ISSUE PAPER ON THE ECOLOGICAL EFFECTS OF METALS

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1. ISSUE OVERVIEW

Regulation of metals in the environment presents many challenges. Meaningful characterization of effects of metals on ecological receptors and humans requires understanding of biochemical, physiological, and ecological processes that reflect an evolutionary history with ties as far back as the origin of life. Organisms have always been exposed to metals, unlike novel synthetic organic substances. Consequently, organisms have developed various means of responding to metals.

Other papers in this collection (U.S. EPA, 2004a, 2004b, 2004c, 2004d) describe physical/chemical processes that determine speciation (stability of particular valence states), environmental concentration, bioavailability, and other dynamic interactions that influence exposure, leading to possible adverse ecological effects. Exposure can be considered to be entirely under the control of abiotic factors that determine the environmental concentration at the point of and entry to the organism. However, for many metals, organisms exert strong regulatory control on the solubility of substances, can access solid-phase substances, and can otherwise alter exposure levels. In such cases, the interplay between exposure and effects becomes more difficult to ascertain. Each of these papers contains useful information on human health effects.

In this paper, we present a summary of biological and ecological responses to metals, and discuss the role of descriptive and predictive methods used to characterize effects of metals in risk assessment. We also examine differences in the mandates and scopes of various regulatory programs that pertain to metals in the environment and suggest language for the Framework document that will rely in part on this and other companion papers.

1.1 Toxicity Literature

Significant literature pertaining to biological/ecological effects of metals in the environment began in the mid-1800s and continues to build. At the beginning of the 20th century, some very good and interesting studies on the aquatic toxicology of metals were reported (Loeb, 1902; Mathews, 1902, 1903; McGuigan, 1903; Lillie, 1904; Woodruff and Bunzel, 1909). Another wave of aquatic toxicity studies on ionoregulation (Smith, 1930; Krogh, 1937, 1938, 1939) and exploration of effects of metal mixtures (Jones, 1939a, 1939b, 1940) occurred in the 1930s. The early focus (before the 1960s) in terrestrial systems was primarily on nutrient requirements for plants, livestock, and humans. The study of toxic effects gained prominence with the advent of modern environmental law, especially with the Clean Water Act of 1970 for aquatic systems and the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) in 1981 for terrestrial systems. One database pertaining to copper holds more than 40,000 reports (ICA, 1996).

Literature reviews on metal toxicity largely have segregated information into media (e.g., freshwater, marine, sediment, or soil) and by taxonomy (algae, invertebrates, vascular plants, birds, small mammals, and large mammals). Arguably, determining ambient water quality criteria is the best starting point for documentation of effects in aquatic systems. For terrestrial systems, the Eco-SSL database

(www.epa.gov/superfund/programs/risk/tooleco.htm)[1] provides a comprehensive analysis of literature prior to 2000.

1.2 Regulatory Context

Risk assessments (both human health and ecological) are performed to inform management on issues pertinent to making decisions under many federal statutes. Different environmental laws and the regulations that have evolved under them differ in breadth and detail from the exposure and effects characterization of risk assessment. Generic risk assessments as carried out in Europe on products of commerce (e.g., all zinc products) call for fundamentally different analyses of data compared to the analysis needed for a hazardous waste site such as a smelter in the United States under CERCLA. Assessing risk due to metals in air emissions such as the estimates provided in the Toxics Release Inventory (TRI) differs from that of releases of metals at RCRA (Resource Conservation and Recovery Act) sites or determining safe discharge levels such as that done under the NPDES (National Pollutant Discharge Elimination System) permitting process.

Some risk assessments are structured to allow for management decisions with different levels of effort appropriate to the project and the information at hand. Examples include decisions to act immediately when obvious serious adverse effects are expected, with no need to explore fine details regarding effects. Similarly, *di minimus* concentrations of metals judged from prior experience are sufficient grounds to conclude likely absence of harm. Complex interactions among metals and across exposure scenarios make up the vast middle ground—the place where detailed examination of likely effects occurring under different levels of exposure is necessary.

2. COMMON ECOTOXICITY ISSUES

Risk assessors often specialize in either aquatic or terrestrial systems. This specialization has, on occasion, led to inaccurate generalizations, as conclusions from one system are presented as applicable to all (e.g., in aquatic systems, aluminum and chromium can be highly toxic, but in terrestrial systems they are usually benign). Despite this being a

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[1] The U.S. EPA led a coalition of scientists representing government, academia, and industry in an effort to establish ecological soil screening levels (Eco-SSLs) for Superfund Ecological Risk Assessments. The effort, which focused on prominent constituents of concern at Superfund sites, included several metals. A comprehensive literature search was conducted, articles were examined thoroughly against established acceptance and evaluation criteria, quality assurance procedures were used to document the usefulness of the toxicity data, and extensive documentation of all steps in the process has been captured in a database maintained by U.S. EPA Midcontinent Ecology Division. The Eco-SSL endeavor is significant for risk assessment of metals in at least three important ways. First, for the elements that were included in the initial effort, data that summarize what is known with regard to toxicity to plants, soil invertebrates, birds, and mammals are compiled and readily accessible. Second, the nature of the literature search that was performed and documented was comprehensive, and it is unlikely that additional relevant information on individual elements would be discovered in accessible literature prior to 2000. Third, the operating procedures that were developed by the work groups provide a prescribed methodology for future efforts that might be done on substances not included in the initial effort. Preliminary Eco-SSLs for 17 metals, for the different receptor groups, are listed in Appendix A.

problem for some aspects of risk assessment, there are common threads that originate from basic biological/ecological foundations. Here we describe some of the most important ecological relationships between organisms and metals in the environment.

2.1 Consideration of Essentiality and Toxicity

Essentiality (a requirement for normal organism metabolic function) of many metals is one of the primary factors that differentiate risk assessment for metals and metal compounds from that of synthetic organic chemicals (Janssen and Muyssen, 2001). Trace elements can be divided into three groups:

- Those known to be essential.
- Those that have beneficial metabolic effects but have not been shown to be essential.
- Those that occur widely in living organisms but seem to be only incidental contaminants, and are not known to be beneficial (Mertz, 1981). Table 1 classifies the metals addressed in this framework by their known essentiality to organisms.

Trace elements such as cobalt, copper, iron, manganese, selenium, molybdenum, and zinc are necessary for the normal development of plants and animals. Extensive research on the determination of essentiality occurred during the last two centuries (Liebig, 1840; Horowitz, 1945; McClendon, 1976; Epstein, 1972), with the focus on determining plant requirements for optimum growth of crops. This involved determining the list of essential minerals, the form of uptake in plants, threshold levels for sufficiency, and toxic levels. Review articles and books have condensed the vast quantity of information into manageable units (Alloway, 1974; Kabata-Pendias and Pendias, 1992). In the 1960s, ecological interests turned to nutrient cycling dynamics, pollution concerns, and food-chain transfers of metals from soil, sediment, or water into plants (Brown et al., 1980; Edmonds, 1982; Howell et al., 1975; Risser et al., 1981; Page et al., 1983; U.S. EPA, 1985a–e; Woodwell, 1967).

In many cases, these metals are added to animal feed and to pharmaceutical products (SRWG, 2002), just as the macronutrients potassium and phosphorous are added to plant fertilizers. Other metals such as arsenic, cadmium, lead, and mercury, have no known beneficial uses.

Table 1. Framework Metals Classified by Their Known Essentiality

Metal [a]	Essential [b]		Beneficial [c]		Non-essential [d]
	Plants	Animals	Plants	Animals	
Aluminum (Al)					x
Antimony (Sb)					x
Arsenic (As)					x
Barium (Ba)					x
Beryllium (Be)					x
Cadmium (Cd)					x [e]
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			

[a] Adapted from a table presented in SRWG (2002) and incorporating data from NRC (1980), Marschner (1995), and Barak (1999). Fairbrother and Kapustka (1997) discussed the roots of essentiality of naturally occurring elements.

[b] Known requirement for health and basic metabolic functions.

[c] Not needed for health or basic metabolic functions, but can have positive effects.

[d] No known metabolic functions and not beneficial for health or basic metabolic functions.

[e] Lane and Morel (2002) reported a biological function for cadmium in marine diatoms.

One of the biggest challenges faced by policy makers and risk assessors is how to address concerns about the risks posed by toxic and bioaccessible metal concentrations without adversely affecting organisms' usage of metals that are known to be essential or beneficial (SRWG, 2002). The essential and generally non-toxic "macro elements," calcium, magnesium, potassium, and sodium, as well as the "micro" or "trace" element, iron (Henry and Miles, 2001), are required for proper organism growth and function and are often screened from the ecological risk assessment process. But assessing risk from elements such as copper, selenium, and zinc—that are essential and beneficial at one dose, and toxic at another—is a more complex matter. Important considerations in assessing risk, while considering essentiality, of metals are discussed in the sections below and in the paper on bioavailability (U.S. EPA, 2004b).

Among the essential or beneficial elements that are metals, some are recognized as macronutrients (i.e., required in high concentrations, such as Fe) and others known as micronutrients (i.e., needed in very low concentrations such as Ni or Mo). When present at high concentrations, even these metals (e.g., cobalt, copper, iron, magnesium, manganese, molybdenum, and selenium) are toxic (Klaassen, 2001). Non-essential metals (e.g. lead, arsenic, and mercury) also are toxic at high concentration.

Organisms expend energy to regulate the internal concentrations of these metals (Epstein, 1972). Regulation may be internal or occur at the point of uptake across an external membrane. For example, metals may be sequestered into inert granules, bound to organic molecules (e.g., metallothioneins in animals or phytochelatins in plants), or shunted into vacuoles away from active enzyme areas. Consequently, in many situations, metals bioaccumulate (U.S. EPA, 2002a). Interpretation of toxicity studies on metals that act as essential elements presents a unique challenge because essential elements exhibit classic bell-shaped, or biphasic, dose-response (or exposure-effect) relationships in which adverse effects are observed both at high and low doses, with an optimal mid-range dose (see Figure 1) (Abernathy et al., 1993; Chapman and Wang, undated).

The concept that many metals are required for organism health at one range of concentrations and are toxic in quantities that may be either more or less than that range has been referred to as the "window of essentiality" (Hopkin, 1989), or the "optimal concentration range" for essential elements (Alloway, 1995; Fairbrother and Kapustka, 1997; Van Assche et al., 1997). This property of certain metals belies one of the common assumptions made in risk assessments (i.e., the lack of exposure to a compound is not deleterious). For anthropogenically derived compounds, this may be a reasonable assumption; however, for essential elements that exhibit biphasic dose-response curves (Figure 1), adverse effects resulting from deficiency also must be considered. Because many metals are essential for proper biological function, organisms are able to metabolize, and thus regulate, both essential and non-essential metals within their internal systems. Essentiality, as well as biochemical and physiological processes that regulate metal concentrations and speciation within living organisms, is among the factors that make ecological risk assessment of metals unique.

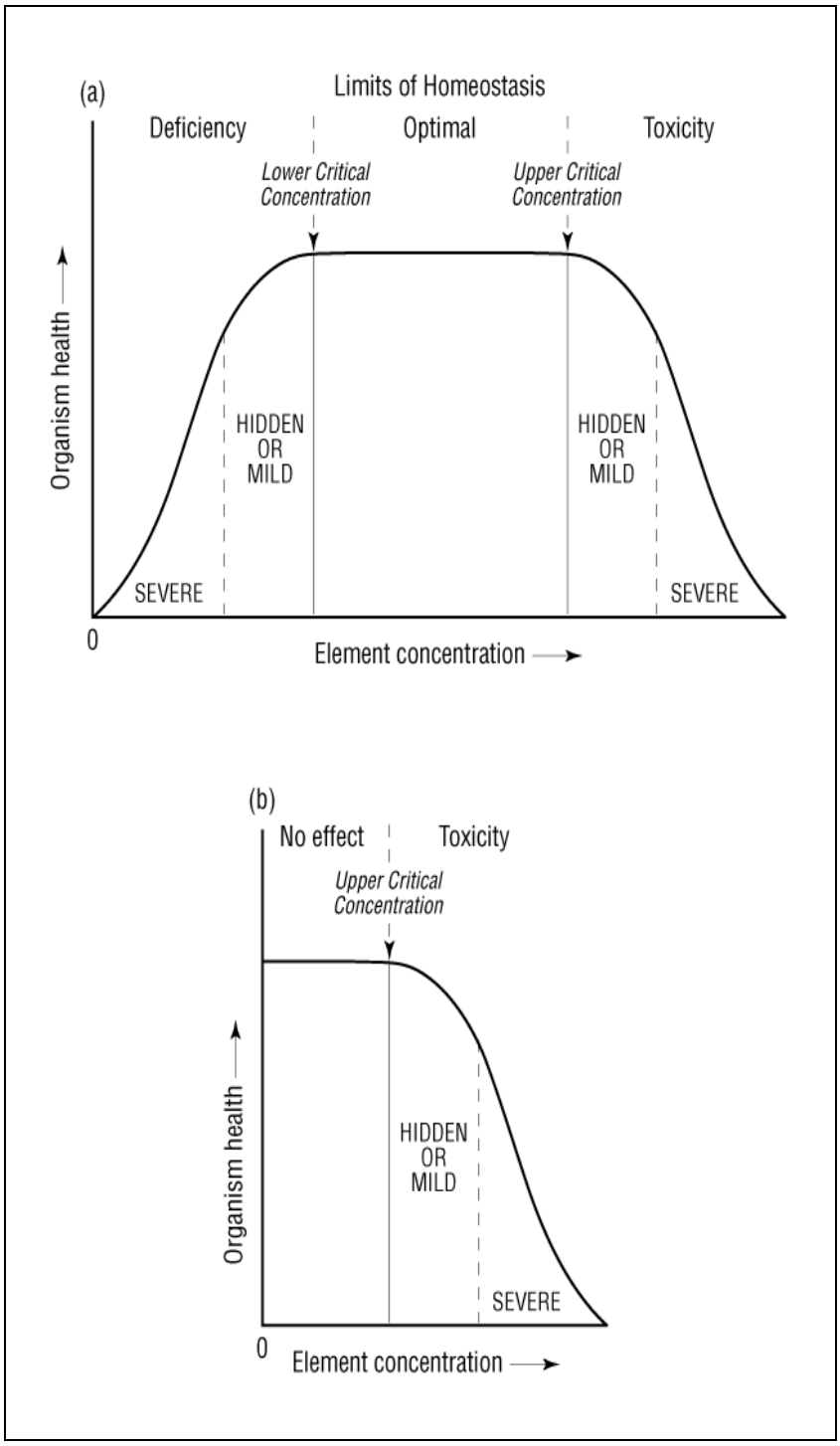

Figure 1. Dose-response curves for (a) essential elements and (b) non-essential elements (Alloway, 1995).

In ecological risk assessment, consideration of essentiality must be incorporated into the analysis phase (U.S. EPA, 1997, 1998) of the risk assessment. The "optimal concentration range" (or safe intake range) should be considered in the effects characterization, ensuring that effects thresholds such as toxicity reference values (TRVs) used to assess risks from metals exposure are not lower than the nutritional requirements for the species being evaluated. This is an area where risk assessment for metals differs distinctly from that for synthetic organic chemicals. Where TRVs or other effects concentrations (or doses) are intended as thresholds for detrimental effects due to excessive intake (Abernathy et al., 1993), care must be taken to ensure that these toxicity thresholds for essential metals are at the upper end of the optimum range or sufficiency range (at the point where toxic effects begin to occur). If set too low (i.e., in the range where deficiency can occur (refer to Figure 1) the determination of risk will be erroneous. Thus, literature on the dietary requirements (NRC, 1980, 1994, for example) and the minimum concentrations required for plant growth (Epstein, 1965, 1972) should be consulted in determining effects concentrations for essential metals.

Factors that influence both the minimum requirements and the maximum tolerable levels for mineral elements must also be considered. These include metal solubility, animal and plant growth potential, physiological function (e.g., growth, egg or seed production), previous health of the organism, and the age, environment, and concentrations of other nutrients (Henry and Miles, 2001).

Regulation of metal accumulation by organisms complicates the interpretation and application of bioaccumulation data for aquatic and terrestrial organisms. Organisms have evolved homeostatic mechanisms that allow metals, as naturally occurring substances, to be stored in non-available forms (sometimes for later use). These mechanisms regulate the uptake and excretion of metals to maintain tissue concentrations within desirable ranges, as well as to prevent toxicity (U.S. EPA, 2002d; Fairbrother and Kapustka, 1997; Chapman and Wang, undated). For certain elements and organisms, bioaccumulation is required for organism health and normal function (e.g., for essential trace elements such as copper and zinc). In other situations, bioaccumulation produces residues in plants and animals that cause direct toxicity to the exposed organism (e.g., copper toxicity to aquatic organisms) or indirect toxicity to consumers (as in selenium accumulation by plants). To further complicate understanding the bioaccumulation and metabolism of metals, the metabolism of an essential element can affect the metabolism of a non-essential toxic metal, as in the case of calcium and lead in the central nervous system (Kern et al., 2000).

Homeostasis should be considered in ecological risk assessment for metals as it influences or regulates bioconcentration factors (BCFs), bioaccumulation factors (BAFs), and exposure concentration (Fairbrother and Kapustka, 1997; U.S. EPA, 1985a; Spacie and Hamelink, 1985). At low concentrations—where organisms experience nutritional deficiency—greater uptake and retention of metals occur to meet nutritional requirements. At concentrations above the nutritional requirement, homeostasis maintains a concentration limit in the organism. However, beyond that range, homeostatic mechanisms (e.g., regulation by excretion) can become overwhelmed, resulting in toxicity (Fairbrother and Kapustka, 1997). Organisms also may compensate for exposure to essential metal concentrations beyond their

nutritional requirements. The BCFs or BAFs could decrease with an increase in exposure concentration (U.S. EPA, 2002a; Adams et al., 2000).

Three patterns reflecting different metal tolerance mechanisms have been recognized for plants (Baker, 1981):

- **Accumulators**—Metals are concentrated in aboveground plant parts from low or high soil levels.
- **Indicators**—Metal concentrations in shoots are approximately equal to soil concentrations.
- **Excluders**—Meal concentrations in shoots are maintained at constant and low levels over a wide range of soil concentrations.

At high levels of metal bioavailability, loss of the capacity to restrict metal uptake can lead to phytotoxicity in excluders. Phytotoxicity also can occur in accumulators and indicators as physiological homeostatic systems become overwhelmed by the internal concentrations of metals.

For these reasons the BCF or BAF is not a good predictor of the relationship between exposure concentration and tissue residue and, therefore, should not be used in metals risk assessment (see the bioavailability chapter for further details). Rather than relying solely on predicted BCFs or BAFs, ecological risk assessment for metals and metal compounds should focus on a suite of field and laboratory measurements and on a variety of species representative of different taxa and trophic levels. They also should reflect site-specific conditions including pH, organic carbon content, water hardness (Ca and Mg preferred), and natural background concentrations of the elements and compounds being assessed (Van Assche et al., 1997; Chapman and Wang, undated).

2.2 Physiology/Toxicology of Metals

In a strict sense, most measured effects used to assess toxicity of metals are not unique. Measurements of mortality/survival, growth, reproduction, and various biochemical/physiological endpoints are common to all toxicity tests. Metal-specific endpoints in animals are limited to a few biochemical or physiological observations such as altered ALAD[2] activity in response to lead, "staggers"[3] in response to selenium poisoning, or diagnostic lesions on foliage in response to nickel. Plants exhibit characteristic patterns of

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[2] ALAD (delta aminolevulinic acid dehydratase) is an enzyme that catalyzes the asymmetric addition of two molecules of ALA to form prophobilinogen in heme synthesis. Lead interferes with the normal functioning of the enzyme and triggers a cascade of physiological responses including elevated blood Pb levels and lowered levels of ALA in plasma.

[3] Staggers is an abnormal condition in animals arising from damage to the central nervous system. Symptoms include incoordination and unsteady gait.

discoloration and malformation of leaves in response to metals (Aller et al., 1990). Ionoregulatory disturbances in fish and other aquatic organisms are frequently found in association with various metals. For example, disturbances of sodium and chloride regulation have been reported to result from exposure to elevated levels of copper or silver (e.g., Wilson and Taylor, 1993a, 1993b; Morgan et al., 1997; Bianchini et al., 2002), and disruption of calcium regulation has been attributed to exposure to cadmium or zinc (Spry and Wood, 1984, 1985; Verbost et al., 1987, 1988). These effects are frequently observed in association with a variety of other, subtler biochemical and physiological effects (Wood, 2001, provides a detailed review of effects on fish that result from waterborne exposure to metals). Despite the limited number of metal-specific toxicity responses, there are features of metals that require special consideration as toxic responses are interpreted. Constructs that were developed to evaluate toxicity of synthetic organic compounds, such as persistence and bioaccumulation, are not fully satisfactory when addressing metals (Tarazona et al., 1999). It is important to consider the evolutionary linkage organisms have with metals in the environment.

The crustal abundance of elements and the physico-chemical properties of metals are related to physiological responses such as essentiality, toxicity, and tolerance (Horowitz, 1945; McClendon, 1976; Fairbrother and Kapustka, 1997). With few exceptions, those elements that appear in greatest abundance were incorporated into enzymes, electron transport chains, and structural features of primitive organisms. Homeostatic regulation of intracellular (or intraorganellar) concentrations of metals evolved to cope with existing conditions. Fairbrother and Kapustka (1997) observed that, generally, the required nutritional levels of essential elements for plants tend to be approximately one order of magnitude less than the average crustal concentration and phytotoxic levels tend to be approximately one order of magnitude greater than the average crustal concentration. This makes good sense from an evolutionary perspective—if nutrient requirements were greater than might typically occur, such species would be restricted to isolated mineralized areas; if phytotoxicity occurred at lower concentrations, species would be relegated to mineral-poor areas.

Toxicity occurs at the point where the capacity of an organism to regulate the internal concentration of metals is lost, resulting in a loss of functions required for normal growth or to sustain life. Generally, this occurs at one or more internal cellular locations or may affect an entire organ. For plants, toxicity may also occur at the root surface without the substance ever entering the internal portions of the plant. Similarly, with microbes, toxic effects may occur at the external membrane surface. Metals may also disrupt extracellular enzyme function.

The site of toxic action could be an enzyme, a membrane, or a co-factor critical to some biochemical pathway. Often, multiple sites of action for a particular substance might exist, and toxicity could be manifested in different ways, depending on how the primary modes of action and the cascade of secondary effects are linked. For many toxicity endpoints, such as reduced growth, fecundity, yield, or survival, multiple disruptions of biochemical functions are likely to occur. For example, a phytotoxic response of reduced growth might be a result of impaired photosynthetic function, impaired respiration, and impaired water uptake by roots. Impaired electron transport or neuro-transmission may lower the capacity of an animal to escape predation.

Toxicity thresholds refer to concentrations above which organisms exhibit adverse effects such as reduced growth or increased mortality. Data from a single experiment or from several studies (either laboratory or field observations) are used to identify thresholds. Literature reviews of toxicity studies are often aimed at identifying the lowest concentration of a substance at which adverse effects were reported (see U.S. EPA, 1985b–f). These values are useful in attempting to find an environmental concentration protective of all species. However, many physicochemical characteristics of soil alter the concentration-response relationships. Most commonly, pH, organic matter content, soil texture, and relative amounts of other substances (e.g., calcium, iron, etc.) influence bioavailability and therefore the threshold concentration for a particular field situation. Also, the chemical form of the substance can be very significant. Because of this, some literature reviews have emphasized ranges of toxicity threshold concentrations. Other disciplines, such as phytoremediation, have focused on the most tolerant species. Though generally there are insufficient data to describe the distribution of species across the range from most sensitive to most tolerant, knowledge of this range will help in anticipating likely responses among diverse groups of species to concentrations in particular field settings. As environmental concentrations increase, it becomes more likely that many more species will be harmed, and the magnitude or severity of the responses will increase.

Metallothioneins (MT) and phytochelatins are small proteins in animals and plants respectively, which regulate and detoxify many metals within the organism. This mechanism of regulation is very effective when the organism is exposed to background or even moderately elevated levels of many metals. The regulation of metals within the organism has limits, however, and plants have demonstrated that the interactive effects of cadmium and arsenate were concentration-dependent and ranged from non-additive to synergistic, as concentrations increased. At high concentrations the ability of the plant to regulate metals collapsed and phytochelatin levels dropped (Sneller et al., 2000). An alternative protective mechanism, the formation of metal granules, has been demonstrated in invertebrates (Rainbow and White, 1989; Vogt and Quinitio, 1994; Walker, 1977).

2.3 Response Surface

The value of nearly all toxicity data is constrained by limitations of the experimental designs used. The overwhelming majority of tests have used an analysis of variance (ANOVA) design with the primary objective being the identification of threshold response concentrations [typically reported as No Observable Adverse Effects Concentration (NOAEL) or Lowest Observable Adverse Effects Concentration (LOAEC)]. The ANOVA design has been criticized for being dependent on concentration intervals chosen, for being insensitive because of inherent variability in responses, and for the fact that most of the information from the test is lost. Comparisons among two or more tests using an ANOVA design are easily erroneous because the reported values for comparisons typically are point estimates. Information regarding the magnitude of the response over a concentration range is missing.

Consensus has formed in the technical community that is in agreement with Chapman et al. (1996) regarding the problems of using “no effect” determinations based on ANOVA designs. The central issues are:

- Study design has marked influence on the interpretation of the "no effect" concentration.

- Important concentration-response relationships are ignored.

The preferred study design for assessing toxic effects is based on regression models. Here, instead of block designs with equal number of replicates spanning three to six concentrations, unequal replicates spread over 10 or more concentrations are preferred (Stephenson et al., 2000; Environment Canada, in review). In such designs, more replicates are desired around the "target" effects level, and few replicates on the tails of the concentration range. For example, if range-finding or other information suggests an EC_{50} at 100 ppm, and one wanted to determine an EC_{20}, then one might have six replicates at 50 ppm, five replicates each at 25 and 75 ppm, four replicates at 15 and 100 ppm, and some of the higher concentrations may have only two replicates. It is important to recognize that the upper and lower ranges in a regression can drive the solution of the equation describing the relationship.

Data may also be analyzed using "hockey stick" regression algorithms, which objectively find the intersection of two distributions. The intersection may be interpreted as the "no effect" level. However, to be useful, "hockey stick" regressions require substantial numbers of different x values in x:y pairs to give meaningful results. Few such data sets exist.

Most data examined for the Eco-SSL effort fell loosely into three groups: 1) lab experiments with amendments, 2) field experiments with amendments, or 3) non-manipulated field studies across gradients. Results from lab or field experiments come from many studies done for purposes other than toxicity determination (e.g., agriculture fertilizer studies, population genetics). Often, such studies report "no adverse effects," even across several concentrations. Alternatively, some studies report effects at all concentrations tested. Either of these introduces problems that limit their utility.

Consider a situation (see Figure 2) in which the solid, curved line represents the "true" concentration response relationship that includes a hormesis response that can only be demonstrated with a well-designed experiment with regression analysis.[4] The "True No Effect" concentration would be approximately 150 ppm. Using the American Society for Testing and Material (ASTM) recommendation in ASTME 1963-98 (ASTM, 2003) in which at least a 10 percent reduction in endpoints is considered to be biological relevant, an equivalent of a LOAEC at approximately 200 ppm could be interpreted. However, a study such as that illustrated by Study Number 1 (Green Box and Whisker Plot with 1 inserted at the mean value), would, with an ANOVA analysis, produce an unbounded NOAEC of 300 ppm. Conversely, a study illustrated by Study Number 2 (Red Box and Whisker Plot with 2

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[4] Note that in plant studies, more often than not, a slight stimulation occurs relative to negative controls. For essential nutrients, chronic exposures would enhance this stimulation effect. Similar hormesis responses are often shown for non-metal test substances. One excellent paper on this is Shirazi et al. (1992).

inserted at the mean value) would produce an unbounded LOAEL of ~700 ppm. If an extrapolation were performed for Study 2 results, a “No Effect” level might be postulated to be as low as 15 ppm.

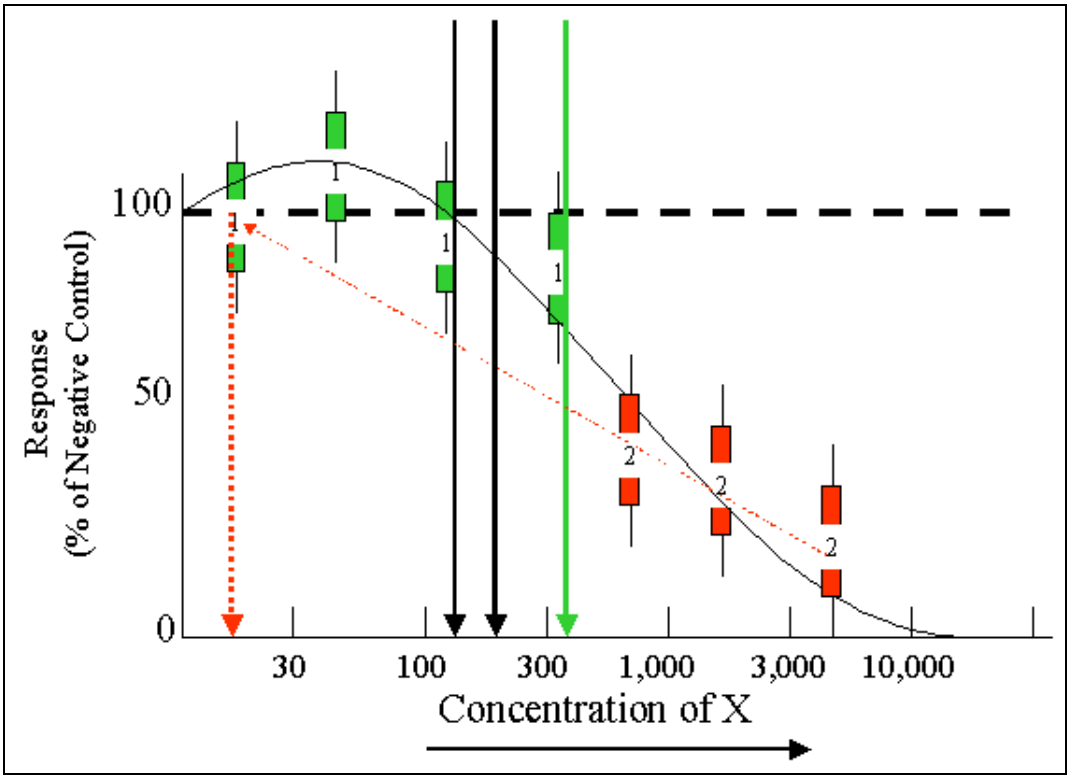

Figure 2. Illustration of potential problems with the interpretation of NOAEL, LOAEL, and regression plots based on limited toxicity response data.

The problem of the ANOVA design for identifying NOAEC and LOAEC values would be further illustrated by additional data in both Study 1 and Study 2. Assume that Study 1 also had the lowest concentration of Study 2, and that Study 2 had the highest concentration of Study 1. Both studies would identify the NOAEC to be 300 ppm and the LOAEC to be 700 ppm.

Ignoring any problems of experimental error that confound detection of differences among treatment, the problems above are encountered when we know the relationship. In studies that did not include sufficient range to establish the response relationship, interpreting the data correctly is not possible.

Finally, it is worth considering that many toxicity studies use log or half-log concentration treatment intervals. Assuming that the results of the study were accurate (i.e., fell exactly along the true concentration response curve), mixing unbounded values with bounded values would result in at least a half-log or log difference in the value used to

interpret the threshold response concentration. This could (most likely would) generate unbounded NOAEC concentrations several concentration steps below the "true" value.

From a practical view, there is one general situation in which an unbounded NOAEC is legitimate. This exception is referred to as a "limit test." Limit tests are appropriate for establishing practical definition of non-toxic substances. There is a point for example, where no practical value is obtained by further testing. Some metals (e.g., Fe, and in some circumstances, Pb) have been shown to have no effect at percent-level concentrations. If the highest expected environmental concentration is substantially less than a verified no-effect level, then further testing is not justified.

A complete surface response profile provides valuable information that can improve risk communication and lead to more informed decisions. The profile illuminates concentration ranges that are stimulatory (i.e., hormesis) due either to attainment of sufficiency levels for essential elements or as "overcompensation" from a sustained low-level stress by non-essential elements. The profile can also highlight the significance of incremental increases in concentrations (Fairbrother and Kapustka, 1997, 2000).

2.4 Ecological Consequences of Toxicity

Plant and animal communities in mineral-rich areas differ from those in surrounding areas. Plant biogeographers have long noted differences in plant community composition related to soil types. Vegetation growing in chalky soils has been distinguished from that growing in sandy soils. Grime and Hodgson (1969) have demonstrated differential toxic responses among species, with calcifuge species showing susceptibility to lime-chlorosis and calcicole species susceptible to aluminum toxicity. Antonovics et al. (1971) reported that characteristics of vegetation growing on high-metal-concentration soils were used at least since the 16th century in prospecting for metals. Metal-tolerant populations of several species growing on serpentine soils were distinguished from non-tolerant relatives growing nearby. Observations of colonization of toxic mine spoils by bentgrass (*Agrostis tenuis*) were described by Antonovics et al. (1971) as "some of the best documented examples of evolution in action." Bradshaw et al. (1965) summarized reports showing emergence of metal-tolerant fescue (*Festuca ovina*) and bentgrass within 50 to 100 years on mine spoils and within 30 years beneath galvanized wire fences. Antonovics (1966) found that metal tolerant races of plants usually were excluded from "normal" soils by non-tolerant ecotypes. Metal-tolerant flora have been described for soils high in zinc, nickel, chromium, and copper (Antonovics et al., 1971; Brooks, 1972). Few soils are completely bare of vegetation solely because of the toxicity of the inorganic ions they contain (Macnair et al., 1993). Metal-tolerant populations of terrestrial invertebrates has been demonstrated conclusively for several animals: *Orchesella cincta*, *Porcellio scaber*, *Isotoma notabilis*, and *Onychiurus armatus* (Posthuma and Van Straalen, 1993).

The Sudbury, Ontario, smelter site is widely recognized for its high levels of metal contamination (elevated levels of aluminum, copper, nickel, lead, and zinc; low pH) (Archambault and Winterhalder, 1995). Clones of Sudbury plants differ statistically from clones of the same species obtained from uncontaminated sites for both germination and growth endpoints. The non-tolerant species displayed reduced germination, and their roots

displayed an observable chemotropic response. A distance of 50 meters or less is sufficient to isolate plant populations from one another, facilitating differentiation in relation to local environmental conditions.

At other highly contaminated sites, adverse effects in small mammal communities have been attributed to a combination of direct and indirect effects. Densities of microtine rodents increased with distance from a copper-nickel smelter in Monchegorsk, Russia (Kataev et al., 1994). Direct effects (e.g., cytogenetic and morphophysiological differences in *Microtus* sp.) were observed near the smelter (Kataev and Popova, 1993), but the primary explanation for the population density gradient was that fewer plants used by microtines were found in the most contaminated areas. In another example, population density of small mammals in woodlands near a lead-zinc smelter in Avon, England, was correlated with copper concentrations in the food chain (Read and Martin 1993).

Two populations of the common shrew (*Sorex araneus*) in the Netherlands were influenced by metal in diets (Denneman, 1990). One population lived in an area surrounding a former Zn smelter and the other was from a national park. Major prey types in all seasons were lumbricids, insect larvae (mainly *Coleoptera* and *Lepidoptera*), adult *Coleoptera*, and *Araneae*. In autumn, *Opiliones* were present in the diet. Soft prey animals contributed about 40 to 50 percent of the total food intake. Both populations showed a preference for *Lumbricidae*, but the shrews at the contaminated site ate more *Coleoptera* than did the reference population. Differences in prey choice appeared to be more connected to differences in availability and preference than to differences in heavy metal content.

Metals in the diet may also alter wildlife behavior patterns. Copper concentrations in plants apparently influenced the selection of nest sites by Abert's squirrels (*Sciurus aberti*) (Snyder and Linhart, 1994). Phloem in twigs of trees with nests had significantly lower copper concentrations than similar trees without nesting squirrels. Snyder (1992) reported a similar relationship in Colorado, where Abert's squirrels selected trees with lower concentrations of specific metals, although the concentrations of copper were not significantly different between selected and non-selected trees.

Not all adverse effects of metals on plants or wildlife result from anthropogenic activities. One example reported recently is that of elk in Yellowstone National Park (Kocar et al., 2004). Elk exposed to elevated levels of arsenic in the park had decreased life expectancy compared to reference elk herds.

Most toxicity test methods are designed to measure the direct response of individuals to test substances or mixtures of test substances. However, in ecological systems, indirect effects could alter the complexity of the system, its composition or species diversity, or dynamic processes such as nutrient cycling and litter decomposition. Efforts to evaluate community responses or system functions were developed to evaluate the effects of pesticides (including metals) using microcosms, mesocosms, and field studies (Hansen and Tagatz, 1980; Meador et al., 1998).

Classic questions in ecology have focused on food webs and patterns in ecological communities. Hairston et al. (1960) proposed the "The world is green" hypothesis wherein

top-down control by herbivores ensures that plant growth is kept in check. The alternate "bottom-up limitation" hypothesis, "The world is prickly and tastes bad" (Pimm, 1991), argued that plants are adapted to predation, and thus plant availability, energetics, and nutrient limitation ultimately control community composition. Parmelee (1995) suggested that contamination of soils, through trophic cascades, could affect ecosystem processes.

Studies of the responsiveness of food webs to different chemicals are recent. Parmelee et al. (1997) evaluated the effects of copper and cadmium in a soil microcosm. Two separate copper tests showed the results to be reproducible, in that the same trophic groups were similarly sensitive to the same concentrations of copper. The omnivore-predator nematodes were most sensitive to copper in both studies; the overall abundance of nematodes was not affected at 100 ppm Cu in the first study, but was reduced relative to the control in the second study. Bogomolov et al. (1996), in a similar study using soil with higher sand content and higher pH, found that copper did not significantly reduce nematode numbers until reaching a concentration of 800 ppm. The differences between these studies emphasize the importance of soil composition and chemistry in affecting the toxicity of chemicals to the soil fauna.

2.5 Microbial Functions

Mycorrhizal relationships as well as bacterial associations with plant roots have largely been ignored in both the scientific community and regulatory circles, despite the well-documented and widespread significance of these plant-microbial relationships. Because mycorrhizae markedly influence the uptake and metabolism of metals by the host plant, failure to account for mycorrhizae can lead to erroneous assumptions of risk to plants and the dietary exposure levels of animals (Kapustka, unpublished SETAC poster). Rhizosphere microbes alter patterns of root growth, affect nutrient relationships of the plant, affect water uptake by plants, change metabolic processes in plant cells, protect plants from pathogens, and differentially influence phytotoxic responses (Harley and Smith, 1983; Smith and Read, 1997). Water, nutrients, and soil contaminants for the most part pass through the fungal hyphae *before* entering the vascular transport stream of the plant. In the field, this has utmost significance as mycorrhizae occur in 83 percent of dicotyledonous plants, 79 percent of monocotyledonous plants, and in all gymnosperms (Marschner, 1995). Enzymes released in the rhizosphere by VAM function as chelating or nutrient-dissolving agents (Gobran et al. 1998). Mycorrhizal associations can result in increased plant uptake of metals including cadmium, copper, zinc, lead (Killham and Firestone, 1983) in plant tissues; but at the same time, these associations alter the compartmentation of ions and distribution among plant tissues (Cantrell, 2000) away from the site of action where toxicity occurs, and therefore confer apparent tolerance. Alternatively, soil hyphae can arrest ions present in the soil solution at high concentrations and prevent their translocation into plant tissues. Mycorrhizal fungi increase the number of uptake sites by increasing the effective sorptive surface area of the plant or by altering the affinity constant for a particular metal ion (Arnold and Kapustka, 1993; Cress et al., 1979; Karunaratne et al., 1986).

The influence of intestinal microbes in modulating metal toxicity in animals has not been explored as extensively as has the influence of microbes at the roots of plants. Differences in gut physiology and intestinal microflora affect the bioavailability of metals

and translate into different apparent sensitivities among animal groups (e.g., earthworms, ruminants, hind-gut fermenters, and carnivores). For example, ruminants are more sensitive to Cu toxicity than are monogastric animals, and sheep are more sensitive to Cu toxicosis than are cattle (Todd, 1962; Osweiler et al., 1985). Protein-bound Cu is less toxic to ruminants than are Cu salts, because it is less toxic to ruminant bacteria. The presence of ciliate protozoa in the rumen of sheep markedly altered the assimilation and metabolism of Cu (Ivan et al., 1986). The protozoa maintain a more stable pH of the rumen and increase the production of sulfate through the metabolism of sulfur-containing amino acids. The sulfate binds to Cu causing it to be less bioavailable.

The importance of microbial process to critical system functions (e.g., nutrient cycling) is undeniable. Toxicity to bacterial or fungal groups can be manifest in many indirect effects to plant community composition and to primary and secondary productivity. Kapustka (1999) discussed the spatial and temporal variability observed in measurements of effects on microbial systems as well as the sensitivity of some groups and resistance of others to metals. Relative to other taxonomic groups (plants, invertebrates, or vertebrates), some microbes exhibit sensitivities approximately one order of magnitude lower than the other groups, and others have tolerance or resistance that is approximately one order of magnitude greater than other groups. With the propensity for functional redundancy among microbes, systems level effects of toxicants, including metals, are often masked. The study indicated that it is exceedingly difficult to relate specific microbial activities with indications of adverse and unacceptable environmental conditions. Moreover, changes in microbial processes do not necessarily result in consequences to plant and animal populations or communities, which in the end are the resources most commonly identified as those to be protected. Therefore, unless more definitive linkages are made between specific microbial effects and an adverse condition for typical assessment endpoint species, microbial endpoints will continue to have limited use in risk assessments; they will not drive the process as primary assessment endpoints.

2.6 Physicochemical Interactions

Experimental approaches require segregation of component parts of the environment to identify causal relationships. With metals, long-standing evolutionary relationships can frustrate such efforts. Moreover, the dynamic interactions within soils can produce a staggering array of complex interactions (Figure 3) that can frustrate efforts to predict response relationships.

← above diagonal: adjacent to roots →

	Li	Ru	Cu	Zn	Cd	B	Al	Si	Pb	V	As	Se	Cr	Mo	W	Mn	Fe	Co	Ni
Li																			
Ru																			
Cu																			
Zn																			
Cd																			
B																			
Al																			
Si																			
Pb																			
V																			
As																			
Se																			
Cr																			
Mo																			
W																			
Mn																			
Fe																			
Co																			
Ni																			

← below diagonal: plants →

synergism	
antagonism	
antagonism or synergism	
possible antagonism	

Figure 3. Complexity of metal interactions in soil with respect to plant uptake and partitioning (adapted from Alloway, 1995).

Most ecological risk assessments rely upon single-chemical laboratory toxicity test results to evaluate the potential for detrimental effects to ecological receptors. No environmental medium has only a single metal present. Spehar and Fiandt (1986) found that metal concentrations considered safe individually, induced chronic toxic responses in selected species when they were exposed to mixtures. Typically in ecological risk assessments, the potential for incorrectly estimating the effects of mixtures is ignored or compensated for using uncertainty factors with little scientific basis. This is not surprising considering there is generally insufficient information to make more refined risk determinations. Differing test conditions, definitions of the interactions, methods of data evaluation and incomplete understanding of the modes of action of some metals have hampered identification of reliable techniques to adequately predict the effect of mixtures (Spehar and Fiandt, 1986; Posthuma et al., 1997; Sharma et al., 1999).

Chemical mixture effects are described as additive (non-interactive), synergistic (greater than additive), or antagonistic (less than additive) (Calamari and Alabaster, 1980; Konemann and Pieters, 1996). The most widely used approach to predict additivity is to calculate and sum the toxic units of the individual components of the mixture, which is the ratio of the concentration of an individual component of the mixture to a specified effect concentration (e.g. LC_{50}) (Sprague, 1970). Non-interactive metals with similar modes of action are assumed to be additive. Metals with dissimilar modes of action (independent) are considered non-additive. Various models to assess and predict additivity exist (Calamari and Alabaster, 1980; Sharma et al., 1999; Liao and Lin, 2001; Lock and Janssen, 2002; Mowat and Bundy, 2002).

Effects of metal mixtures and are generally variable or contradictory (Sharma et al., 1999). Similar problems have been shown for fish, crustaceans, and algal species. The traditional approach to dealing with metal mixtures involves factorial experimental designs, which may be useful for site-specific assessments but have limited usefulness for generalized assessments, because of the overwhelming number of combinations of receptors and metal mixtures. Experiments of this design generally do not attempt to elucidate the mechanistic underpinnings of the observed results, and conclusions are limited to comparisons of tests conducted under very similar conditions.

Metal interactions, according to Calamari and Alabaster (1980), occur at three levels:

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

Much of the difficulty in interpreting the available information on the toxic effects of metal mixtures is due to differing measures and definitions of the bioavailable fraction of metals, whether it is the fraction that’s available for uptake from the environment or at the site of toxic action. Some measure of the bioavailable metal fraction in the exposure media is needed to predict the effects of metals and metal mixtures accurately (Ankley et al., 1996; Posthuma et al., 1997; Sauvé et al., 1998; Weltje, 1998; Di Toro et al., 2001). A review of the

effects of metal mixtures on freshwater aquatic organisms was conducted in 1987 by the European Inland Fisheries Advisory Commission (EIFAC, 1987). More recent discussions of ecological effects of metal mixtures appear in Norwood et al. (2003) and other papers in that issue.

Weltje (1998) evaluated data from several studies conducted on the toxic effects of soil metal mixtures to earthworms. Several measures of metal concentrations available in the data (total, $CaCl_2$–extractable, and water-extractable) indicated that interactions were antagonistic as total soil metals but additive as extractable metal fractions, assuming additivity of the metals is only slightly conservative (Weltje, 1998). The characterization of the effects of metal mixtures has also been reported to be concentration-dependent for other organisms (Spehar and Fiandt, 1986; Herkovits et al., 2000; Fargašová, 2001; Mowat and Bundy, 2002).

The antagonistic effect of selenium on the toxicity of mercury has been studied extensively in many species. The exact mechanism of this interaction, still an area of active research, appears to be species dependent. Selenium apparently reacts with mercury *within* the organism to detoxify mercury and allow storage of non-toxic mercury-selenium complex or facilitate its excretion (Magos and Webb, 1980; Caurant et al., 1996; Bjerregaard et al., 1999; Hoffman, 2002). An interesting application of this phenomenon was demonstrated by adding selenium to a mercury-contaminated lake for a period of 3 years. The results were an approximate 10-fold reduction in the tissue concentrations of Hg in pike (Paulsson and Lundberg, 1989).

Insufficient dietary quantities of essential metals has long been known to cause adverse effects in animals. Copper is an essential element that plays an important role in the functioning of enzymes involved the immune system, oxygen use, and energy production (Frank, 1998; Minatel and Carfagnini, 2000). Elevated levels of molybdenum have been shown to interfere with the ability of ruminants to absorb copper leading to a clinically complex array of symptoms (molybdenosis) that are potentially fatal to the animal (National Academy of Sciences, 1977). The disease is suspected to have caused the mysterious moose disease in southwest Sweden, killing an estimated 3 percent of the moose population each year. This region of Sweden has been affected by acid rain, and the discovery of molybdenosis in moose coincided with efforts to counteract the effects of acid rain by widespread application of lime (Frank, 1998; Frank et al., 2000; Frank et al., 2002). While naturally alkaline soils can cause molybdenosis in ruminants, soil affected by mine spoils has also been shown to deleteriously alter the copper: molybdenum ratios in forage species to potentially toxic levels (Erdman et al., 1978).

In some limited situations, sufficient descriptive or mechanistic information may be available to assess the effects of metal mixtures, but unifying theories are not ready for regulatory application. Significant advances in the understanding of the chemistry, bioavailability, and toxicity of metals have produced promising results that may lead to mechanism-based theories, which will explain the complexities of metal mixtures.

Predictive models based on metal speciation have had some success with individual metals and recently metal mixtures (see the papers on exposure and bioavailability for

detailed discussions of hard acid–soft acid characteristics, Quantitative Ion Character-Activity Relationships [QICARs], Free Ion Activity Model [FIAM], Biotic Ligand Model [BLM], Gill Surface Interaction Model [GSIM], Acid-Volatile Sulfide [AVS], and Simultaneously Extracted Metals [SEM]). Though much work remains, recent developments (U.S. EPA, 1999a, 2002b) indicate that the prediction of the toxic effects of metal mixtures is likely to become more useful for criteria development purposes. The various approaches hopefully can be merged into a unifying theme that can reliably address the toxic interactions of metal mixtures in all media.

2.7 Tissue Residue Levels

Metals are not distributed uniformly throughout an organism. Rather, metals preferentially accumulate in selected organelles, tissues, and organs. Further, the accumulated metal may exist in a variety of chemical forms in different internal locations, and the form at a particular site may be more or less toxic than at other sites. Such variations in chemical form could also alter bioavailability in subsequent trophic levels. Though it is technically feasible to characterize internal locations with high levels of precision, the cost of doing so makes such work impractical for most risk assessments.

Bioaccumulation models have been used, in lieu of field measurements, to evaluate tissue chemical accumulation levels (Thomann et al., 1974; U.S. EPA, 1978; Gobas et al., 1986; Gobas, 1993). This use has in part been dictated by a need to predict future tissue levels that will result from alternative site cleanup scenarios (a situation in which field measurements are not possible). Although there are exceptions, most of the bioaccumulation models that are available for use were originally developed for applications to organic chemicals and have only relatively recently been applied to metals (see Paquin et al., 2003). Kinetic models have been used to estimate accumulation of metals by a variety of aquatic organisms, including bivalves, copepods, and fish (Reinfelder et al., 1997; U.S. EPA, 2004b). When used in conjunction with kinetic coefficients that are measured in laboratory studies, such models have been shown to have good predictive capabilities (e.g., Luoma et al., 1992; Wang et al., 1996, 1997). The current model formulations do not include features that are specific to the unique characteristics of metals. This limitation reflects, in part, an incomplete understanding of homeostatic processes and intracellular speciation of metals. Ongoing advances in scientific understanding and continued refinement of available models will likely lead to the development of more mechanistically based models. Such models should have an improved capability to predict tissue metal levels and related effects. As with any model, care must be used in their application and in the interpretation of their results. Use of model results, in conjunction with other information at a site, should lead to an improved ability to make quantitative evaluations and related decisions pertaining to site remediation. Site- and scenario-specific verification should be performed to the degree possible, as this will enhance the validity and credibility of any analyses that are performed.

A potential limitation of using either measured or modeled tissue metal accumulation levels is that the effects assessment requires the accumulation level to be related to a benchmark effect level. This can be problematic, especially when whole body tissue levels are involved, because it is the metal concentration at the site of action of toxicity that is most relevant. If the concentration at the proximate site of action of toxicity (e.g., a particular

organ, a cytosolic fraction, or an affected enzyme) is proportional to the whole body concentration, then this point is of lesser concern. However, if the concentration at the site of action is not proportional to the whole body concentration, then a more direct measure of the concentration of a metal at the site of action is expected to be more indicative of the potential for effects (e.g., Din and Frazier, 1985). More direct measurements might include organ-specific tissue metal levels or even measures of the intracellular distribution of a metal. Since measurements of this type are relatively costly and difficult to obtain in comparison to whole body measurements, use of this approach is most appropriate as part of a relatively high-tier assessment. As a result, while the literature related to characterization of intracellular metal distributions and how this may be related to effects is extensive (see Viarengo, 1989; Mason and Jenkins, 1995; Langston et al., 1998; and Rainbow, 1998, for excellent reviews on this subject), examples of the practical applications of such methods in the context of a site assessment have been limited to date (e.g., Harrison and Lam, 1982). Physiologically based toxicokinetic (PBTK) models, which are designed to evaluate organ-specific tissue levels, might serve as an alternative to measurement of organ-specific metal levels, though the application of PBTK models to metals has been limited to date (e.g., Thomann et al., 1994, 1997; Paquin et al., 2003).

A further complication associated with using total metal levels in tissues to predict effects is that, in addition to the concentration of metal that is present in the tissue, the rate of uptake of the metal should also be considered (Kraak et al., 1992; Roesijadi, 1992; Andres et al., 1999; Hook, 2001; Hook and Fisher, 2002). Effects related to metal accumulation are mitigated by the ability of some organisms to sequester metals that enter the cell (e.g., by inducing the synthesis of metallothionein [MT], or by incorporation of the metal into intracellular granules). 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 sufficiently high, the rate of synthesis and complexation capacity of the binding ligand (e.g., MT) will be exceeded, cytosolic levels of the reactive forms of the metal will become unacceptably high, and adverse effects could ensue.

There is considerable evidence in support of the idea that information on the form of the metal that is present in a tissue, MT complexes serving as just one important example, may be a more reliable indicator of the potential for effects than total metal concentration (Roesijadi, 1992; Mason and Jenkins, 1995; Langston et al., 1998). However, attempts to extend such techniques from laboratory test conditions to field conditions are limited in number, making it difficult to arrive at a definitive conclusion at this time about the potential for routine use of this approach..

In addition to sequestration by MT, metals may be incorporated within organo-phosphorus granules, thereby rendering them non-available to bind with other intracellular target enzymes (Coombs and George, 1978; George, 1982). While either of these methods of sequestration will lead to an increase in tissue metal levels, they do not necessarily result in adverse effects. Methods have been proposed for measuring the intracellular distributions of metal, and it is entirely plausible that such methods may prove useful in future assessments of the potential for effects (e.g., Winge et al., 1974; Brown and Parsons, 1978; Benson and Birge, 1985; Roesijadi and Klerks, 1989). As an example, one measurement scheme attempts

to operationally differentiate between the particulate fraction and the low molecular weight (LMW < 3000), intermediate molecular weight (IMW on the order of 10-12,000), and high molecular weight (HMW > 70, 000) of intracellular cytosolic fractions (Roesijadi and Klerks, 1989). The metal of the HMW fraction interacts with target enzymes and results in adverse effects (Winge et al., 1974; Brown and Parsons, 1978). While models that can be used to perform this sort of evaluation are currently under development, the ability of such models to be used as a tool in performing a meaningful effects assessment remains to be demonstrated. Measurement of intracellular speciation is probably limited to the highest risk assessment tiers.

2.8 Acclimation and Adaptation

The genetic makeup of an organism defines its ability to cope with environmental conditions. Genes can be expressed or remain "silent," and shifts in gene expression can occur when the environment changes. Furthermore, organisms use different portions of the total array of genetic information in different life stages. This shifting of tolerance within the genetically defined limit of the organism is *acclimation*; modification of the limits of an organism through changes in heritable genetic material is *adaptation* (Wallace and Srb, 1961). Physiological changes induced by acclimation may be reversed if the environment reverts to the original conditions. Tolerance acquired through physiological acclimation processes is not always passed on to offspring. However, the same genetic information that allowed acclimation to occur in the parents will be passed on so that the offspring will retain the ability to acclimate in a similar fashion. If the offspring develop in the altered environment, they will express the set of genes most appropriate for tolerance of those conditions.

Populations chronically exposed to metals often exhibit enhanced tolerance relative to unexposed or naive populations (Klerks and Weis, 1987; Klerks and Levinton, 1993). Genetic adaptation results from increased survival of tolerant genotypes and subsequent changes in gene frequencies. Genetic changes in aquatic populations exposed to metals may explain the greater tolerance of some organisms (Klerks and Weis, 1987; Benton and Guttman, 1992). However, linking these genetic changes to increased tolerance in the field and identifying the specific mechanisms responsible has proven challenging. Laboratory experiments conducted with F_1 generations obtained from metal-contaminated habitats provide the strongest evidence to support a genetic basis of tolerance (Klerks and Levinton, 1993).

Overall genetic variation increases if rare tolerant traits increase in frequency in response to selection (Posthuma and Van Straalen, 1993). Metal tolerance is a result of the influence of major genes for tolerance with initially low frequencies. The alleles of metal tolerance genes are present in the genome of the population mixed with non-metal tolerant genes. This state can be described as a heterozygous condition. There is reduced variation if selection ultimately drives the metal tolerance alleles to fixation, i.e., in small or non-isolated populations with random genetic drift or immigration from neighboring populations. When the alleles are driven to fixation, the genes for metal tolerance remain in the population without the old, non-metal tolerant alleles. The duration of selection influences the chance to show the opposite evolutionary responses towards non-tolerance. This is dependent on

whether or not alleles are driven to fixation, and can carry tolerance in the heterozygous conditions for long periods (Posthuma and Van Straalen, 1993).

Although there is considerable evidence that previously exposed populations may be tolerant to metals, establishing a clear determination of cost for physiological acclimation or genetic adaptation to contaminants has been elusive. Though induction of metal-binding proteins that increases tolerance to subsequent metal exposure uses metabolic energy, this does not necessarily translate to reduced growth, survival, or reproduction with demonstrable effects on the populations. Reduced genetic diversity has been reported in populations exposed to contaminants and may result in population bottlenecks. As tolerant genotypes are eliminated from a population, the reduced genetic diversity may increase the susceptibility of this population to other stressors.

2.8.1 Plants

A population of metal tolerant plants can emerge quickly, even within a single generation (Antonovics et al., 1971; also see the discussion in Section 0 above and references therein). This is particularly true for species producing relatively large numbers of seeds, even if the allele frequency for tolerance is quite low. Consider, for example, a plant living 10 years and producing 100 seeds per year. To maintain a constant population, only one seed would have to be established per plant. With 1,000 seeds over the 10-year life of the plant, a success rate averaging only 0.001 is needed to maintain the constant population. Viewed differently, this means that if one individual can establish new seedlings at a success rate of 50 percent, only one individual out of a population of 500 would be needed to maintain the population. In this simple example, a tolerance allele frequency of 0.002 would be sufficient to establish a tolerant population without any decline in numbers of individuals. An even lower allele frequency would be needed by plant species that also reproduce vegetatively by ramets.

Evidence of adaptation (convergent evolution) for metal tolerance comes from the fact that plants of diverse taxonomic relationships grow on soils high in metals. Metal tolerant floras have been described for soils high in zinc, nickel, chromium, and copper (Antonovics et al., 1971; Brooks, 1972). These reviews indicate that some species are restricted to the high metal soils, but other species exist across a broad concentration range. These represent differences in niche breadth (i.e., those restricted to high metal soils versus those occurring in soils that have either high or low concentrations of metals). Interference mechanisms (either competitive exclusion or allelochemic limitation) in the species with narrow niche breadth could accelerate the pace toward reproductive isolation and speciation.

Koch et al. (1998) studied the evolutionary history of *Thalaspi caerulescens*, a plant in Western Europe that has both metal tolerant and non-metal tolerant populations. Using an isozyme analysis, they showed the two different types were still the same species. This means that the different populations contained the same genetic information for tolerance, so the metal tolerant plants were not specially adapted. Traits of zinc tolerance and hyperaccumulation frequently are found in relatives of this species, which suggests this trait has been established and manifested in different populations from metalliferous sites for a long time (Koch et al., 1998).

2.8.2 Animals

Metal adaptation in plants often is accompanied by metal adaptation in animals (Posthuma and Van Straalen, 1993). Avoidance behavior has not precluded the occurrence of adaptation in animals. Selection for metal tolerance is expected to improve fitness in exposed conditions (Posthuma and Van Straalen, 1993). Metal exposure is regarded as a strong and stable selective force compared to other environmental parameters, which may affect both tolerance and life-history characteristics (Posthuma and Janssen, 1995). The role of genetic variation for evolutionary responses can be evaluated from the combination of heritability estimates for reference sites and evolved characteristics in metal-tolerant populations. Selection by metals appears to favor animals that grow fast, mature early, and have high excretion efficiency for cadmium (Posthuma and Janssen, 1995). Mechanisms that underlie toxicity of metals in soil have been identified in animals and may be common to other types of organisms (Van Straalen and Donker, 1994).

Metal tolerance also has been demonstrated in ticks and a fly species in response to the application of a metal-based pesticide. There is evidence for increased metal tolerance in other species, but acclimation and adaptation could not be distinguished (Posthuma and Van Straalen, 1993). Metallothionein is an induced metal-binding protein that exists in most tissues (Klaassen and Liu, 1998). Another mechanism used by invertebrates is concentrating metals in the exoskeleton, thereby losing the metals when they molt (shed their exoskeleton).

The first indications for divergence between populations usually are obtained when laboratory-reared strains of the same species, cultured under identical conditions, respond differently to exposure to metals (Donker and Bogert, 1991). This type of evidence has been obtained for *P. scaber* (Donker and Bogert, 1991). The results of the study showed that isopods from a mine site did not experience any toxic effect of Cd up to a 41 µg/g (dry weight) concentration in the food, while reference isopods decreased their growth rate when the food concentration exceeded 4.4 µg/g (dry weight). Isopods from the zinc (Zn) smelter site were stimulated by intermediary concentrations and did not grow well when the food did not contain cadmium (Donker and Bogert, 1991).

2.8.3 Microbes

There also is evidence that soil microbes adapt to increased concentrations of metals. Pennanen et al. (1996) studied phospholipid fatty acid (PLFA) composition of soil microbial communities. Increases in numbers of metal-tolerant bacteria, actinomycetes, and fungi have been seen as the result of elevated metal levels. The researchers studied two different metal concentration gradients, one around a smelter in Finland with a simple concentration pattern (mainly copper emitted) and one around a primary smelter in Sweden where a mixture of metals was emitted (Pennanen et al., 1996). The changes in the PLFA patterns at the two sites were similar, suggesting that the elevated metals had resulted in similar changes in the microbial community structure at both sites, despite the fact that different situations were present. This is an indication that the microbial community structure will change in similar ways because of the different metal concentrations in different coniferous forest soils (Pennanen et al., 1996). The trees also were visually affected near the Sweden smelter. The strong reduction in PLFA represented a decrease in the ratio of fungal to bacterial biomass.

Smelter emissions increased the copper (Cu) tolerance of the bacterial communities in both sites, and bacterial community tolerance was the most sensitive technique to detect elevated metal levels of the techniques used, compared to a thymidine incorporation technique (Pennanen et al., 1996). The increased metal tolerance of the bacterial community could be due to either an acquired tolerance by adaptation, a genetically altered tolerance, or a shift in species composition in which organisms already tolerant become more competitive and thus more numerous.

An increased tolerance to metals other than the principle contaminant (multiple tolerance) is a phenomenon reported several times (Pennanen et al., 1996). Using amended arable soil, others have revealed tolerance couplings for the bacterial community between Zn, Cd, and Pb, as well as Zn and Pb. In contrast, they did not find that Cu tolerance automatically led to community tolerance to other metals. Only Cu tolerances were found in this study, with no evidence of multiple tolerance to Cd, nickel (Ni), and Zn (Pennanen et al., 1996).

Copper tolerance of *Thiobacillus ferrooxidans*, a soil bacterium, also has been researched (Das et al., 1998). Copper tolerance by *T. ferrooxidans* is stress-dependent; in other words, the resistance to copper is induced in progeny after prolonged exposure to copper and is not a permanent trait of the adapted strain. In the absence of Cu, progeny of adapted strains lose their ability to tolerate Cu, and show that adaptation is possible in a single sub-culturing, provided the cells are able to oxidize all the ferrous iron in the medium. The researchers demonstrated changes in the cell surface properties that allow the bacteria to become more tolerant, namely the addition of proteins on the surfaces of Cu-adapted cells (Das et al., 1998).

Another study found that pre-exposure of *E. coli* to sub-lethal concentrations of cupric sulfate induced Cu tolerance (Rowbury and Hussain, 1992). The authors surmised that tolerance is not dependent on the selection of Cu-resistant mutants, but rather the result of a phenotypic change in the organisms.

2.8.4 Community Level

Most research examining tolerance to metals has focused on population-level responses, and few studies have examined consequences at the community level. As noted above, the most common explanations for increased tolerance of populations include acclimation and selection for resistant genotypes. These same intraspecific mechanisms also may account for increased resistance of communities to metals. However, because communities consist of large numbers of interacting species, it is likely that other mechanisms, unique to these systems, will contribute to increased tolerance. Pollution-induced community tolerance (PICT) has been proposed as an ecotoxicological tool to assess effects of contaminants on communities (Blanck et al., 1988). PICT is generally tested by comparing responses of communities collected from contaminated and reference sites. The replacement of sensitive species by tolerant species, termed “interspecific selection” (Blanck et al., 1988), is a common response in contaminated systems and one of the most consistent indicators of exposures to metals.

The increase in community tolerance at a contaminated site that results from the elimination of sensitive species is considered evidence that this restructuring was caused by the elevated metals. Proponents of the PICT argue that while differences in communities between field sites can be attributed to a large number of environmental factors in addition to contaminants, increased tolerance observed in communities is less sensitive to these confounding factors and is most likely a result of exposure to contaminants (Blanck and Dahl, 1998).

The PICT hypothesis was originally tested in marine periphyton, but community-level tolerance has also been measured in other aquatic communities. Protozoan communities developed under low levels of Zn stress were more tolerant of Zn than naive (e.g., unexposed) communities (Niederlehner and Cairns, 1992). Resistance to Zn in acclimated communities increased by >3X compared to naive (e.g., non-acclimated) communities. Tolerance of nematode communities collected from sediments along a contamination gradient increased with concentrations of Cu (Millward and Grant, 2000). Benthic macroinvertebrate communities collected from a site with moderate levels of metals were significantly more tolerant to subsequent Cd, Cu, and Zn exposure than those from pristine sites (Clements, 1999; Courtney and Clements, 2000). These differences were most likely a result of population-level (acclimation or adaptation) and community-level (interspecific selection) responses.

According to Gustavson and Wangberg (1995), the use of PICT as an ecotoxicological tool to assess impacts of contaminants at the level of communities is based on three assumptions:

- Sensitivity to contaminants varies among species.
- Contaminants will restructure communities, with sensitive species replaced by tolerant species.
- Differences in tolerance among communities can be detected using short-term experiments.

The first two assumptions are relatively straightforward and easy to verify with field sampling. The third assumption is more problematic and significantly constrains application of the PICT as an assessment tool. While tolerance at the population level can be assessed using a variety of species, logistical considerations will limit the types of communities where tolerance can be investigated experimentally. For obvious reasons, most of the original research on PICT has been conducted using small organisms with relatively fast life cycles.

The ability of organisms to acclimate or adapt to metal exposure complicates field assessments of effects. Predicting effects of acclimation or adaptation to effluents on field populations is complicated by the fact that these processes may be contaminant specific, even within classes on contaminants. There is theoretical support for the hypothesis that populations adapted to contaminants have higher metabolic costs (Wilson, 1988) or are more susceptible to other stressors; however, few empirical studies have demonstrated increased costs. Studies have also been conducted in which metal-resistant cell lines, genetically

selected for their ability to synthesize MT, grew at the same rate as metal-sensitive cells that were limited in their ability to synthesize MT (Hildebrand et al., 1979). It was also shown that the relative rate of non-MT protein synthesis by the Cd-resistant line was not altered when MT synthesis was induced by exposure to Cd (Hildebrand and Enger, 1980). These results suggest that the energy required for MT synthesis, at least in the case of these cells and under laboratory test conditions, is a relatively minor component of their overall metabolic needs.

2.9 Influence of Exposure Pathway on Toxicological Effect

The exposure pathway can be an important factor in determining the adverse effect that may be expected from an environmental exposure of a metal to an organism. Organisms potentially are exposed to metals through a variety of pathways throughout their lives. Many metals have multiple target organs and mechanisms of toxicity (Friberg et al., 1990a, b). Some exposure pathways do not exist at certain life-stages; for example, dietary exposure does not occur in developing embryos or early development stages of fish, and air exposures do not occur in aquatic stages of amphibians, but may occur in the adults. Other exposure pathways may be specific to a particular life-stage for physiological, developmental, or behavioral reasons. Understanding the relationships of exposure pathways and related adverse effects is complicated by the fact that many exposure pathways are specific to the chemical form of the metal involved as well as the exposure intensity. Therefore, exposure pathway specific toxic responses need to be evaluated, from multiple points of reference, rather than simply the exposure pathway.

Aquatic organisms may be exposed to metals through the gills or from their diets (Mount et al., 1994; Lanno et al., 1987; Julshamn et al., 1988; Cockell et al., 1991; Miller et al., 1993; Szebedinszky et al., 2001; Pedlar et al., 2002; Farag et al., 1994; Woodward et al., 1995). Quantitative determination of the level and consequences of dietary exposure has proven challenging. Nutritional quality between diets from field-collected invertebrates may mask metals' toxic effects. This variability may be reduced by rearing invertebrates in controlled laboratory conditions using different levels of contaminated sediments (Hansen et al., 2004). Dietary toxicity of metals might be enhanced when metals are naturally incorporated into biological tissues of prey items (e.g., bound to proteins).

Some examples of specific toxicological effects resulting from different exposure pathways include inhalation exposure to Al dust, which can result in fibrosis of the lung (Elinder and Sjogren, 1990), while dietary exposure to soluble Al can affect phosphate metabolism in the digestive track by binding with the phosphate. The latter mechanism is a possible explanation for depressed egg shell-breaking strength and the mechanism for the use of Al salts to induce forced molting in avian farming (Hussein et al., 1988).

Exposure to relatively high concentrations of dissolved forms of several metals to fish can result in damage to the gills and respiratory functioning, while exposures to lower concentrations or alternate exposure pathways (dermal adsorption and dietary exposure) may result in effects on other target organs such as the kidney (Sorensen, 1991). Dietary metal may affect toxicity across the gill in aquatic toxicity tests (Dang et al., 2001; Pyle et al., 2000).

2.10 Extrapolations

Many risk assessments require extrapolation of data to fit the specific features of a risk assessment. The following sections discuss issues pertaining to risk assessments of metals.

2.10.1 Among Chemical Forms

Knowing which form of an element is likely to be present is necessary for successful extrapolation of effect levels that have been characterized in the laboratory to those that are expected from one site to another under field conditions. Only a limited number of metal species are considered to interact at the biochemical/physiological site of action. Additionally, the fraction of the total metal that is present in these bioreactive forms may vary markedly with water quality, representing only a small fraction of the total concentration of metal that is present in some situations. As an example, for metals such as copper or silver, which may be present in the form of the free metal ion and as a variety of inorganic and organic complexes, or metal species, not all forms are of equal importance when assessing the potential for a given total metal concentration to result in a toxic effect. The free metal ion, such as Cu^{2+} or Ag^{+}, is typically viewed as being the principal bioreactive form, though it is not necessarily the only bioreactive form (Campbell, 1995). Depending on conditions at a specific site, these reactive metal fractions may vary widely, representing from less than 0.1 percent to more than 50 percent of the total concentration of the metal that is present. The form of the metal is also known to influence uptake and accumulation of a metal by an organism, whether the route of exposure is via waterborne metal (via the gill or other exterior surfaces) or via dietary metal (via the gastrointestinal tract) (U.S. EPA, 2004a, 2004b). Once a metal has entered the organism, it will also be present in a variety of forms. This leads to a continuing need to consider the form of the metal that is present within the organism when assessing the potential for effects (e.g., Winge et al., 1974).

Computational models (e.g., the aquatic biotic ligand model [aBLM]) were developed specifically for assessing effects of metals under acute exposures in aquatic systems (U.S. EPA, 1999a). The aBLM is based on principles of the FIAM, a conceptual framework that was first formally described by Morel (1983). The first practical application of the FIAM concepts was performed by Pagenkopf and coworkers, who used the GSIM to interpret toxicity data for metals (Pagenkopf et al., 1983). The aBLM considers:

- Total metal concentration in the water taking into account chemical speciation of the metal in the water (*exposure*, including complexation of the metal by inorganic and organic ligands).

- The interactive effects of the bioreactive forms of the metal (e.g., the free metal ion and perhaps other metal species) with cations that are in competition with the metal ions for interaction at the site of uptake or toxicity (in combination, concentration, complexation, and competition control a metal's *biological availability*).

- The actual *accumulation* of metal by the organism at the site of action of toxicity.

Playle and coworkers conducted a series of experiments that demonstrated how complexation and cation competition actually affect metal accumulation at the gills of fish, using chemical equilibrium calculations to simulate these interactions (Playle et al., 1992, 1993a, 1993b; Janes and Playle, 1995).

Each of these aspects (*exposure*, *bioavailability*, and *accumulation*) is discussed in detail in the companion issue papers. The essence of the FIAM as well as the aBLM, however, is that it is not the form of the metal in the water that is directly related to a fixed effect, but the level of metal accumulation at the site of action of toxicity. Pursuant to this idea, Di Toro and coworkers (2001) extended the earlier results such that the focus was on the evaluation of the biotic ligand accumulation level and the associated dissolved effect level. In the context of the aBLM, this site of action of toxicity is represented in the same way as any other dissolved ligand that is present, as a reactive biotic ligand that has a prescribed concentration (i.e., binding site density) and metal binding strength (characterized by a Log K value) that control the level of accumulation of the metal at the biotic ligand. The computations that are performed are entirely consistent with those performed by any of a number of chemical equilibrium programs (e.g., MINTEQ, MINEQL, etc.), with the key output being the concentration of the metal-biotic ligand complex (M:BL). The M:BL concentration is key, because this accumulation level at the proximate site of action of toxicity is assumed to be uniquely associated with a fixed biological effect (U.S. EPA, 1999a; Di Toro et al., 2001). While the requisite chemical speciation calculations in prior aBLM applications have often been performed by the CHemical Equilibrium in Soils and Solutions model (CHESS) (Santore and Driscoll, 1995), and they employ metal-organic matter interactions patterned after those developed for the Windermere Humic Aqueous Model (WHAM) (Tipping, 1994), this is not a necessary requirement for use of the BLM approach (e.g., McGeer et al., 2000). Despite the significant advances in this area, the aBLM may overestimate the potential for toxicity under some field conditions, since it does not take into account the release of organically enriched exudates (so-called living ligands) by some organisms, a process that increases the degree of metal complexation and hence decreases metals' bioavailability (Janssen et al., 2003).

Assessing the potential adverse effects of metals in sediments is also challenging. A common approach used for both organic chemicals and metals has been to compare bulk sediment concentrations (a similar discussion applies to soils) to threshold effect levels (TELs) and probable effect levels (PELs) (U.S. DOC, 1991). Similar bulk sediment concentrations are associated with a wide range of effects, depending on the sediment that is considered (Di Toro et al., 1990, 1992; Ankley et al., 1991, 1993; Berry et al., 1996; U.S. EPA, 2000). Equilibrium partitioning methods, which consider the chemistry and partitioning of organic chemicals and metals in aquatic sediments, have been developed to address the limitations associated with using bulk sediment chemical concentrations (Di Toro et al., 1991; Ankley et al., 1996; Di Toro et al., 1999; U.S. EPA, 2000). Acid volatile sulfide (AVS, measured with a standard cold acid extraction) is the principle binding phase for the simultaneously extracted metals (SEM, including Cu, Cd, Ni, Pb and Zn). The fraction of the SEM metals that react with the AVS are reduced in bioavailability and therefore exert less toxicity than they would otherwise. Hence, the original approach was to determine the molar ratio of the concentration of SEM/AVS; if it was less than unity, adverse effects were unlikely to occur. It was concluded that adverse effects may result from SEM/AVS >1, but in

this case the presence of other binding phases would determine if this was in fact the case. This is because these other binding phases would complex a portion of the excess SEM, thereby helping to maintain relatively low levels of the free metal ion in the sediment pore water.

The underlying conceptual approach of the AVS and SEM paradigm is similar to the aBLM, though the level of detail that is required to represent the order one chemical interaction of metals with AVS is considerably less than is called for in the BLM. Additionally, no attempt is made to evaluate an accumulation level at the site of action of toxicity. More recently, in an effort to further refine the AVS approach for metals, the method has been modified to consider the carbon normalized difference of SEM and AVS, i.e., $(SEM – AVS)/f_{oc}$, where f_{oc} is the organic carbon fraction of the sediment (U.S. EPA, 1999a, 2000). Here, if the carbon-normalized ratio is less than about 130, the potential for effects is considered mitigated, if not entirely eliminated. This consideration of f_{oc} serves as an initial refinement to the original AVS and SEM paradigm. It is adopted as a way to consider the effect of another important binding phase (i.e., sediment organic matter) on metal availability, in situations where SEM is present in excess of AVS. Silver was also added to the list of SEM metals in this latest implementation of the approach, and Cr is undergoing active consideration for inclusion as well (Berry, 1999; Berry et al., 1999a, 1999b; U.S. EPA, 2000).

Most recently, an approach for development of an aquatic sediments BLM (sBLM) has been proposed (Di Toro et al., 2004). The idea would be to develop a version of the aBLM that would incorporate a more detailed representation of pore water chemistry in an effort to more accurately evaluate the potential for effects in situations where SEM exceeds AVS, or where the carbon-normalized ratio of $(SEM – AVS)/ f_{oc} > 130$.

However, in some cases in which AVS was present in excess of SEM, such that bioavailability would be expected to be minimized, metal accumulation has still be observed. This would seem to be a clear indication that the metals were in fact available to the benthic invertebrates. The AVS model was mostly validated with laboratory experiments where the acute and chronic toxicity of bivalent metals (cadmium, copper, nickel, lead, and zinc) was predicted. In the field, studies of Warren et al. (1998) and Hare et al. (2001) showed that the AVS model, which considers only sedimentary metals, was more effective in predicting cadmium concentrations in pore waters than those in most animal taxa studied. In fact, at negative (SEM-Cd)-AVS, there was a concentration-dependant increase of cadmium levels in several aquatic invertebrate species, which is in disagreement with the model. This result arises in part because invertebrates studied in their experiment were not exposed to bulk anoxic pore water. Most of the invertebrates studied can irrigate their borrows with oxygenated water, which is pumped from the overlying water (Wang et al., 2001). In classic sediment toxicity tests, the AVS model likely predicts metal accumulation in all invertebrate species because the overlying water may be contaminated by upward diffusion of metals from the contaminated sediments, or simply because tested organisms do not irrigate their tunnels. Hence, the AVS model can be used for invertebrates that cannot irrigate their tunnels (e.g., Tubificidae) and are exposed to anoxic pore water.

It should be recognized that a potential complication with application of the SEM/AVS approach is that conditions at the sediment water interface may vary over time, leading to potential changes in metal bioavailability and toxicity. Hence, guidelines have been developed to facilitate sampling to be performed during what would be considered relatively critical conditions or times (U.S. EPA, 1999a). This complication is not limited to the SEM/AVS approach alone, as effect levels that have been evaluated based on bulk sediment metal concentrations would also be expected to vary in a similar manner over time.

The approach of using the aBLM in aquatic settings has had broad appeal, and many industry- and regulatory agency-supported efforts are in progress to further its development. (See Paquin et al., 2002a, for a detailed overview of the historical development of the BLM and Gorsuch et al., 2002, for a compilation of manuscripts that describe many of the more recently completed and ongoing efforts). More recently, industry-sponsored efforts have been initiated to extend the applicability of this general conceptual approach to terrestrial settings, by the development of what has been referred to as a terrestrial BLM, or tBLM. The conceptual approach is very much the same as for the versions of the BLM that have been developed for aquatic settings, but in this case the somewhat different conditions that control metal speciation, bioavailability and effects that are prevalent in unsaturated soils need to be addressed (Allen, 2002). Experimental testing and model development programs are under way in the hopes of providing a tool that will be of great practical utility in the coming years.

2.10.2 Among Species

The selection of species used in the derivation of water quality criteria for metals is based on historical and practical factors. Consequently, the list of species typically used in routine laboratory toxicity tests is only a small subset of species that are potentially exposed to contaminants in nature. Because the selection of test species is not a random subsample of all species, extrapolation among species is potentially one of the greatest sources of uncertainty when assessing the toxicological effects of metals.

Responses of aquatic organisms to metals vary significantly among taxonomic groups (Suter and Rosen 1986; Suter et al., 1983; Barnthouse et al., 1986; Doherty, 1983; Volmer et al., 1988). It is well established that mayflies (Ephemeroptera), caddisflies (Trichoptera), and stoneflies (Plecoptera) are relatively sensitive to organic enrichment, whereas some chironomids (Diptera) are generally tolerant. While most mayflies are generally sensitive to metals, caddisflies and many stoneflies are relatively tolerant (Clements et al., 1992). In fact, these species-specific differences in sensitivity to contaminants have motivated the development of numerous indices of water quality based on composition of benthic communities (Hilsenhoff, 1987; U.S. EPA, 1989; Barbour et al., 1992). Because responses of aquatic organisms to chemical disturbances are usually contaminant-specific (Slooff, 1983), it may be difficult to assign causality when more than one contaminant is present, as is typical of field assessments. In such cases, biotic indices should be used cautiously. In some situations, it may be necessary either to develop chemical-specific indices based on sensitivity to specific classes of contaminants or to calibrate metrics used in individual field assessments.

Because aquatic organisms differ greatly in their sensitivity to contaminants, natural variation in community composition and abundance of dominant taxa between locations will influence results of field assessments. For example, metals discharged into a system dominated by highly sensitive taxa (e.g., heptageniid mayflies) will have greater effects than the same effluent discharged into a system dominated by tolerant organisms (e.g., chironomids). Kiffney and Clements (1996) showed that benthic communities from headwater streams were more sensitive to metals than communities from mid-elevation streams. These results show that criteria protective of mid-elevation streams may not be protective of headwater streams.

The derivation of WQC involves the use of species sensitivity distributions (SSD), a method that provides a probabilistic characterization of variation in species sensitivity. The acute and chronic WQC are then derived on the basis of the 5 percentile level of this SSD. The draft copper WQC document, which makes use of the aBLM, does so by evaluating a lethal accumulation level (i.e., an LA50) for each of the organisms in the species sensitivity distribution. These results are in turn used to evaluate an LA50 for an organism that is sensitive at the 5-percentile level. This 5-percentile LA50 is in turn used to evaluate a BLM-based acute WQC for a given set of water quality characteristics.

2.10.3 Among Test Methods

The papers on chemical speciation, exposure, and bioavailability describe many parameters that influence the magnitude of concentration-response reactions observed in toxicity tests or in field conditions. Consideration of these parameters is important in higher-tier risk assessments. Though many parameters are understood to some level, control of each in experimental conditions can be extremely difficult and rarely are they addressed in toxicity test designs. Therefore, these issues are addressed only qualitatively in the uncertainty sections of risk assessments.

Questions of toxicity test validity arise from a number of angles. In aquatic tests, the routes of exposure (e.g., food versus dermal absorption) have shown important differences that can be controlled in test designs. However, analogous comparisons for terrestrial systems are lacking. For example, in assessing the risk of industrial emissions, what is the relevance of toxicity data obtained from studies that used soluble salts added to sediment, soil, or food? Is there a reasonable way to extrapolate concentration response relationships from tests that used metal halides to expected field conditions where the constituent of concern is likely to exist as metal oxides, metal sulfates, metal phosphates, or organo-metal complexes? In feeding studies, what are the differences in concentration response relationships between studies in which the test substance was blended into feed versus one in which the test substance was incorporated into the tissues (e.g., foliage, larvae) of food items? Unfortunately, to our knowledge, there has not been a comprehensive comparative study performed to establish how these differences would alter the observed responses. Therefore, current practice cannot account for many important chemical speciation issues.

Predictive models derived from physico-chemical properties that quantify the reactive fraction of metals in soil generally fail to account for the non-equilibrium conditions that prevail when organisms are introduced to the systems, especially at levels where the metals

cause toxicity. Currently, the best we can do regarding predictions of toxicity from soil metal concentrations is to develop descriptive statistical models that are valid for the particular soil type (defined by parent material and texture), within specified soil parameters (e.g., organic matter content, pH). Such descriptive statistical models generally do as well or better using total metal levels than using various measures or estimates of *exchangeable*, *extractable*, or *bioavailable* fractions. It remains an open question whether successes seen in aquatic systems will be realized in sediment or soil matrices. Perhaps the efforts to develop a terrestrial biotic ligand model (discussed above) will improve upon this situation.

There are other major impediments in risk assessments of metals stemming from test limitations. These include a disconnect between theoretical or predictive chemistry and observations of effects; the generally low statistical power of most standardized test designs; and the general reluctance to move from ANOVA-based study designs to regression designs.

Partly related to the complexities of soils and sediments that have so far frustrated predictive modeling, experimental designs that seek to establish concentration-response relationships require extraordinary procedures. The controlling feature of concentration-response relationships is the link between the substance of interest in the test matrix and the test organism. Intuitively, as the quantity of the test substance increases in the test unit, there should be a corresponding increase in exposure, resulting in a proportional response (effect). That intuition, however, derives from experience with aquatic tests, where experimentally, the test substance is diluted to desired concentrations. “Dilutions” using soil or sediment often do not result in changes of concentration at the scale that matters to the test organism. To illustrate the point, consider two experiments, one having constant soil mass of 500 g with the concentration of metal from 100 units to 25 units and another having a corresponding decrease in soil mass (Table 2). Though it would seem logical that the first experiment, with decreasing metal concentration, should yield better survival or growth of test organisms, the organisms may respond solely to the initial concentration (i.e., 100 units). This is because the mixture behaves differently than a homogeneous solution. Consequently the roots, the hyphae, the enchitrids, or other test organisms may experience identical (i.e., undiluted) exposure levels. Alternatively, differences across treatments may be due primarily to differences in the volume of the test matrix. To overcome such problems, it is necessary to have nearly identical starting soil, ensure that the test substance is truly integrated uniformly throughout the test matrix, not merely blended, and provide an adequate period for aging of the metals in the new test matrix.

Table 2. Illustration of Two Experiments Using Soils or Sediments That May Not Reflect Concentration-Responses Expected in a "Dilution" Series

Treatment	I	II	III
Experiment 1	500 g @ 100 Me mg kg^{-1}	250 g @ 100 Me mg kg^{-1} 250 g @ 0 Me mg kg^{-1}	125 g @ 100 Me mg kg^{-1} 375 g @ 0 Me mg kg^{-1}
Experiment 2	500 g @ 100 Me mg kg^{-1}	250 g @ 100 Me mg kg^{-1}	125 g @ 100 Me mg kg^{-1}

To address concentration response relationships of a metal in a specific soil type, two alternative approaches can be successful. A bulk quantity of a soil may be separated into aliquots and to this, specified quantities of the test metal may be introduced using precautions to use appropriate carriers *and* establishing an aging/weathering regime that allows the test metal to become incorporated into the soil matrix (Van Assche et al., 2002). Alternatively, one can collect site soils (taking precautionary steps to ensure that samples are collected from the same soil type) that are suspected to have a range of concentrations of the test metal. The concentration of metal is then determined for each sample, and post-classification of samples may be required to handle outliers (e.g., different texture, organic matter, pH, co-contaminants, etc.). Regression analyses can then be used to describe the concentration-response relationship. The particular statistical model used to establish the relationship is dictated by the experimental design and the descriptive characteristics of the data set. It may be necessary to employ weighting factors for co-variants, transformations, principal components, or other statistical modeling techniques to achieve maximum understanding of the relationships.

Much of the toxicity literature is dominated by studies that used an ANOVA design intended to identify NOAECs and LOAECs. These studies had relatively few concentrations, low statistical power, and usually are not conducive to compute regression lines across the concentration ranges of interest. Only recently have there been concerted efforts to encourage toxicity testing using experimental designs intended to characterize the full spectrum of concentration-response relationships (Stephenson et al., 2000; Environment Canada, in review). Having the full response surface is critical as the rate of response across a concentration gradient may be more important in risk assessment than knowing the threshold.

Moreover, unraveling relationships of metals ultimately may require knowledge of the changes across response surfaces defined by several parameters. These at a minimum include interactions with pH, organic matter, and clay content, but also may require expansion to include other metals. Such information can only come from carefully constructed studies using regression designs as recommended by Stephenson et al. (2000).

2.10.4 Among Direct Endpoints

Responses of communities and ecosystems to contaminants may be divided into two general categories: structural and functional. Structural endpoints typically include changes

in abundance, species richness, and community composition. In contrast, functional endpoints focus on ecosystem processes, such as the rates of primary productivity, nutrient cycling, energy flow, and decomposition. Ecosystem level processes are closely linked to structural characteristics, and experimental research has demonstrated a relationship between structural characteristics such as biodiversity and ecosystem function (Tilman and Downing, 1994). Results from model ecosystem experiments showed that reduced species diversity in an ecosystem due to anthropogenic disturbance may have important consequences for ecosystem function (Naeem et al., 1994). We often measure structure as a surrogate of function under the assumption that the system has functional redundancy. However, this is not always the case (Chapman et al., 2003), and thus we should be wary of making errors as a result of such assumptions.

One of the key advantages of functional measures is that they integrate responses of component populations. However, because of functional redundancy, lower sensitivity, and high variability, there is some evidence that functional measures may not respond to subtle perturbations (Schindler, 1987; Stay et al., 1988). In studies conducted in the Experimental Lakes Area, Schindler (1987) concluded that functional measures were relatively insensitive to acidification, and that shifts in community composition were better early warning signs of ecosystem stress. In contrast, Hill and coworkers (1997) reported that sediment microbial community respiration was a sensitive indicator of exposure to metals in the Eagle River, Colorado.

Despite the uncertainty in the literature concerning the usefulness of structural or functional responses in assessing effects of metals, these measures are so intimately related that a distinction between them is somewhat arbitrary. Lamont (1995) describes an approach to test the effects of structural responses on ecosystem function. Newman et al. (1987) reported that reduced litter processing in experimental streams dosed with chlorine resulted primarily from the elimination of shredders: organisms that break down leaf litter. Changes in community composition, such as elimination of grazers, may have cascading effects on primary productivity and contaminant transport. Wallace et al. (1987) showed strong agreement between structural indices and ecosystem processes such as leaf decomposition and secondary production. A holistic perspective of ecotoxicology requires that, where possible, we measure structural and functional endpoints simultaneously.

Identifying direct structural and functional responses to metals would greatly facilitate field assessments of metal contamination. Early work by LaPoint et al. (1984), and more recently by Rapport et al. (1998), shows that ecosystem indicators respond to stresses in similar ways. Characteristics of the ecosystem distress syndrome (Rapport et al., 1998) include changes in nutrient cycling, primary productivity, species diversity, species composition (sensitive species replaced by tolerant species), and size composition. Howarth (1991) also reported similar responses to contaminants among different ecosystems in how they respond to contaminants. Although some ecologists will be reluctant to embrace Rapport's ecosystem stress syndrome, identifying common responses to metals has advantages. Because it is impractical to measure every possible indicator of exposure to metals, characterizing general patterns of responses to disturbance across a diverse array of ecosystems and disturbance types is essential.

2.10.5 Indirect Effects of Metals and Species Interactions

The focus in aquatic and terrestrial ecotoxicology over the past 30 years has been on understanding and predicting the direct effects of contaminants on organisms, with a few notable exceptions (e.g., Winner et al., 1980). Understanding the effects of contaminants on species interactions has been largely ignored. However, considerable research in basic ecology has been devoted to understanding the relative importance of competition and predation, and many ecologists recognize the importance of species interactions (e.g., predation and competition) in structuring communities (Connell, 1961; Dayton, 1971; Menge and Sutherland, 1987). The lack of information on effects of metals on species interactions is surprising, given the prominent role that research on species interactions has played in basic aquatic ecology. Furthermore, the inability to detect effects of contaminants on species interactions is cited as a major limitation of single species toxicity tests (Cairns, 1983). Some evidence indicates that effects of contaminants on species interactions may occur at lower concentrations than those eliciting direct toxic effects. Sandheinrich and Atchison (1989) reported that exposure of bluegill to sublethal levels of metals significantly reduced prey detection and capture. Clements et al. (1989) reported that filter-feeding caddisflies (Trichoptera: Hydropsychidae) were relatively insensitive to low-levels of Cu in experimental streams. However, susceptibility of these animals to predation by stoneflies increased when both groups were exposed to metals. Alternatively, some studies have led to conclusions that single-species toxicity tests were protective of community endpoints (Arthur, 1988).

Results of experiments designed to assess the effects of contaminants on species interactions indicate that exposure to contaminants may either increase or decrease intensity of species interactions. Warner et al. (1993) found that interspecific competition between anurans was influenced by acidification. Dunson and Travis (1991) observed that competition for food between killifish was influenced by salinity. Clements et al. (1989) reported that susceptibility of net-spinning caddisflies (Trichoptera: Hydropsychidae) to stonefly (Plecoptera: Perlidae) predation increased in streams dosed with copper compared to control streams. Finally, Wipfli and Merritt (1994) reported that reduced black fly density in a larvicide-treated stream altered species interactions and indirectly affected community structure.

In the field, the effects of contaminants on species interactions may vary depending on the level of the stress and the relative sensitivity of predators and prey. Menge and Sutherland's (1987) model of community regulation predicts that the relative importance of species interactions decreases with increased environmental stress. Menge and Olson (1990) predicted that relative sensitivity of predators and prey to stress would influence the outcome of predation. If predators are more sensitive to stress than their prey, the consumer stress model predicts that predation rates will be lower in high stress environments. This pattern has been observed in rocky intertidal habitats subjected to extreme wave action (Menge and Sutherland, 1987), high gradient streams (Kiffney and Clements, 1996), and acidified lakes (Locke and Sprules, 1994). In contrast, if prey were more sensitive to stress, the prey stress model predicts that predation rates will be greater in stressful environments. This pattern was observed in predator-prey interactions between stoneflies and caddisflies (Clements et al., 1989; Kiffney, 1996; Clements, 1999).

The lack of studies on effects of contaminants on species interactions is most likely related to the difficulty in conducting the necessary field experiments. Separating these effects in the field requires demonstrating that species interactions are important, and then demonstrating that these interactions are affected by contaminants. For example, by conducting caging experiments in reference and contaminated locations, one could evaluate the effects of predation on both communities. The best examples of studies documenting the role of species interactions in aquatic ecosystems have been experimental studies, where abundance of one or both species is manipulated directly. If predation effects were greater in the contaminated sites, this would support the hypothesis that contaminants increase the susceptibility of prey organisms to predation. Alternatively, microcosm experiments using communities obtained from reference and contaminated locations could be compared to measure effects of contaminants on species interactions. In experimental streams, Clements (1999) measured effects of stonefly predation on benthic invertebrate communities obtained from reference and metal-contaminated locations. Results show that communities from contaminated sites were more susceptible to stonefly predation than were communities from reference sites.

Understanding indirect effects of contaminants may help reconcile differences between laboratory and field responses. For example, loss of a particular species from a system may not be a direct result of toxicity, but rather caused by loss of a prey resource or increased susceptibility to predation. Indirect effects have been demonstrated in aquatic ecosystems where addition or removal of top predators has cascading effects on planktivorous fish, zooplankton, and algae (Carpenter and Kitchell, 1993).

2.10.6 Across Ecological Levels (Cascade Effects)

Effects of contaminants on aquatic organisms are manifested at all levels of organization, from molecules to ecosystems (Adams et al., 1992; Clements, 2000), and ecotoxicologists have become increasingly aware of the need to measure a diverse array of endpoints to assess ecologically significant effects. The rationale for this approach is that indicators at different levels of biological organization provide different types of information necessary for ecological risk assessment. Responses at lower levels of organization are often specific to a particular contaminant (e.g., metallothionein induction and metal exposure) and generally have a well-understood mechanistic basis. In addition, some endpoints at lower levels of organization may be linked to exposure (e.g., chemical residues and metabolites) and are direct measures of contaminant bioavailability.

Despite our greater mechanistic understanding of molecular and biochemical indicators, the ecological consequences for populations and communities of most biochemical, physiological, and individual responses have not been demonstrated. This is especially true for residue levels and metabolites, which are excellent indicators of exposure, but poor indicators of ecological effects. Consequently, some researchers have argued that responses at higher levels of organization (populations, communities, and ecosystems) measured in the field are more ecologically relevant than traditional toxicological indicators (Cairns, 1983; Clements, 2000). The emerging field of ecotoxicology generally emphasizes effects of contaminants on populations, communities, and ecosystems. However, despite their greater ecological relevance, responses at higher levels of organization are more complex and

often lack mechanistic explanations. Thus, establishing a cause-and-effect relationship between stressors and responses at higher levels of organization is not straightforward. Procedures using weight-of-evidence criteria are used in lieu of cause-effect relationships to explain ecological responses to agents (U.S. EPA, 2002c). The challenge is to narrow the list of candidate measurements to those that are most logically relevant to ecological effects.

2.10.7 Laboratory to Field

The greatest source of uncertainty in assessing effects of metals is the extrapolation of laboratory results to the field. This uncertainty can result in either overestimates or underestimates of field effects. For example, concentrations of dissolved organic materials (DOM), particulates, and other constituents that reduce metal bioavailability are likely to be greater in the field than in the laboratory. This is because laboratory dilution water must contain very low DOM in order to meet test method specifications. Often laboratory tests are designed to eliminate the influence of ligands in order to evaluate toxicant effects at the greatest bioavailability level.

Differences in physicochemical characteristics between the laboratory and field should be considered when predicting field effects based on results laboratory toxicity tests (Hansen, 1989; Winner et al., 1975; Janik et al., 1982; Geckler et al., 1976). The use of hardness-adjusted water quality criteria is one way in which laboratory effect levels have been modified to reflect site-specific water quality characteristics. Recognition that water quality characteristics other than hardness were also important factors that modified metal bioavailability led to EPA's adoption of the water effect ratio (WER) methodology as a more refined approach to deriving site-specific WQC (U.S. EPA, 1994a). Most recently, the BLM has provided a chemistry-based approach for evaluation of site-specific WQC based upon a chemical characterization of the water of interest (U.S. EPA, 2003).

While consideration of these physicochemical factors has improved our ability to predict effects of metals in the field, ecotoxicologists have made considerably less progress accounting for biotic factors that determine effects in the field. As described above, acclimation or adaptation may increase the tolerance of organisms to metals, resulting in differences between field and laboratory responses. Predicting the consequences of acclimation or adaptation will be complicated if these responses result in greater susceptibility to other stressors.

Aquatic organisms in the field are exposed to metals from both aqueous and dietary sources. Because laboratory toxicity tests focus primarily on aqueous exposure, dietary effects of metals generally are not considered. Organisms in the field will be exposed to higher levels of metals because of dietary uptake; however, there is considerable uncertainty over the relative importance of dietary effects of metals.

Organisms used in laboratory toxicity tests are generally not exposed to starvation, disease, or other natural stressors. If these natural stressors increase sensitivity to contaminants, it is likely that populations in the field will show greater effects than predicted by laboratory toxicity tests using healthy organisms.

Differences in the sensitivity or susceptibility of natural populations will complicate extrapolation from the laboratory to the field. Some populations and communities are inherently more sensitive to stressors than others. If some communities are inherently more fragile than others, identifying characteristics that increase fragility and the mechanisms responsible are important areas of research. Howarth (1991) speculated that communities with fewer opportunistic species, lower diversity, and closed element cycles would be sensitive to contaminants. Kiffney and Clements (1996) speculated that the greater effects of metals on benthic communities from small headwater streams compared to similar communities from larger rivers resulted from abundance of small and highly sensitive early instars in the headwater streams. Alternatively, resilience (e.g., the rate of recovery from disturbance) of larger streams may be greater because of a larger pool of potential colonists.

2.10.8 Acute to Chronic

An important question to be addressed in performing a risk assessment for metals is how to evaluate the potential for effects due to longer-term chronic exposures to metals. The BLM approach that was described previously has been developed specifically for application to acute toxicity. While methods to be discussed below are directed at application of a similar approach to assess chronic toxicity, these methods are at present still in the developmental stages. What approach may be adopted in the interim? EPA guidelines outline procedures for evaluating chronic water quality criteria, including minimum data requirements and the like (U.S. EPA, 1985a). While the method is for the most part very similar to the approach used to evaluate acute WQC, a common problem is that the requisite data are not typically available for use. In lieu of this, the recommended method is to employ an acute-to-chronic toxicity ratio (ACR) to modify an acute WQC to a chronic WQC.

While this approach offers a workable solution, it does not address the need to evaluate a chronic WQC on a site-specific-basis. Methods are under development by EPA to incorporate the BLM into updated WQC for metals (the copper WQC is the prototype), and once completed, the procedure should provide a way to evaluate a site-specific acute WQC—one that reflects the consideration of site-specific chemistry as determined with the BLM (U.S. EPA, 1999b, 2002b). However, this method will not initially offer a way to evaluate a site-specific chronic WQC. With regard to the chronic WQC, the envisioned short-term approach is to apply an ACR to adjust from a site-specific acute WQC to a site-specific chronic WQC. This procedure offers an expedient and practical alternative, one that is consistent with the application of an ACR to an acute WQC to evaluate a chronic WQC. This method is already used in the absence of the requisite chronic data that would be needed for the direct derivation of a chronic WQC (U.S. EPA, 1985a; U.S. EPA, 1994b). Sources for data to be used in these evaluations for aquatic settings are the EPA WQC documents for metals; the European Union has also compiled selected data as part of the EU risk assessments for metals that are under way.

During acute exposures, it is not clear that tissue accumulation levels will have sufficient time to increase to internal levels that will result in adverse effects, as lethality will often intervene in advance of the onset of these other chronically induced effects. However, during relatively low-level, longer-term exposures, organisms may accumulate tissue metal levels in internal organs, and the potential for a wide variety of effects to be manifested

increases. While it is less clear that models such as the BLM are of direct applicability in such cases, the analogous approach has been applied to data from chronic exposures with some success (e.g., de Schamphelaere and Janssen, 2002; de Schamphelaere et al., 2002).

As a minimum, one would expect that a chronic BLM should be a viable approach to the degree that longer term chronic effects may be related to the effects of chemical speciation on metal bioavailability. Since the BLM considers metal speciation in the abiotic environment, and the concentrations of the biologically available metal forms in the abiotic environment may vary widely, such an approach should at least be able to reflect such differences in any attempt to predict chronic effect levels. This approach might be difficult in situations where the kinetics of uptake and accumulation become important, and where the internal concentration at some yet-to-be-determined site of action of toxicity is not necessarily directly related to the external metal concentration. However, it is not clear at the present time how much of a problem this will be, and further testing of the chronic BLM approach will be required before a firm conclusion can be drawn in this regard.

Another approach for extending the applicability of the BLM to chronic toxicity is use of the Ion Balance Model (IBM) (Paquin et al., 2002b). The IBM uses the BLM to predict accumulation levels at the biotic ligand, and then explicitly represents the degree of the physiological response of the organism to metal exposure over time (i.e., disruption of ionoregulation, and gradual loss of plasma sodium). While the approach, which was initially applied to silver, may ultimately provide a way to predict effects due to metals over varying exposure durations, further development and testing is required.

2.10.9 Domestic Animals vs. Wild Animals

The toxicological data used to assess the risk of many metals to wildlife is largely based on test animals (e.g., laboratory species such as rats or mice) or domestic species (e.g., cattle, chickens) exposed to soluble metal salts. Extrapolating the results of such tests to evaluate toxicity to wildlife is necessary because of the paucity of data on the toxicity of metals to wildlife. However, extrapolation of results should be approached with caution due to the large amount of uncertainty they could introduce into the risk assessment process.

Estimating exposure levels can be exceptionally difficult for wildlife due to their uneven use of their local environment. Foraging patterns for many birds and mammals change with season, age, and reproductive status. To the extent that different forage items may carry markedly different metal concentrations, such changes in wildlife use patterns can have significant influence on the magnitude of risk expected through dietary exposures. Considerations of wildlife use patterns become more important in higher tiers of the risk assessment continuum. Several groups are making progress in this area with the use of

spatially explicit risk assessments (see Kapustka, 2004; Kapustka et al., 2001, 2004; Shumaker, 1998).[5]

Laboratory and domestic species may be more or less sensitive to chemicals than the selected receptor. This is due to differences in physiology, especially gut physiology, but also because of differences in methods of sequestration/excretion of metals, such as metallothionein induction, renal excretion rates, or egg production. These differences place substantial constraints on which types of wild animals can be modeled from existing laboratory and domestic animal data. For example, mammal studies should not be extrapolated to birds and rats to ruminants would require much larger uncertainty factors than rats to canids, and so on. Due to the often-large differences in sensitivity among species, interspecies extrapolations are one of the largest sources of uncertainty in effects (Suter, 1993). And in the case of metals, where some species are able to regulate or store metals residues in their tissues without experiencing toxic effects (i.e., biota specific detoxification), extrapolations between species used to assess metal bioaccumulation and toxicity can be especially problematic.

Methods for extrapolating effects-data among species are not unique to metals risk assessment. Some of the methods for extrapolating effects-data among species include:

- Use of uncertainty or safety factors to adjust for uncertainties associated with interspecies extrapolations (Calabrese and Baldwin, 1994; Calabrese, 1985; Chapman et al., 1998, for example). This approach has been commonly used in human health risk assessments, to extrapolate effects data from test animals to humans (e.g., Dourson et al., 1996; Naumann and Weideman, 1995; Renwick and Lazarus, 1998; Vermeire et al., 1999). However, uncertainty factors for essential metals, if applied, should account for lower levels, below which deficiencies can occur (Abernathy et al., 1993; Van Assche et al., 1997). Such extrapolations are often based on the results of testing with the most sensitive organisms, which can result in thresholds that are below the nutritional requirements for most organisms (Chapman and Wang, undated).

- Toxicity threshold distribution-based approaches (Van Straalen, 2001), or other statistical or probabilistic methods, such as analysis of extrapolation error (Suter, 1993).

- Body-weight normalization (described, for example, in Sample et al., 1997; Calder and Braun, 1983; and Nagy, 1987).

In situ and *ex situ* methods, where organisms are exposed to site media (water, sediment, soil) under field or laboratory conditions, have been used to develop site-specific

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5 See U.S. EPA PATCH model (www.epa.gov/wed/pages/models.htm). Also, the U.S. Army Risk Assessment Modeling System (ARAMS) (www.wes.army.mil/el/arams/arams.html) is developing modules that use habitat quality assessments to improve the realism of exposure assessments that can be used to characterize risk.

and receptor-specific toxicity values. This has been used with success in site-specific risk assessments for metals including Murray Smelter, Colorado; Palmerton Zinc, Pennsylvania; Jasper County, Missouri; Smuggler Mountain, Colorado; and the Kennecott site in Utah (U.S. EPA, 2002a).

2.10.10 Non-transferability of Information

Not all information that is available for use in a risk assessment is readily transferable from one setting to another. Clearly, the local environment, whether it is an aerobic water body, anoxic aquatic sediment, or a soil that undergoes periodic wetting and drying and has zones that are both aerobic and anaerobic, will affect the form and availability of the metal of interest. For example, in terrestrial settings most of the chromium that is present will be in the form of CrIII, which is relatively non-toxic to earthworms, birds, and mammals. This is not necessarily the case in an aquatic setting. Further, when earthworms ingest CrVI, it is almost immediately converted to CrIII within the gastrointestinal tract (Arillo and Melodia, 1991). Effect levels for CrIII are not applicable to a CrVI exposure, and vice versa. At the same time, it is not unreasonable to make a worst-case assumption in a Tier I evaluation, by assuming that all of the Cr is present in the form of CrVI. If the potential for concern can be ruled out, then the level of detail in the analysis may be adequate. If, however, it is concluded that there would be a significant potential for adverse effects, then it may be necessary to proceed with a more detailed analysis, one where the form of the chromium is evaluated, prior to arriving at a conclusion about the potential for risk.

Other methods for making site-specific evaluations and for extrapolating from laboratory to field conditions are available, with many having been discussed above. Procedures for measuring water effect ratios (WERs) have been available for some time as a way to adjust nationally applicable WQC for metals to site-specific conditions (U.S. EPA, 1994a). The BLM has been developed expressly for this purpose, as it considers variations in chemical speciation that occur with changes in water quality. The use of carbon-normalized excess SEM applies a somewhat related approach in sediments, with plans to develop BLM applications to sediment pore water and terrestrial settings promising to provide risk assessors with additional tools to use in site-specific assessments in what is hoped is the not too distant future. Methods of relating accumulation to effects are also available, and with their increasing frequency of use, it is expected that the scientific community will continue to develop improved ways of relating exposure levels to accumulation levels and effects. With the fate and effects of metals currently receiving much attention in the scientific and regulatory communities, and steady advances being made with regard to our current level of understanding, it will be important for those performing risk assessments for metals to remain informed of the continuing advances that are regularly being made in this rapidly evolving area.

3. SUGGESTED FRAMEWORK-SPECIFIC LANGUAGE

Assessing the risks of metals in the environment requires a clear understanding of the dynamic interplay among biological, chemical, and physical components. Organisms have evolved in the presence of metals and have acquired many physiological and behavioral mechanisms that enable them to cope with differing environmental and internal

concentrations of metals. Consequently, few relationships between organisms and specific metals are linear. Our collective knowledge of ecological effects to metals often leaves us with considerable uncertainty. The many controlling factors that can influence adverse ecological responses to metals change in relative importance from one situation to another. This is perhaps understood best in aquatic systems as evidenced by the derivation of site-specific Water Effects Ratios (WERs) used to refine broad-based Water Quality Criteria (WQC).

The need to establish levels of metals that are generally protective at a national level necessarily errs on the side of maximum protectiveness. This is done with the full realization, that in some areas higher concentrations would cause no harm. But here lies one substantive concern that pertains specifically to assessing risks from metals—namely low concentrations of essential elements needed for protection in some waters are below nutritional requirements in other waters, due to factors that limit bioavailability. Though there are fewer data for sediments and soils, the inherently greater spatial heterogeneity in sediments and soils suggests even greater ranges of responses across the span of conditions found nationally.

Great advances have been made in the past decade with the development of computational models such as the aquatic biotic ligand model (aBLM). The value of such models stems from their ability to consider simultaneously an array of physicochemical parameters and forecast likely toxicity responses. Research appears headed toward expanding the utility of the aBLM to accommodate more metals. It also looks promising that analogous models will emerge for sediments and soils. Nevertheless, for the foreseeable future, site-specific calibration of models will be needed in order to strike a balance between Type I and Type II errors. It continues to be important to recognize that our capacity to generate descriptive statistical models far exceeds our capacity to produce predictive models based on first principles. Considerable work remains before reliable predictions of toxic effects from chronic exposures are routine; even more work is needed to predict adverse effects at the various ecological levels of organization (e.g., population, community, system, landscape).

The risk assessor should be cautioned about a number of points:

- Be cognizant of the potential effects of site water, sediment, and soil chemistry on the bioavailability and toxicity of the metals (total and dissolved carbon fractions, hardness, pH, redox, competing anions/cations including sulfates, phosphates, calcium, etc.).

- Beware that effects levels are often determined by ratios of two or more metals that may be additive or antagonistic, rather than by the absolute concentration of a metal of concern (e.g., Mo:Cu).

- Recognize that dietary exposure to metals may have a profound influence on effects.

- Understand the important role that site-specific or scenario-specific calibration of models can play in defining effects responses.

- When possible, avoid use of point estimates of toxic effects and rely on more reasonable surface response relationships that characterize the full range of stimulation and inhibition of measurement endpoints.

- When considering body-burden or tissue concentrations, make an effort to understand the relationship between the measured levels and the likely site of action, because most organisms have elaborate physiological mechanisms that effectively isolate the metals away from the active site thus affording some level of protection against toxicity.

- Recognize the individual organism's capacity for acclimation to metals, and that the rate of metal uptake, in addition to the absolute concentration of metal in tissues, may be an important factor to consider.

- Understand that adaptation of populations to metal concentrations can result in markedly different toxicity response profile within a species and across species.

- If viewed at a level of ecological processes, recognize that metal-tolerant communities may provide essentially identical ecological goods and services as metal-intolerant communities.

These points should not be seen as threatening to any regulatory process, but rather providing recognition of the complexity of biological and ecological systems. Statutory requirements or stakeholder interests may not permit addition of metals under some industrial activity, even if metal-tolerant communities would likely replace extant metal-intolerant communities. However, for areas that are naturally high in particular metals, it seems best to acknowledge the breadth of ecological systems that occur, and, therefore, prepare the risk assessments accordingly.

4. RESEARCH NEEDS

Relevant ecological topics to pursue are abundant, including those in the realm of understanding the basic interactions of organisms and metals. Chapman and Loehr (2003) specified four rules to guiding the selection of scientific studies: environmental relevance, remaining unanswered questions, likely publishable results, and having explicitly stated and relevant hypotheses. Clearly these rules should be helpful in sorting through the many issues remaining with respect to effects of metals in the environment. As our capability to handle large, complex data sets continues to expand, especially as spatially explicit displays of naturally occurring metals become possible on continental scales, we will undoubtedly move toward development of ecoregional criteria for water quality parameters, as well as sediment- and soil-screening levels. We can imagine criteria that also reflect extant populations, communities, and ecological systems. However, in the near term, tackling many less ambitious problems could lead to improved estimates of adverse effects due to metals in the environment. Consequently, the following potential research topics are ordered from short-term (and lower cost) to long-term (and higher cost) within two categories: toxicology and ecology.

4.1 Toxicology

Test matrix—One area of research that should prove to be fruitful is to perform tests under conditions that are representative of environmentally relevant exposure conditions. These data will augment the already existing database of test results that have been obtained under relatively consistent laboratory test conditions. In combination, such test results will form a basis for development of methods to extrapolate from laboratory to field conditions, and from site to site in the field in situations where the water-, sediment-, or soil-quality characteristics are quite variable. Even so, extrapolation may still have substantial limitations. Two concurrent efforts could be embarked upon for each medium: 1) Development of descriptive statistical models that establish the boundaries for which the results are applicable and 2) Pursuit of predictive models along the lines of the aBLM.

Test design—Clearly, after several concerted efforts to mine the toxicity literature to develop threshold response concentrations (e.g., Eco-SSLs), we must conclude that the historical toxicity data have limited value for robust ecological risk assessments. In the aquatic arena, tests have been performed on numerous enough species that the apparent response relationships can be deciphered, despite the errors inherent in the individual tests. However, in sediments, and especially in soils, the data are too few to hope to achieve similar insights into the response profiles. Therefore, a concerted effort to generate reasonably complete concentration-response surfaces for metals in major soil and sediment types representative of larger areas of the continent would be very useful. Test designs should be based on regression models and strive to depict the range of responses from relatively low concentrations to relatively high concentrations.

Measurement endpoints—Because different metals evoke different responses in various organisms, multiple endpoints should be scored over the course of the in-life portion of tests. Data on growth parameters, overall healthiness of test organisms, behavioral, and reproductive endpoints should be explored. Such data could be useful in developing descriptive statistical models, including multiple regressions, clustering analyses, and such. In addition, such data could be used to calibrate predictive models that attempt to relate effects across exposure periods.

Interspecies extrapolation—The debate over which species are most sensitive, most representative, or most relevant continues to be one that is largely not confused by facts. Again, there are sufficient data for aquatic species to make reasonable conclusions as to the sensitivity or tolerance of a wide diversity of species. Benthic species or terrestrial species have not been tested adequately to provide good characterizations of sensitivity or tolerance to metals. Because the typical species that are tested are not drawn randomly from the pool of potential test species, it is not possible to know with reasonable accuracy how other species might fall across the sensitivity-tolerance spectrum. Data from studies designed to fill this critical gap would be invaluable.

Interactions among metals—Another area where further research is needed is the area of metal interactions when organisms are exposed to metal mixtures. The presence of multiple metals can lead to the competition amongst these metals for the complexation capacity of the water, resulting in decrease in complexation capacity relative to what would

be available for any single metal alone. This has direct implications to the evaluation of metal availability and the potential for adverse effects. Such interactions are most important when considering low effect levels for the metal of interest, increasing in importance as the concentrations of competing metals increases. Another complication with multiple metals is that metal interactions could either exacerbate or mitigate effects on the target organism. This could be in the form of a single effect being exacerbated, as would be the case when the two metals have the same mode of action (e.g., Cu and Ag affecting Na regulation, or Zn and Cd affecting Ca regulation), or it could result in an organism being affected in different ways at the same time when the modes of action differ. Metal interaction might also lead to a decrease in the rate of uptake by one at the expense of the other, not only when trace metals such as cadmium, strontium, or zinc interact with a hardness cation such as calcium, but when metals such as Pb and Cu interact with each other as well. Methods for representing the joint effects of metal mixtures on the organism, as well as mixtures of metals and organics, in the context of the BLM, have been proposed, but these methods have not been implemented nor tested to date.

Relationship of accumulation level to effects—Greater understanding of the effective concentration at the specific site(s) of action for metals would improve the ability to predict effects. Current methods of analyzing concentrations in tissues, organs, or whole organisms generally preclude any useful linkage of accumulation level to magnitude of effect. Rate of uptake could be one of the variables included in such studies. Ultimately, an improved understanding of the biochemical response to metals at the molecular level, including identification of the enzymes and other target sites of action of toxicity, should enhance our capability to assess the potential for adverse effects.

4.2 Ecology

Extrapolations from the laboratory to the field—There is a considerable amount of uncertainty associated with extrapolating findings of laboratory toxicity tests to field populations. Differences among species in sensitivity to metals, between direct and indirect endpoints, and across levels of biological organization complicate our ability to predict responses in the field based on laboratory bioassays. More studies documenting the correspondence or lack of correspondence between simple laboratory toxicity tests and field assessments are necessary. In situations where laboratory and field results are inconsistent, research is necessary to identify factors that contribute to these differences. It may be productive to encourage the development of effective field-based toxicity tests methods. Similarly, attention should be devoted toward development of field-validated BLM, which would consider both waterborne and dietary metal uptake.

Indirect effects of metals and species interactions—If species interactions such as competition and predation play an important role in structuring communities as suggested in the ecological literature, it is likely that metals will influence the outcome of these interactions. More consideration of the effects of metals on species interactions is necessary. Assessing the significance of these effects will be challenging and may require experimental manipulation.

Integrating responses across levels of biological organization—Because of the difficulty of identifying ecologically significant responses at lower levels of biological organization and our poor mechanistic understanding and lack of specificity of responses at higher levels, studies that integrate responses across several levels of organization are necessary. Biochemical and physiological endpoints ought to be linked with more ecologically significant population and community responses. Establishing these linkages will help elucidate mechanisms responsible for changes at higher levels.

Acclimation and adaptation to metals—There is considerable uncertainty regarding how previously exposed populations will respond to metals. Although tolerance to metals may be achieved in previously exposed populations, some have posed theoretical arguments that there is a cost to tolerance and that such exposed populations may be more susceptible to other stressors. Several studies exploring this theoretical cost have been unable to demonstrate that a cost related to tolerance exists. Additional research is necessary to understand the cost of tolerance and potential consequences of exposure to multiple stressors.

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APPENDIX A. DRAFT ECOLOGICAL SOIL SCREENING LEVELS (ECO-SSLS) IN MG/KG DRY WEIGHT SOIL FOR 17 METALS AS OF MARCH 14, 2003

Metal	**Plant**	**Invertebrate**	**Bird**	Mammal
Aluminum (Al)	Narrative statement			
Antimony (Sb)	No	78	0.3	No
Arsenic (As)	31	No	P	P
Barium (Ba)	No	330	450	No
Beryllium (Be)	No	40	30	No
Cadmium (Cd)	28	150	0.4	2.0
Chromium (Cr-III)	No	No	290	7.9
Chromium (Cr-VI)	No	No	94	No
Cobalt (Co)	32	No	190	240
Copper (Cu)	95	54	P	P
Iron (Fe)	Narrative statement			
Lead (Pb)	210	1,700	25	15
Manganese (Mn)	152	450	P	P
Nickel (Ni)	48	No	P	P
Selenium (Se)	1.0	No	P	P
Silver (Ag)	No	No	P	P
Vanadium (V)	No	No	P	P
Zinc (Zn)	130	120	P	P