity can be predicted reliably (when $\sum$SEM<AVS) but the occurrence of toxicity (when $\sum$SEM>AVS) cannot be because of other factors that are not accounted for, which reduce metal toxicity.

Risk assessors should also consider the QICAR approach (described in Section 3.1.1) for addressing the toxicity of metal mixtures. 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 develop a model of joint independent action (Finney, 1947). They predicted that the joint action of combined metals will increasingly deviate from independent action as their ligand-binding chemistries become more and more similar. Although Microtox is considered to be a useful tool for organic contaminants, its sensitivity for evaluating metal toxicity has been called into question (Willemson et al., 1995).

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, 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, then these models may provide more reliable estimates of individual metal bioavailability, which then can be combined in more accurate effects addition models. However, at present, these possibilities remain theoretical. Furthermore, this approach, 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 the joint toxicity of metal mixtures to aquatic organisms remains a significant challenge for the risk assessor. For site-specific assessments, risk assessors are encouraged to assess mixture toxicity using *in situ* measurements (i.e., bioassays using site water or sediments). This approach is the foundation of the WER procedures used by EPA for making site-specific bioavailability adjustments to metals criteria. For site-specific assessments involving sediments, risk assessors should consider using the $\sum$SEM-AVS approach as a no-effect threshold. For national-level assessments, there is some precedence for assuming additive toxicity of mixture constituents, particularly when considering acute effects. However, the risk assessor should carefully consider the limitations to assuming strict additivity (i.e., potential for overprediction or underprediction of toxicity) and highlight these uncertainties in the Risk Characterization phase of the aquatic risk assessment.

5.3.4. Critical Body Residues

The bioavailability of metals from multiple exposure routes (water column, food, sediments) should be considered in aquatic risk assessments to account for relative contributions to overall toxicity. In concept, expressing toxicity on the basis of tissue residues is an attractive

approach to accomplishing this because it integrates chemical uptake from different routes of exposure, accounts for differences in bioavailability from exposure media, and addresses differences in toxicokinetics that occur for different species.

Expressing toxicological effects on the basis of internal (tissue) concentrations (e.g., use of critical body residues [CBR] or residue-response relationships) has gained significant attention in the aquatic ecotoxicology literature, particularly for organic chemicals (e.g., Landrum et al., 2005, 2004; Escher and Hermens, 2002; McCarty and Mackay, 1993; Cook et al., 1993, 1989; McCarty, 1986; Veith et al., 1983; Könemann, 1981). For many nonionic organic chemicals, available data indicate that whole-body burdens of chemical (normalized to lipid content) can serve as useful metrics of toxicological dose, and these relationships appear to be independent of whether exposure was via water or diet. A major strength of the CBR 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 the CBR approach does not appear to be a robust indicator of toxic dose. One reason why the CBR approach currently appears more limited for metals relates to differences in their mechanisms of uptake, distribution, and disposition in aquatic organisms. Specifically, the distribution of nonionic organic chemicals in organisms is largely influenced by passive partitioning. In contrast, the uptake, distribution, and disposition of metals are typically governed by highly-specific biochemical processes that alter the metal form and involve facilitated or active transport. 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. As a basis for improving residue-response relationships, 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).

Other researchers have suggested that CBR 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; therefore, the effects are governed by factors that influence the rate of uptake. When uptake rate 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). Because different species of aquatic organisms invoke different "accumulation strategies" (i.e., involving combinations of regulation, detoxification, and storage), considerable difficulties arise among species when interpreting the toxicological significance of metal whole-body residues.

Therefore, risk assessors should ensure that a toxicologically valid residue-response relationship supports the CBR threshold before using tissue residues as indicators of toxicity.

Although many toxicological studies report measurements of metal residues in multiple tissues along with adverse effects, these tissue residue values may not be appropriate for use as a CBR threshold because metal concentrations in some tissues may have little or no relationship with toxicity. Furthermore, risk assessors are cautioned against extrapolating CBRs across differing exposure routes (food vs. water), durations, tissues, or species, because the potency of metal residues often differs depending on these factors.

5.3. RISK CHARACTERIZATION

As described in Section 2.4, Risk Characterization is the final phase of the risk assessment and is the culmination of the Planning, Problem Formulation, and Analysis of predicted or observed adverse effects. Risk Characterization produces a detailed description of the risk estimate(s), evaluates and summarizes the lines of evidence that support or refute the risk estimate(s), describes the uncertainties, assumptions and qualifiers in the risk assessment, and reports the conclusions of the assessment to risk managers (U.S. EPA, 2000c, 1998a).

While there are no metal-specific methods in the Risk Characterization, there are aspects that are important to metals risk assessments. For example, considering multiple lines of evidence such as results from *in situ* toxicity testing or biological assessments can be valuable for supporting the conclusions of a risk assessment. Care should be taken, however, to evaluate and present the limitations associated with each line of evidence, as discrepancies may not always indicate underlying differences; rather, they may reflect inherent limitations of each of the methods. For example, biological assessment methods may not be sufficiently sensitive to detect the level of effects or exposures that are of concern in the risk assessment. Documenting assumptions and uncertainties (e.g., use of background metal concentrations rather than added metal or specific metal species) becomes increasingly important the closer hazard thresholds are to background concentrations. Risk assessors also should document all assumptions and uncertainties in the methods used, such as how metals bioavailability was addressed. Because data may not be adequate to conduct a quantitative uncertainty analysis, risk assessors should describe the *sensitivity* of the risk assessment results to key assumptions and the *direction of bias* introduced by these assumptions (i.e., under- or overestimation of risks). This is particularly important for national or regional assessments, where results often are intentionally based on organisms and conditions that enhance exposure, bioavailability, and toxicological sensitivity. For essential metals, risk assessors should describe the relationship of the risk threshold to nutritionally required levels for the organisms of concern. Risk assessment results that fall below nutritionally required levels are an indication that some methods or assumptions require additional refinement. Risk assessors should carefully document the form(s) of metals used in the exposure and effects assessment, as they frequently differ due to data limitations. Additional issues and questions that should be addressed in the risk assessment are listed in Section 2.4.