Utility of Tissue Residues for Predicting Effects of Metals on Aquatic Organisms

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EDITOR'S NOTE

This paper represents 1 of 6 review articles generated from a SETAC Pellston Workshop entitled ''The Tissue Residue Approach for Toxicity Assessment (TRA)'' (June 2007, Leavenworth, Washington, USA). The main workshop objectives were to review and evaluate the science behind using tissue residues as the dose metric for characterizing toxic responses and to explore the utility of the TRA for mixtures, guidelines or criteria, and ecological risk assessment.

ABSTRACT

As part of a SETAC Pellston Workshop, we evaluated the potential use of metal tissue residues for predicting effects in aquatic organisms. This evaluation included consideration of different conceptual models and then development of several case studies on how tissue residues might be applied for metals, assessing the strengths and weaknesses of these different approaches. We further developed a new conceptual model in which metal tissue concentrations from metal-accumulating organisms (principally invertebrates) that are relatively insensitive to metal toxicity could be used as predictors of effects in metal-sensitive taxa that typically do not accumulate metals to a significant degree. Overall, we conclude that the use of tissue residue assessment for metals other than organometals has not led to the development of a generalized approach as in the case of organic substances. Species-specific and site-specific approaches have been developed for one or more metals (e.g., Ni). The use of gill tissue residues within the biotic ligand model is another successful application. Aquatic organisms contain a diverse array of homeostatic mechanisms that are both metal- and species-specific. As a result, use of whole-body measurements (and often specific organs) for metals does not lead to a defensible position regarding risk to the organism. Rather, we suggest that in the short term, with sufficient validation, species- and site-specific approaches for metals can be developed. In the longer term it may be possible to use metal-accumulating species to predict toxicity to metal-sensitive species with appropriate field validation. Integr Environ Assess Manag 2011;7:75–98. © 2010 SETAC

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INTRODUCTION: WORKSHOP PERSPECTIVE

A goal of the tissue residue approach (TRA) for toxicity assessment is to determine the dose–response relationship for various toxicants and evaluate the range of tissue residues that would likely lead to adverse effects. Although considerable effort has been expended over the years to relate tissue metal residues that result from waterborne or dietary exposure to effects, these efforts have achieved only limited success.

Interpretation of results has been confounded by a limited understanding of the manner in which aquatic organisms store, detoxify, or eliminate metals and by lack of standardized exposure periods, standardized test protocols, and specified test species. In particular, advancement in the science of using tissue residues has been hampered by a failure to recognize that metals in whole body or muscle do not reflect the biologically or metabolically active portion of metal that is available to contribute to toxicity at the site of action and, furthermore, that total metal is at best a surrogate for the fraction of metabolically active metal at the site of action.

In this paper, we explore the strengths and limitations of using metal tissue residues to assess the potential for biological effects. Case examples are provided in which tissue residues for specific metals and organisms have evolved to an extent that the tissue assessment approach is becoming robust. Finally, an approach is provided to demonstrate how

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the results of laboratory tissue residue experiments for specific aquatic organisms can be combined with field validation, to derive tissue residue metrics to assess the potential for population-level and potentially community-level effects.

STRENGTH AND LIMITATIONS OF A TISSUE RESIDUE APPROACH FOR METALS

Metals and organisms

The toxicity of metals to aquatic organisms strongly depends on exposure scenario and exposure time, factors that make the derivation of environmental quality criteria (i.e., water–sediment quality guidelines) for metals a complex exercise (Chapman et al. 2003). In principle, environmental quality criteria can be set on the basis of external exposure concentrations or internal body or tissue residues. Both approaches have their strengths and limitations. In the real world, organisms are exposed to metals via different routes, and, depending on an ecosystem's structural and functional organization, metal exposure can occur via one dominant source (e.g., food or water) or a combination of different sources. Metals entering a given environment are distributed among different phases depending on their specific chemical characteristics and the nature and availability of binding sites. For example, in the aquatic environment, metals occur in solution, in or on suspended particles, and in sediments, from which they are taken up by different types of organisms and transferred throughout ecosystem food webs. Within each of these phases, metals occur in different chemical forms or species. A metal in solution forms ion pairs and complexes with the inorganic and organic ligands present, resulting in the formation of a multitude of metal species.

Aquatic organisms are isolated from their environment by different types of barriers such as cell walls or epithelia. Exchange of material with the environment occurs largely through specialized tissues or organs forming epithelial structures that are involved in the uptake of essential nutrients and minerals and the excretion of waste materials (Randall et al. 2002). For example, exchange of material in fish occurs largely across the gill and gut epithelium. The ultimate barriers between an organism and its environment are the cell membranes surrounding the cells. These are phospholipid bilayers embedded with proteins that have a hydrocarbon core forming an almost impermeable barrier to water-soluble or hydrophilic substances. The transport of hydrophilic substances across these membrane barriers therefore involves specialized transport systems that are embedded in the membranes. For example, the exchange of essential elements such as Na or Ca is mediated by specific transmembrane protein carriers and channels.

Most metal species present in the environment are hydrophilic, and only a limited number are lipid soluble or hydrophobic. This implies that metal uptake by an organism requires the involvement of more or less specific transporters that take the metal across the membrane interface separating the ambient from the intracellular environment (Simkiss and Taylor 2001). The fact that these transporters tend to be specific for certain elements and may be limited in distribution across all biota explains why some metal species are easily taken up by an organism whereas others seem to be almost biologically inert. For example, the divalent Cd ion (Cd^{2+}) resembles the divalent Ca ion (Ca^{2+}) in terms of radius and charge. It is therefore easily taken up by biological systems via Ca^{2+} transporters. Formation of inorganic and organic Cd complexes changes the structure of the Cd species in such a way that their uptake via these Ca transporters can be markedly reduced in comparison with uptake of Cd^{2+}. In contrast, some organometal compounds, such as methylmercury, are lipid soluble. As a result, these same membranes do not necessarily serve as a strong barrier to the diffusive uptake of these types of metal species.

The basic chemical and biological principles outlined above explain why it is difficult to establish environmental quality criteria for metals on the basis of total metal exposure concentrations. However, a fundamental understanding of the processes controlling the chemical speciation of metals in the environment and the uptake of metals by organisms makes it possible to develop criteria that take into account differences in environmental chemistry and their effect on metal availability for the exposed organisms (Di Toro et al. 2001; Niyogi and Wood 2004; Luoma and Rainbow 2005). Such models have been developed for a number of metals, organisms, and environments and have demonstrated their usefulness for the setting of site-specific environmental quality criteria for metals. However, despite their clear advantages, even these models do not fully reflect the true complexity of a real environment in which different exposure routes, conditions, and organisms have to be considered.

A complementary approach, therefore, is to monitor the metal exposure by measuring whole-body or tissue residue of the metal in the exposed organisms. The clear advantage is that the determination of metal residues in the exposed organisms provides a relatively direct and unambiguous measurement of the internal exposure, one that integrates the effects of the different chemical and biological factors influencing metal availability to organisms (Vijver et al. 2004). The utility of this approach for effects-assessment purposes is premised on the assumption that critical metal whole-body or tissue residues can be defined, for one or a series of indicator organisms, above which adverse effects may occur in the most metal-sensitive organisms present in a given ecosystem. Note that the indicator organisms do not necessarily have to correspond to metal-sensitive organisms, and, as discussed below, it may be advantageous for them to be relatively insensitive to metals.

In summary then, different types of organisms are differentially exposed to metals in a given environment and will accumulate the metals to varying degrees. This is partially the result of differences in metal exposure routes and environmental chemistry-related effects. Equally important are the differences in metal uptake, detoxification, or metabolism, and elimination kinetics of the organisms, as discussed in the next section.

Trace metal accumulation patterns: An aquatic invertebrate perspective

Aquatic invertebrates take up and accumulate trace metals whether essential or not, all with the potential to cause toxic effects. Subsequent tissue and whole-body residues of accumulated trace metals vary enormously across metals and invertebrate taxa (Eisler 1981; Rainbow 2002). Accumulated metal can be classified into two components, 1) metal that has been detoxified, and 2) metal that is metabolically available to satisfy essential needs or, in

extreme circumstances, to interact in a way that manifests itself as a toxic response (Rainbow 2002, 2007). This distinction has implications for any relationship between accumulated residues and metal toxicity. The reason is that differences in metal uptake rate can lead to the same tissue residue that in one case results in an adverse effect and in another case does not (see, e.g., Kraak et al. 1992; Andres et al. 1999; Hook and Fisher 2002). This observation does not necessarily rule out the potential utility of tissue residues being indicative of effects but clearly will complicate any such interpretation.

All aquatic invertebrates will take up trace metals into the body from solution through permeable body surfaces and from the gut. It is now appreciated that uptake of trace metals from the diet may be the major source of metals for many aquatic invertebrates (Wang 2002). When metal first enters the body of an invertebrate after uptake either from solution through permeable ectodermal surfaces or across the endoderm of the gut, it will initially be metabolically available; that is, it has the potential to bind to molecules in the receiving cell or elsewhere in the body after internal transport via the hemolymph. In the case of an essential metal, it is available to bind to sites where it can satisfy an essential need (e.g., Zn in the enzyme carbonic anhydrase or Cu in hemocyanin) or, if present in excess (caused by entry at too high a rate relative to the rate of detoxification or elimination), to sites where it may cause toxic effects. Such an excess of an essential metal (and all nonessential metals) must be detoxified, i.e., bound tightly to a site from which escape is limited, often in an organ that differs from the original site of uptake. The metal has now entered the second compartment of accumulated metal, the detoxified store, which may be temporary or permanent. Trace metals taken up into the body might or might not be excreted, either from the metabolically available component or from a detoxified store, depending on the accumulation pattern of a particular animal for a particular metal (Rainbow 2002).

The second compartment of accumulated metal is that of detoxified metal. Many trace metals are detoxified in the form of various insoluble granules in invertebrate tissues (Hopkin 1989; Marigomez et al. 2002; Wallace et al. 2003). Hopkin (1989) described 3 types of intracellular granules: type A, consisting of concentric layers of Ca and Mg phosphates that can contain trace metals such as Mn and Zn; type B, more heterogeneous in shape and always containing S in association with metals that include Cu and Zn; and type C, often polyhedral with a crystalline form, mainly containing Fe, probably derived from ferritin. In crustaceans, the most commonly reported metal-rich granules are type A and B granules, whereas large ferritin crystals are characteristic of the ventral caecum cells of stegocephalid amphipods (Rainbow 2007). Detoxification also occurs in the soluble phase. Some trace metals (e.g., Ag, Cd, Cu, Hg, Zn) are associated with, and induce, metallothioneins (MT), low-molecular-weight cytosolic proteins involved in the cellular regulation and detoxification of these metals (Amiard et al. 2006). These S-rich proteins have a high percentage of cysteine residues (~30%) that have high affinity for metals, which they sequester in the cytoplasm, reducing the metabolic availability. It is the lysosomal breakdown of MT that probably gives rise to the S-rich type B granules described above, as in the amphipod *Orchestia gammarellus* from Cu-contaminated sites (Nassiri et al. 2000).

Table 1. Copper and zinc concentrations in crustaceans from uncontaminated sites (Rainbow 2002)

	Concentrations ($\mu g\, g^{-1}$)	
Species	**Zn**	**Cu**
Barnacles		
Lepas anatifera	552	63.0
Tetraclita squamosa	2245	14.9
Balanus amphitrite	2726	59.3
Amphipods		
Orchestia gammarellus	188	77.5
Talorchestia quoyana	133	15.6
Decapods		
Systellaspis debilis	46.9	49.0
Palaemon elegans	80.6	110

Copper, Zn, and 3 crustacean taxa (barnacles, amphipods, and caridean decapods) can be used to exemplify some general principles of trace metal accumulation (Rainbow 2007). Barnacles have Zn concentrations an order of magnitude above those of amphipods and decapod carideans, even when the barnacles are from uncontaminated sites (Table 1). Zinc concentrations can increase greatly in barnacles and to a lesser extent in amphipods from contaminated sites (Table 2). On the other hand, barnacles from uncontaminated sites have whole-body Cu concentrations usually below those of amphipods and carideans (Table 1). As malacostracans, they have significant body contents of the Cu-bearing respiratory protein hemocyanin, which is absent from barnacles. However, barnacles have the potential to increase their whole-body Cu concentrations well

Table 2. Copper and zinc concentrations in a barnacle and an amphipod collected from uncontaminated (background) and contaminated sites (Rainbow et al. 1999; Rainbow 2002, 2007)

	Concentrations ($\mu g\, g^{-1}$)	
Species	**Zn**	**Cu**
Balanus amphitrite		
Background	2726	59.3
Hang Hau, Hong Kong	11990	486
Chai Wan Kok, Hong Kong	9353	3472
North Point, Hong Kong	7870	1010
Orchestia gammarellus		
Background	120–190	50–90
Millport, UK	186	64
Dulas Bay, UK	126–151	105
Restronguet Creek, UK	169–392	136–362

above those of amphipods and carideans when at Cu-contaminated sites (Table 1; Rainbow 2007). Therefore, the accumulation pattern of a particular invertebrate for a particular trace metal determines the whole-body concentration, and these accumulation patterns correspondingly vary within and between crustaceans and other invertebrates (Rainbow 2002).

The first accumulation pattern to be considered is that of the essential trace metal Zn in caridean decapods. The caridean *Palaemon elegans* is able to regulate its whole-body Zn concentration (to about 90 μg Zn g^{-1}) when exposed to a wide range of dissolved Zn concentrations, an accumulation pattern for Zn also shown by related caridean decapods (Rainbow 2007). Zinc is taken up by *P. elegans* in significant quantities (14% of whole-body Zn content per day at 100 μg Zn L^{-1} under defined physicochemical conditions at 20 °C), but the uptake rate is balanced by the excretion rate so that the body concentration remains unchanged. Eventually, at a high enough dissolved Zn concentration, the excretion rate fails to match the uptake rate, and there is a net increase in whole-body Zn concentration to only about double the regulated body concentration, with lethal effect (Rainbow 2002, 2007). The implication here is that much of the Zn remains in a metabolically available form without detoxification, the concentration of metabolically available metal building up sufficiently to cause toxicity. It can also be concluded that, in caridean decapods at least, whole-body concentrations of Zn are regulated to approximately those required to meet metabolic demand, with relatively little stored in detoxified form (Rainbow 2002, 2007). Barnacles occupy the other extreme of the range of Zn accumulation patterns (Rainbow 2002, 2007). Zinc taken up from solution (Rainbow and White 1989) and the diet by barnacles is accumulated without significant excretion, having a half-life of 3.7 y in *Elminius modestus* (Rainbow and Wang 2001). Correspondingly, accumulated whole-body Zn can reach very high concentrations (50 000 μg Zn g^{-1} or more; Rainbow 2002, 2007), and most of this accumulated Zn inevitably is in a detoxified form, in fact, bound in Zn-pyrophosphate granules (Rainbow 2002, 2007).

Thus, accumulation patterns for Zn in these 2 taxa of crustaceans could not be more different: 1) regulation to a constant whole-body concentration with apparently little stored in detoxified form (caridean decapods) and 2) storage in detoxified form without significant excretion (barnacles), resulting in some of the highest accumulated concentrations of any trace metal in any animal tissue (Eisler 1981; Rainbow 2002, 2007).

The Cu accumulation pattern of amphipods appears to be intermediate between these 2 examples (Rainbow 2002, 2007). Amphipods exposed to a range of dissolved Cu exposures typically show net accumulation at most if not all exposures (Rainbow 2007). Copper is accumulated in the cells of the ventral caeca (equivalent to the hepatopancreas of carideans), in the form of type B Cu-rich granules presumed to be derived from MT. Copper detoxified in these granules is excreted on completion of the cell cycle of the ventral caeca epithelial cells (Galay Burgos and Rainbow 1998). This is not a regulation process, because the whole-body concentration of Cu in the amphipods will reach a new steady-state level as Cu bioavailability changes, the availability of Cu being reflected in the number of granules in (and hence the Cu concentration of) the ventral caeca. As the Cu concentration increases, the proportion of accumulated copper in the detoxified component increases, from a starting point at which most Cu in an amphipod from an uncontaminated site will probably be in metabolically available form (Table 1; Rainbow 2007). Thus, amphipods are weak accumulators of Cu.

Although trace metal accumulation patterns differ between organisms and between metals, even for the same organism, it is possible to identify some further accumulation patterns. Other decapod crustaceans and several other taxa, including fish, are considered to be regulators of whole-body concentrations of essential metals, especially Zn. Strong accumulators of trace metals include oysters in addition to barnacles. Weak accumulators (sometimes referred to as partial regulators) such as talitrid amphipods for Cu and Zn include mytilid mussels (Cu) and the polychaete worm *Nereis diversicolor* (Zn; Luoma and Rainbow 2008).

Strategies for metal regulation

Inside an organism, metals are distributed among different compartments and are handled in different metal- and tissue-specific ways. In general, 2 main metal pools can be distinguished, metals that are present in biologically active forms and metals that are present in inactive forms (Rainbow 2002). Metals in biologically or metabolically active forms participate in or can interact with the biological machinery of cells and tissues (Figure 1). Metals in biologically inactive forms are immobilized in some way so that they cannot participate with or interact with normal biological functions. Some metals, such as Cu and Zn, are essential; others, such as Ag and Pb, do not have a clearly defined biological function and are considered nonessential.

Whether essential or not, all metals become toxic above certain internal exposure levels. Therefore, it is crucial for a biological system to maintain metal levels in the biologically active pools below a certain threshold to avoid disturbance or

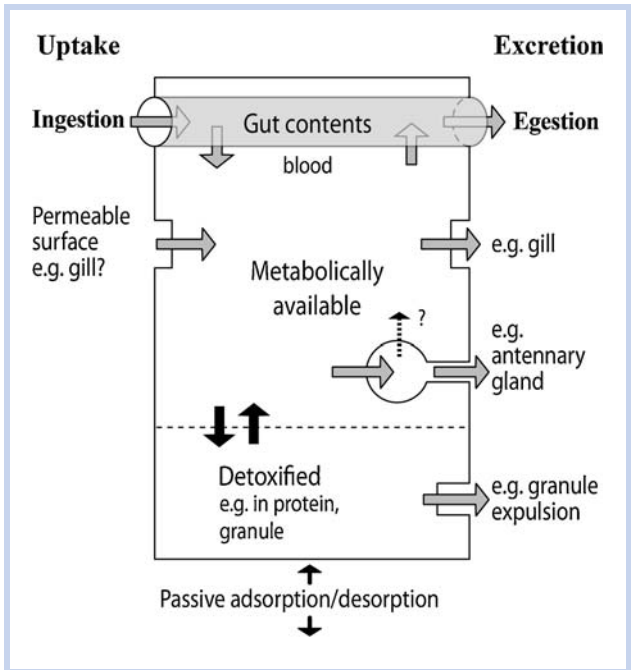

Figure 1. Schematic overview of metabolically active and detoxified metal pools in aquatic organisms. From Luoma and Rainbow (2008).

damage. This can be achieved by controlling the whole-body or tissue residue by regulating uptake or elimination from a specific tissue compartment or the whole organism. Alternatively, the organism may transfer part of the metal to a biologically inactive compartment that so it cannot interfere with normal functions.

Organisms have developed different types of metal-accumulation and -handling strategies, and in many cases metal regulation and detoxification are the result of a combination of the 2 main strategies mentioned above. Thus, some organisms can accumulate rather high metal concentrations without apparent negative effects, whereas other organisms show signs of toxicity with much lower whole-body or tissue-specific metal residues. This means that the whole-body or tissue residue above which toxicity occurs is organism specific and that a common critical metal threshold residue does not exist.

Metal accumulation is a dynamic, time-dependent process characterized by an initial phase of increasing tissue concentration that gradually levels off when elimination balances uptake to reach equilibrium. Metal uptake and elimination rates are not necessarily constant, even under constant exposure conditions, but might increase or decrease depending on the metal-regulatory capacity of the organism (Blust 2001). Metal toxicity occurs when biologically active metal in 1 or more sensitive tissue compartments reaches a threshold concentration. How fast this concentration is reached depends on three main processes: first, the rate of metal accumulation within the metal-sensitive compartments inside the organism; second, the capacity of the organism or specific tissue to translocate the incoming metal from biologically active to biologically inactive compartments; and, third, the ability of the organism to excrete the metal.

Metal exposure can trigger several physiological responses resulting in an increased metal-processing capacity so that, given time, the organism can activate several detoxification systems. This means that, for a given species, the whole-body or tissue metal residue associated with the toxicity threshold depends on the exposure scenario. For example, a short-term exposure to a relatively high concentration might result in a rapid accumulation of metal in the most sensitive tissue compartments without an appropriate detoxification response of the organism, resulting in a low tissue-specific toxicity threshold. A long-term exposure to a lower concentration might result in a continued accumulation of metals in the most sensitive tissue compartments (for some organisms), but with appropriate detoxification response, resulting in a considerably higher toxicity threshold concentration. This might vary as a function of the exposure route. Nonetheless, it appears that, at least for some invertebrate species and fish, the concept of a whole-body toxicity threshold seems to hold true under a series of different exposure situations provided that exposure is long enough.

Several studies of invertebrates (Borgmann et al. 2004; Ma 2005; Simpson and King 2005) and fish (Marr et al. 1996; Hansen, Lipton, et al. 2002) demonstrate relationships between metal accumulation and chronic toxicity across a range of conditions. However, these and other results also demonstrate that the relationship becomes more consistent over time and that accumulation rate, in combination with the concentration of accumulated metal, will determine whether there is a toxic response. This is also what can be expected from a basic understanding of bioaccumulation kinetics and physiological responses to metal exposure; i.e., a relatively short-term exposure to a high concentration might lead to a physiological response that differs from the response to a long-term exposure to a low concentration, even though the resulting tissue concentrations might be the same.

The availability of tissue-concentration data that can be coupled to the relevant endpoints varies considerably from metal to metal, and not all taxonomic groups are equally represented. The 2 main databases that list these data are the Environmental Residue-Effects Database (ERED; Bridges and Lutz 1999) and the Linkage of Effects to Tissue Residues: Database for Aquatic Organisms (Jarvinen and Ankley 1999). For example, searching the ERED database (June, 2007) results in 1149 hits for Cd and 103 species, 844 hits for Cu and 74 species, 409 hits for Pb and 44 species, 63 hits for Ni and 11 species, and 324 hits for Zn and 54 species. The endpoints reported include survival, reproduction or growth effects, and other behavioral and physiological responses and are generally reported in terms of effect tissue concentrations causing percentage response or no-observed-effect concentrations (NOEC) or lowest-observed-effect concentrations (LOEC).

Compared with the toxicity databases for effects as a function of waterborne-exposure concentrations, the tissue-concentration information currently available is more limited. However, for some of the more extensively studied metals and taxonomic groups, the data sets are probably sufficiently large and diverse to analyze and derive whole-body or tissue-effect concentrations for certain species or groups of species. However, the quality of the data sets available is an important issue, and criteria for screening the information are needed (e.g., analytical and experimental procedures and conditions, including route of exposure, background information on the history of the test organisms, statistical treatment, and reporting of the data). Two approaches are possible for data selection, 1) an a priori selection based on preset conditions so that studies that do not meet these criteria are removed from the final compilation, or 2) an a posteriori selection in which all data present in the database are considered in an initial screening analysis. This latter approach would identify the most crucial results, which would then be subjected to a more rigorous quality and consistency assessment. The advantage of the first approach is that from the start, on the basis of the predefined conditions and information provided in the original reports, the analysis is based on high-quality data. The disadvantage is that, in the selection process, intrinsically valuable information might be discarded before the effect on the overall analysis has been evaluated. An example of the a priori approach was used to develop a set of long-term NOECs and LOECs for Cd based on survival, reproduction, and growth endpoints, which are expressed as cumulative probability distributions (see below under *Cadmium case study*).

Perspectives on metal accumulation and metal toxicity indicator species

Kinetic accumulation models allow one to analyze and model the uptake and elimination of metals under complex exposure scenarios. Different exposure routes can be considered simultaneously and their relative importance determined. The effects of changes in metal speciation and other processes such as cation competition can be incorporated in

the models. By coupling the accumulation models to dose–response toxicity models, metal exposure can be directly related to effects in a dynamic manner. However, further work is required to generate high-quality data to parameterize the dose–response models under a broad range of ecologically relevant conditions.

The species-sensitivity-distribution (SSD) approach provides information on the dose–response relationships and provides an intuitive way to identify the most sensitive species in terms of the tissue-residue approach. However, the whole-body or tissue data used to generate an SSD have to be interpreted with caution, because a single critical body or tissue concentration does not exist for metals for reasons previously discussed (i.e., the rate of metal uptake, metal detoxification and storage, and rate of metal elimination). The SSD combines species with various strategies for metabolizing metals unless carefully screened and thus could be misleading.

By combining all the information and data on metal accumulation kinetics and toxicity, it becomes possible to identify the most ecologically relevant species for use as metal accumulation indicator species (MA species). These species must integrate metal exposure in terms of bioavailability over time and as such provide an indication of metal exposure within a given time frame. Because whole-body or tissue metal concentrations do not provide information on the exposure route, selection of a suite of sentinel species that are differentially exposed to metals may help to characterize this important exposure aspect. When the proper MA species have been identified, kinetic accumulation models that can be applied to these species can then be developed. The species themselves can then be used for monitoring purposes and for the development of species-specific models for the site-specific prediction of metal availability and accumulation in a given system.

Along the same lines of reasoning, metal toxicity indicator species (MT species) can be identified. These are the organisms that have fast metal uptake kinetics or limited elimination or detoxification capacity, so that a toxic metal concentration is reached rapidly inside the body in terms of both exposure intensity and time. These species should be selected on the basis of information concerning their metal-accumulation patterns and sensitivities. After identification of the MT species, kinetic accumulation–toxicity models can be developed for them. These models can then be used to take into account environmental chemistry and other metal accumulation- and toxicity-modifying factors. Relationships should be established between metal accumulation in MA species and toxicity in MT species to evaluate the extent to which information from metal-accumulation monitoring can be used to predict toxicity in MT species. Finally, tissue-residue criteria should be developed for MA species to protect MT species, and these criteria could then be further used to set environmental quality standards.

APPROACHES FOR DEVELOPING TISSUE RESIDUE APPLICATIONS FOR METALS

Background

Few examples exist of the tissue residue approach being applied within a regulatory framework for metals. The example of Se is a comprehensive fish tissue-based criterion and is currently in draft form (USEPA 2004). Other examples include a review of the biotic ligand model (BLM) approach for metals, an intracellular speciation approach for bivalves, a recent assessment of tissue-based effects for Cd to evaluate derivation of a critical body residue (CBR), and a case study of Ni using tissue residue effects data for the amphipod *Hyalella azteca* to assess potential effects resulting from sediment contamination. This section concludes with a conceptually novel approach for the development of a framework in which tissue residue thresholds might be applied in a regulatory setting.

Biotic ligand model: Modes and mechanisms of toxic action

The modes of action of acute toxicity are relatively well understood for most waterborne metals. Notably, diet-borne metals are highly unlikely to cause acute toxicity (Meyer et al. 2005). At very high waterborne concentrations that result in death within a few hours, virtually all metals act as respiratory toxicants, causing a blockade of respiratory gas exchange and suffocation because of inflammation, edema, and cellular disruption of the gill or its analogue. As the branchial epithelium breaks down and mucus cells discharge their contents, high concentrations of metals may accumulate on the respiratory surface shortly before death.

For most metals, such exposures would rarely be environmentally realistic or of regulatory interest, and the associated tissue residues are useful only for post hoc assessment, e.g., for diagnosing the cause of a sudden fish kill. However, 2 interesting exceptions are Al, which causes respiratory toxicity (gill smothering) at circumneutral pH and by ionoregulatory toxicity at acidic pH, reflecting its complex pH-dependent speciation and solubility (Playle et al. 1989), and Ni, which appears to cause mainly respiratory toxicity in fish (Pane, Richards, et al. 2003), though not in daphnids (Pane, Smith, et al. 2003). For these 2 metals only, respiratory effects occur at concentrations close to those of regulatory interest for short-term exposure (i.e., 48–96-h LC50s).

For most waterborne metals at acute regulatory concentrations, respiratory toxicity is usually replaced by a second mode of action, ionoregulatory toxicity. It has been demonstrated that this mode of toxicity can be predicted by using a BLM (Figure 2). At least in freshwater organisms, it is now clear that many metals act as fairly specific ionoregulatory toxicants, competing with osmoregulatory ions for normal active uptake processes (ionic mimicry; Bury et al. 2003) and often inhibiting or blocking key proteins responsible for ion transport (for review see Wood 2001). For example, based mainly on research with freshwater teleost fish, the toxic mechanism of Cu and Ag involves competition with Na^+ at uptake sites (Na channels) on the apical surface of the ionocytes and ultimately inhibition of the basolateral Na^+/K^+-ATPase and cellular carbonic anhydrase. The BLM provides a means to predict when toxicity by this mode of action will occur. For active Cl^- uptake, at least in daphnids, which are among the most sensitive organisms, acute toxic mechanisms appear similar (Bianchini and Wood 2003). Interestingly, Ni might specifically block active Mg^{2+} uptake in daphnids, although the exact mechanism is unknown (Pane, Smith, et al. 2003).

This mechanistic understanding has provided significant benefits for acute toxicity prediction. For example, the understanding that metals in general are taken up through specific transport processes has justified the incorporation of Michaelis–Menten kinetics into bioaccumulation models such

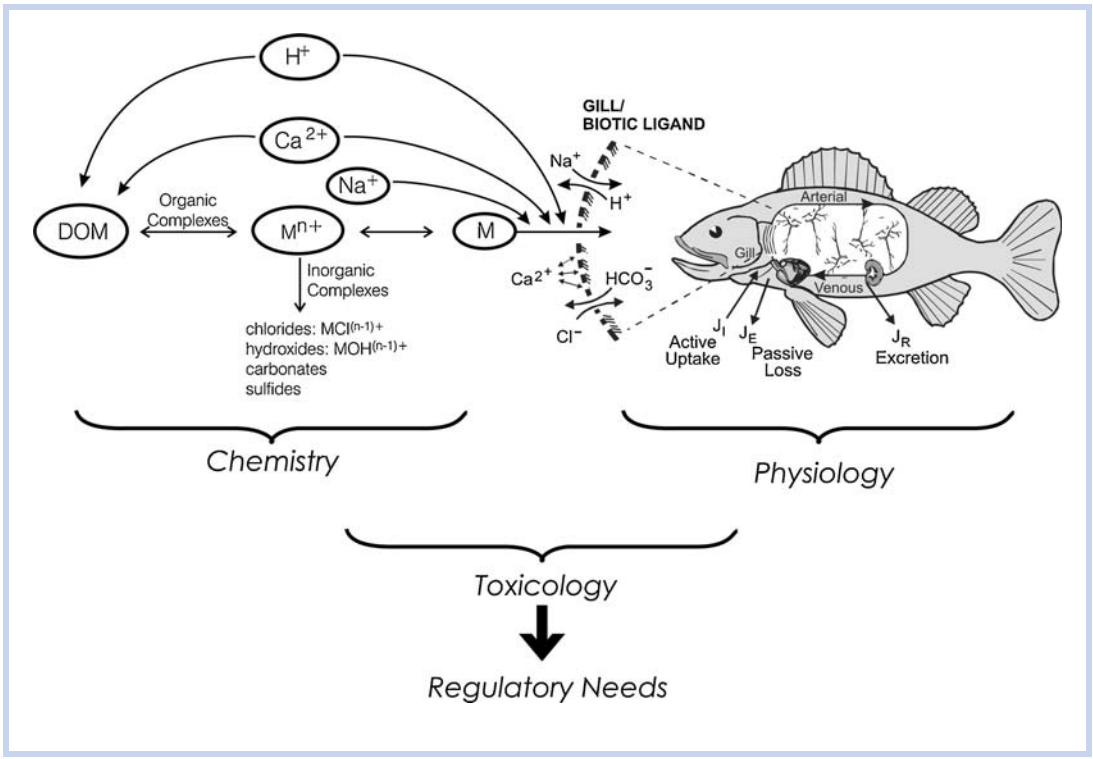

Figure 2. Schematic diagram, from Figure 1 of Paquin et al. (2002), describing the integration of chemistry, physiology, bioaccumulation, and toxicology within BLMs.

as BIM-BAM (Steen-Redeker and Blust 2004). Identification of sites of acute toxicity has helped provide a firm scientific underpinning for BLM development (Di Toro et al. 2001; Paquin et al. 2002; Niyogi and Wood 2004). At present, there appear to be at least 3 toxic sites or biotic ligands in the ionocytes of the gill, one for Na^+ (targeted by Ag, Cu, and probably also Al and inorganic Hg), one for Ca^{2+} (targeted by Zn, Cd, Co, and Pb), and one targeted by Ni (perhaps an Mg^{2+} transport site).

Original BLM development hinged on a tissue residue measurement, whole-gill metal burden accumulated over a short period of either 3 or 24 h, as a predictor of 96-h or 168-h toxicity (Playle et al. 1993a, 1993b; MacCrae et al. 1999). The threshold residue for toxicity was termed the LA50 (lethal accumulation above background associated with 50% mortality). This short-term tissue residue, although not a steady-state measurement of accumulation at the gill, does reflect the concentration needed to inhibit specific proteins involved in ion transport that result in toxicity at 48 or 96 h and so is a CBR. More recent BLMs no longer use an actual tissue residue but, rather, an inferred or operationally defined tissue residue determined by iteration to provide the best fit to measured toxicity data (see, e.g., USEPA 2007, Deleebeeck et al. 2007). Therefore, the LA50, when inferred, is not a true CBR, but nevertheless, it is the critical concept that facilitated development of the BLM and is based in principle on a tissue residue approach.

In contrast to acute toxicity, the modes and mechanisms of toxic action remain poorly understood for chronic toxicity of most metals. In part this might reflect the fact that much less work has been carried out to date on this topic relative to acute toxicity. However, it might also reflect the fact that, for chronic toxicity, there may be no single key toxic mechanism or site of action for a particular metal but rather a suite of effects contributing to physiological deterioration.

In general, metals have very high affinity for S- and N-based moieties. Therefore, once they have entered cells, they probably bind to a variety of proteins and thereby perturb their function, unless the metal can be compartmentalized in a way that prevents this (e.g., into MT or storage granules) or excreted (Vijver et al. 2004). In addition, many metals react with H_2O_2 and can undergo redox reactions to form reactive oxygen species (ROS), a process known as the Fenton reaction. The resulting cellular damage can be in the form of membrane lipid peroxidation, DNA damage, or protein carbonyl production (see, e.g., Farag et al. 2006).

Although rarely measured directly, an important chronic effect might be the increased metabolic cost of damage repair to combat this nonspecific damage. Certainly, there is evidence of impaired metabolic capacities in organisms chronically exposed to elevated metals throughout their lives in the wild (Rajotte and Couture 2002; Couture and Kumar 2003; Levesque et al. 2003) and evidence of increased metabolic costs in animals chronically exposed to metals in the laboratory (De Boeck et al. 1997; Ricard et al. 1998). These costs and reduced metabolic scope could contribute to the classic chronic endpoints of accelerated mortality, depressed growth, and reduced reproduction that are commonly seen during chronic exposures in the laboratory (Hansen, Lipton, et al. 2002; Hansen, Welsh, et al. 2002; De Schamphelaere et al. 2006). The nonspecific nature of such effects combined with the fact that some aspects (e.g., ROS production) might not involve binding of metal to a biological site creates considerable challenges for the interpretation of tissue metal residues.

Specific mechanisms of chronic toxicity might occur in combination with the above mentioned nonspecific effects and might be more amenable to the tissue residue approach, at least from a modeling perspective (e.g., inferred LA50 for chronic toxicity). Some of these may simply be extensions of acute toxicity mechanisms. For example, reductions in growth rate commonly observed with chronic sublethal exposures to metals that compete at Ca uptake sites (Hansen, Welsh, et al. 2002; Perceval et al. 2004) might be explained by decreased bone or shell calcification (Grosell and Brix 2009), and reductions in reproduction might be associated with decreased Ca^{2+} availability for vitellogenin and egg production (Hook and Fisher 2001a, 2001b, 2002). Chronic Ag toxicity in daphnids (reproductive endpoints) appears to be associated with a failure of whole-body Na^{+} regulation (Bianchini and Wood 2002); likewise, chronic Ag toxicity in fathead minnows (growth and mortality endpoints) is similarly associated with a failure of whole-body Na^{+} regulation, just as in acute exposures (Naddy et al. 2007). In trout chronically exposed to sublethal Ni concentrations, there are no effects on resting physiology, but exercise capacity is reduced, associated with a thickening of the O_2 diffusion barrier at the gills (Pane et al. 2004). Again, this suggests a subtle extension of the acute toxicity mechanism of respiratory blockade in trout (Pane, Richards, et al. 2003). Chronic Ni toxicity in daphnids (survival, growth, and reproduction) is associated with a failure of Mg^{2+} regulation, as in acute toxicity in these animals; however, there are additional pathologies in respiratory parameters that are not seen during acute exposures (Pane, Smith, et al. 2003). In contrast, whereas acute Pb toxicity and chronic Pb toxicity in snails appear to be the results of disruption of Ca homeostasis (Rogers and Wood 2004), chronic Pb toxicity to fish is associated with developmental abnormalities, anemia, and neurological disorders (Davies et al. 1976; Mager et al. 2008).

Thus, chronic mechanisms of toxicity may be similar to acute mechanisms for some metals and organisms and may be quite different from acute mechanisms in other cases. Mechanisms that are unimportant in acute toxicity may become very important in chronic toxicity and ecosystem-level responses for some metals; there are no generalizations to be made, because these mechanisms are metal specific and organism specific.

A major factor that must be addressed is the role of metal uptake from food (see, e.g., Dallinger et al. 1987; Clearwater et al. 2002; Meyer et al. 2005). Although not often incorporated into laboratory toxicity tests, in the real world, food-borne uptake (including sediments for deposit feeders) can be an important exposure pathway (Meyer et al. 2005; Pyle et al. 2005; Farag et al. 2007). In fact, both modeling scenarios (see, e.g., Luoma and Rainbow 2005) and case studies (see, e.g., Farag et al. 1994, 1999; Munger and Hare 1997) suggest that, for many organisms in nature, the food provides the major source of loading and sometimes the major source of toxicity. For example, there is now laboratory evidence for some marine invertebrates that low waterborne metal levels that are safe for reproduction in themselves may result in food-route exposures (contaminated phytoplankton) that inhibit reproduction (Fisher and Hook 2002; Bielmyer et al. 2006). However, it is also apparent, at least for fish, that metal taken up through the gastrointestinal tract is distributed differently among tissues from metal taken up from the water (Harrison and Klaverkamp 1989; Szebedinszsky et al. 2001), providing a further challenge to the interpretation of tissue residues.

Several other mechanisms of chronic metal toxicity have emerged in recent years that are completely different from those seen in acute toxicity. These include disturbance of immune function by a variety of metals (Sanchez-Dardon et al. 1999); disruption of endocrine function by Cd (Ricard et al. 1998), Cu (Handy 2003; Gagnon et al. 2006), and mixed metal exposures (Norris et al. 1999; Levesque et al. 2003; Gravel et al. 2005); and blockade of chemosensory function by low concentrations of Cu (Julliard et al. 1993; Hansen, Marr, et al. 1999; Hansen, Woodward, et al. 1999; Pyle and Mirza 2007). The latter has also been seen in fish exposed to polymetallic conditions in the wild (Mirza et al. 2009). This might be important ecologically, because organisms that cannot sense their food or detect the presence of their predators will be at a competitive disadvantage. Interestingly, Pyle and Mirza (2005) and Mirza et al. (2009) have proposed that a BLM-type approach to chemosensory disruption might be feasible based on either the measured or the inferred metal residue at the olfactory epithelium, although this has not yet been developed.

Although a few BLMs have now been developed to predict chronic toxicity in a few species, these are applicable to water-only exposures, do not consider toxic mode of action, and rely entirely on inferred rather than measured LA50s. Indeed, in those few instances in which gill metal residues have been directly measured in chronically exposed animals, they are many times greater than acute LA50s, and log K and B_{max} values are altered, yet toxicity at the whole-animal level is not observed (Niyogi and Wood 2003). This illustrates that, when organisms are allowed to accumulate metals slowly during chronic exposures, much higher metal burdens may be tolerated, and the BLM constants are altered. At least in part, this might be explained by the induction of detoxification mechanisms, although other types of acclimation might also be involved. Again, this argues against the use of a single CBR for all exposure conditions.

Given all these uncertainties in the interpretation of tissue metal residues in organisms chronically exposed to metals, is there a way forward? One very promising development is the concept of the metabolically reactive pool (Rainbow 2002; for review see Vijver et al. 2004) that can be separated from stored and detoxified metal by classical subcellular fractionation techniques. Another promising approach is understanding the metabolism and storage mechanisms of various organisms and using this information to select specific species for monitoring metal exposure (e.g., insensitive organisms producing granules) and other species for assessing potential for effects (e.g., sensitive organisms that have limited detoxification abilities). This latter approach is discussed further below.

Intracellular speciation modeling

The ultimate success of the CBR approach will depend on the existence of reasonably definitive metal residue–effect relationships. The establishment of a definitive set of CBRs, for various metals, organisms, and endpoints continues to be an elusive goal. The BLM was developed as a tool for evaluating the effects of water chemistry on dissolved metal bioavailability and the potential for adverse effects on aquatic organisms (Di Toro et al. 2001). As discussed above, the BLM

predicts the metal concentration at a somewhat generically defined biotic ligand. This approach is consistent with the concept of CBR and tissue residues, but at a more fundamental level of cellular organization. Some important concepts that were highlighted by the BLM development effort are relevant to consider. First, it is important to consider water chemistry and speciation when evaluating the toxicity of metals to aquatic organisms. Additionally, when considering tissue metal concentrations, it is not simply the environmental chemistry of the metal that is important but also the internal biochemistry and intracellular chemistry. Finally, knowledge of metal accumulation at the site of toxic action should add explanatory power to any such evaluation. Therefore, a physiologically based pharmacokinetic (PBPK) model that simulates metal accumulation at the organ-specific level could serve as a logical refinement to the whole-body–based CBR approach (Thomann et al. 1997).

Initial efforts to develop a PBPK model for Cu accumulation by bivalves encountered several difficulties. First, the tissue Cu concentrations for the zebra mussel (*Dreissena polymorpha*) and the blue mussel (*Mytilus edulis*) appeared to be well-regulated (i.e., they remained nearly constant), insofar as dissolved or particulate Cu varied over a range of relatively low Cu concentrations. Second, during somewhat longer duration studies with *Mytilus* (>82 d), the increase in tissue Cu was more than proportional to dissolved Cu when the concentration exceeded the upper limit of Cu regulation, about 3 μg/L. With respect to the bioaccumulation factor (BAF), it decreased as dissolved Cu increased from <1 μg/L to 3 μg/L and then increased as dissolved Cu increased above 3 μg/L. At the same time it was also observed that the measured gill and digestive gland biota-blood accumulation factors (BBAF; the ratio of organ Cu to hemolymph Cu) for Cu increased markedly over the course of the 82-d exposure (Paquin et al. 2007; Salazar and Salazar 2007). Neither the whole-body kinetic model nor the PBPK model that had been applied to these data sets had the capability to simulate these observations. An exploratory modeling effort was therefore initiated to gain an improved understanding of these observations and how they might be modeled.

The CBR approach, which is based on the idea that a tissue residue concentration can be related to an effect, is expected to facilitate the establishment of relationships between exposure and effects. The reason is that it makes use of the concentration of metal in the tissue, metal actually taken up by the organism, regardless of how external factors have affected the bioavailability of ambient dissolved and particulate metals. It would seem to follow that increasing the level of specificity, by evaluating target organ-specific residue concentrations, should serve as a refinement to use of the CBR approach on a whole-body basis. When the PBPK model was used to simulate results for bivalves from San Diego Bay, California, USA, it provided information on concentrations in individual organs. The model was set up to maintain a constant ratio of organ Cu to hemolymph Cu (i.e., a constant BBAF). At the sampling station with the largest increase in tissue Cu levels, the data indicated that this was a reasonable approach for most of the tissues but not for the bivalve gill and digestive gland. The Cu concentrations in these two organs increased more than 34-fold, compared with only about a 5- to 10-fold increase in the other organs, and the BBAF increased nearly 10-fold, while remaining nearly constant in the other organs. It would seem reasonable to expect that this response was probably related to metal detoxification processes, and MT induction in particular, a process that is more effectively induced in the gill and digestive gland than in other organs (Viarengo et al. 1981).

The preceding result was one of several studies that led to the development of an intracellular speciation (ICS) model as a way to understand better what was happening to the bivalves. The conceptual framework that was used is an adaptation of the description provided by Mason and Jenkins (1995) in their review of metal detoxification processes. It is also similar in concept to the framework described below, in that it attempts to quantify and differentiate between biologically inactive metal (BIM) and biologically active metal (BAM). As shown, 3 general classes of ligands have the potential to interact with the accumulating metals within this internal milieu. The first group consists of ligands that are activated by an essential metal such as Cu and require some minimum Cu concentration to function properly and elicit their intended beneficial effect. These same ligands can also be inactivated by nonessential metals, leading to adverse effects. A second pool of susceptible target ligands is analogous to the biotic ligand in the BLM. This pool includes high-molecular-weight (HMW) proteins and intracellular enzymes that are inactivated by either a nonessential metal or an excess of an essential metal. Finally, the third class of intracellular ligands can bind the excess cytosolic metals, rendering them biochemically inert and, potentially at least, limiting their interaction with target ligands. This latter pool includes glutathione (a ubiquitous ligand that is present at relatively high concentrations but binds metals relatively weakly), MT (an inducible, sulfhydrylrich ligand that binds metals relatively strongly), insoluble granules, and other intracellular particulate material (e.g., nuclei, mitochondria, and the endoplasmic reticulum, which may be potential biotic ligands themselves). As long as these metal-binding ligands are present at concentrations high enough to prevent the accumulation of excess bioreactive forms of essential metals and nonessential metals, toxicity is mitigated. At least for some metals, the metal:MT complex is thought to be transferred into membrane-bound vesicles (lysosomes) that are then excreted from the organism. Although the binding affinity of the metal:MT complex is high enough to decrease intracellular free Cu concentrations markedly, residual free or labile Cu is still expected. After being transported into the lysosome, however, Cu is effectively sequestered from the metal-sensitive biotic ligands and is no longer in equilibrium with free metal ions within the cytosol. Although details of how the preceding processes are linked are somewhat uncertain, this is in general the way in which the ICS model has been structured to date.

The most useful level of specificity for the purpose of representing a cytosolic biotic ligand is unclear at this time. One approach is to follow the lead of Winge et al. (1974), who were the first to associate the onset of adverse effects with what they termed spillover of Cd from the MT pool in rats. Similarly, Din and Frazier (1985) also worked with rats that had been injected with Cd and showed that inhibition of protein synthesis in rat hepatocytes could be related to non-MT–bound cell Cd in a dose-dependent manner. Results of other investigations have shown that additional specificity may be provided by associating effects with the concentration of metals in an HMW pool (Brown and Parsons 1978;

Harrison et al. 1983; Brown et al. 1990) or in a metal-sensitive fraction (containing organelles and heat-sensitive proteins, and described as enzymes; Wallace et al. 2003). As an example, the 3-week survival of *M. edulis* was found to vary with the cytosolic Cu in the HMW enzyme pool of the digestive gland in a dose–response manner (Harrison et al. 1983). Finally, it might be possible to associate effects with the activity of one or more highly specific proteins such as Na^+/K^+-ATPase and/or carbonic anhydrase. The activities at the gill of these ionoregulatory proteins are inhibited by some metals, and the response is directly related to the degree of adverse effects (Morgan et al. 2004).

In summary, various levels of specificity can be considered in metal bioaccumulation models, including whole-body, organ-specific, and intracellular levels of detail. Ultimately, for models to predict the effects of metals accurately, the amount of detail at the cellular level must increase. Although increasing the level of detail will be associated with an increased level of effort and complexity, it also provides additional information and insight about the underlying processes and mechanisms of accumulation and effects. The appropriate level of detail that will be required to perform a meaningful evaluation of metal accumulation and an assessment of the potential for adverse effects remains to be determined. It might ultimately depend on the nature of the specific problem at hand. Consideration of intracellular interactions in an effort to relate accumulation to effects probably will produce benefits beyond gaining an improved understanding of the mechanisms of toxicity. As one example, it should provide further insight into the kinetics of metal accumulation and the differences observed among species in response to that accumulation.

Selenium case example

The US Environmental Protection Agency's draft fish-tissue-based criterion for Se (technically a metalloid; USEPA 2004) provides an example of a tissue-based criterion approach. Selenium represents a strong candidate for a tissue-based criterion because multiple inorganic and organic forms can occur in the aquatic environment, each of which is differentially bioavailable, bioaccumulative, and toxic. The relative amounts of each Se form are in turn driven by site-specific factors, such as biological productivity and oxidation-reduction potential. Furthermore, dietary exposure to organoselenium is the most important exposure route for fish, the most sensitive taxa in aquatic systems (DeForest et al. 1999). Thus, substantial uncertainty exists in trying to regulate or interpret the likelihood of Se-related impacts based on a single aqueous-based Se criterion broadly applied over a wide range of site types.

In fish, adults may be unaffected by Se exposures, but Se maternally transferred to the eggs can cause embryo or larval mortalities and deformities if egg Se concentrations are sufficiently high (DeForest et al. 1999). Additionally, juvenile fish directly exposed to dietary organoselenium can also be sensitive. The USEPA (2004) selected the whole-body tissue residue for deriving the draft tissue-based criterion for reasons of sampling practicality (tissue available year-round, easier to obtain adequate sample mass) and because a larger toxicity database could be compiled. In compiling whole-body–based toxicity values for Se, the USEPA also estimated whole-body Se from concentrations in individual tissues (liver, muscle, ovaries), when necessary, based on regression relationships for other species (predominantly bluegill, *Lepomis macrochirus*). Summary statistics for the complied Se toxicity data were expressed using on the following hierarchy depending on data availability: 1) EC20 (20% effect), 2) geometric mean of the NOEC and LOEC, 3) NOEC (when no adverse effects were observed at the highest concentration), or 4) LOEC (when adverse effects were observed at the lowest concentration).

The USEPA derived 9 species mean chronic values (SMCVs; the geometric mean of chronic toxicity values for each species) ranging from 9.32 and 9.50 μg/g dry wt for rainbow trout (*Oncorhynchus mykiss*) and bluegill, respectively, to >23.28 μg/g dry wt for flannelmouth sucker (*Catostomus latipinnis*). Thus, the range in fish sensitivities to Se, expressed on a whole-body Se basis, is not large. However, given the narrow range in Se essentiality and toxicity, and the range in background Se concentrations across the United States, even small differences become important in determining the broad applicability of a single tissue concentration. Ultimately, the USEPA lowered the draft whole-body criterion to 7.91 μg/g dry wt based on the sensitivity of bluegill simultaneously exposed to cold stress, which includes a summer–fall monitoring trigger of 5.85 μg/g dry wt.

Based on the above overview, the following observations and conclusions can be made. First, the tissue residue approach is scientifically defensible for Se and decreases much of the uncertainty in regulating Se in aquatic systems. Although there is still disagreement in the scientific community on the appropriate tissue-based criterion, the possible range in appropriate fish tissue-based criteria is much less than the range in similarly protective water Se concentrations across a range of site types.

Second, in order to derive 9 SMCVs, tissue-based Se toxicity data were compiled from a wide range of studies with exposures based on differing Se forms (dietary selenomethionine, dietary selenite); different exposure routes (diet, maternal transfer); and different endpoints (larval deformities, juvenile mortalities), life stages, and exposure durations. Furthermore, the draft criterion is based on a single study with a single Se exposure and cold-water temperature regime. These uncertainties in identifying and interpreting tissue-based toxicity values will be relevant to other metals and metalloids as well, because such studies have not been standardized. Thus, one must consider a variety of studies with varying exposure routes, durations, and endpoints and use the weight-of-evidence and professional judgment to identify possible tissue-based criteria for metals and metalloids.

Third, although much of the Se toxicity data suggest that whole-body Se correlates reasonably well with adverse effects, including reproductive effects, ratios between whole-body and reproductive-tissue (ovaries, eggs) Se concentrations have largely been measured only for bluegill. It is unclear how well these relationships hold for other fish species. For example, Holm et al. (2005) found that the ratio between muscle Se and egg Se was much lower in rainbow trout than in brook trout. It is unclear whether there are species-specific differences, because timing of sampling probably also influences these ratios. The tissue residue approach is more reliable and useful if the tissue measured is closely related to the site of action (i.e., ovaries for Se).

Fourth, an important consideration in developing a tissue-based criterion is the ability to monitor the relevant effect in

the field. For Se, a key endpoint is embryo–larval deformities following Se exposure via maternal transfer. The fact that adult fish are relatively insensitive to Se provides assurance that there is an organism to sample, whether it be whole-body tissue, ovaries, or eggs (assuming that potential Se impacts are detected before large-scale population declines). An additional benefit is that many larval deformities are Se specific and, thus, can be used to confirm the predictions based on Se measured in whole-body or reproductive tissues.

Overall, therefore, the tissue residue-based approach appears to be more defensible and reliable for assessing potential Se-related impacts than measuring Se concentrations in surface water. A key challenge in the USEPA's approach for deriving the draft tissue criterion for Se, an approach that probably would apply to other metals and metalloids as well, is the wide variation in toxicity studies linking tissue concentrations to adverse effects and deciphering the relative environmental relevance of each (for example, studies vary in terms of the exposure routes, Se forms, endpoints, and life stages evaluated). Additionally, this case example demonstrates that surrogate measures for assessing the likelihood of Se toxicity, such as measurement of whole-body Se, can vary by species. Finally, when selecting appropriate tissues and endpoints to monitor in the field, consideration should be given to the feasibility of implementation. The relative insensitivity of adults, for example, results in this life stage being a good Se monitor in the field, whereas directly measuring Se in a sensitive life stage may preclude the ability to determine whether impacts have occurred. The application of this line of reasoning to different life stages is analogous to the approach proposed herein, to monitor tissue concentrations in specific MA species as a way to protect MT species.

One final point regarding the Se case example should be considered in the context of applying tissue residue thresholds to metals in general. It is important to recognize that the tissue residue approach for Se considers only fish because they are the most sensitive taxa. Fish all have the same general accumulation, elimination, and detoxification strategy for Se, and this similarity probably drives the relatively narrow range of thresholds estimated for different species. This will not be the case for most metals, for which invertebrates, with their wide variety of metal-handling strategies, will create many of the complications in applying a tissue residue approach that were discussed above.

Cadmium case study

Cadmium was used as a case study for evaluating the feasibility of linking whole-body and individual tissue residues to adverse effects in aquatic biota in the laboratory and as a mechanism for identifying and evaluating uncertainties in translating this approach into the field for metals. Cadmium was evaluated specifically for this workshop because it is nonessential and poorly regulated by organisms, thereby increasing the likelihood that whole-body residues will increase with increasing exposure concentration. It is also a relatively common contaminant of concern that has been extensively studied. The salient results and observations from this review study are summarized here.

Laboratory toxicity studies in which Cd concentrations were measured in the tissue of exposed organisms were compiled based on a review of the scientific literature. Data were compiled from subchronic and chronic studies with endpoints such as survival, growth, reproduction, and development. Studies that evaluated only unrealistically high aqueous Cd concentrations (e.g., >1 mg/L) were not considered. For each study reviewed, Cd tissue concentrations and corresponding response data were compiled, and NOECs and LOECs were identified. A sufficient number of studies was found to present a case study for discussion. The following highlights some of the key observations and conclusions.

Most of the laboratory toxicity studies in which Cd was monitored in the tissue of exposed organisms are based on water-only exposures, a few are based on diet-only exposures, and fewer still are based on combined water and dietary exposures. The prevalence of water-based exposure studies might underestimate the relationship between Cd toxicity and bioaccumulation in a natural scenario, however, there is conflicting evidence on whether diet-only exposures may over- or underestimate toxicity, because data are limited and the response may be species specific. It is apparent that, for tissue residue effect concentrations to be applicable to the field, laboratory toxicity studies should address combined waterborne and diet-borne metal exposures.

The results of this assessment are presented as an SSD for discussion purposes. Based on the available data for Cd, fish as a group have the lowest whole-body LOECs, followed by crustaceans, amphibians, molluscs, and polychaetes (Figure 3). Because aquatic taxa have different methods and capacities for detoxifying and storing metals, a whole-body Cd concentration associated with toxicity in one species is not necessarily toxic in another species, so it is unlikely that a single critical whole-body or tissue concentration would apply to all species (see below under *Developing models of metal accumulation for sensitive and surrogate species* for further discussion of this issue).

A true sensitive species is an organism that shows toxicity at a low external (bioavailable) exposure concentration and low internal body concentration, i.e., high uptake rate constants, comparatively low elimination rate constants, and limited detoxification capacity. In interpreting the NOEC and LOEC values associated with tissue residues, it is crucial to understand that the relationship between tissue concentrations and toxicity effects depends on several factors. Therefore, whole-body- or tissue-based species sensitivity

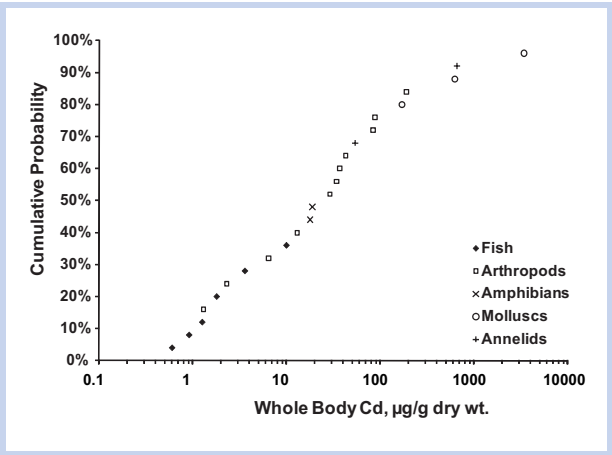

Figure 3. Species sensitivity distributions of whole-body Cd LOECs for different groups of aquatic taxa.

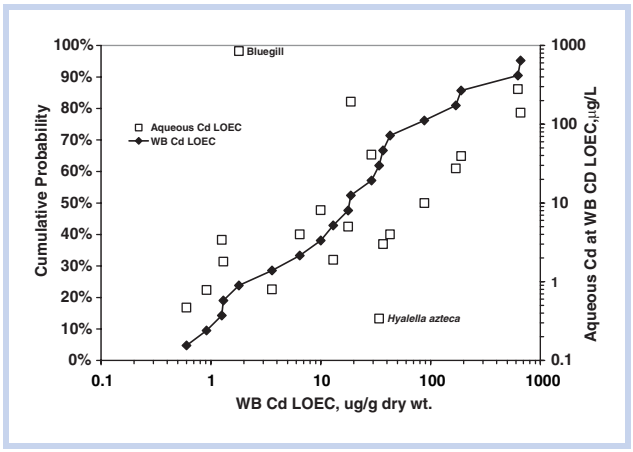

Figure 4. Cumulative distribution of whole-body Cd LOECs and associated aqueous exposure concentrations. Lozenges = LOECs expressed as the whole-body Cd concentration for the same data; squares = LOECs expressed as the aqueous Cd concentrations. For example, based on the whole-body Cd LOEC, *H. azteca* ranks approximately in the 60th percentile of the distribution, but the associated aqueous Cd concentration in this study was 0.35 μg L^{-1}, making it the most sensitive taxa in the distribution based on aqueous Cd exposure.

distributions, such as the Cd example in Figure 3, should be interpreted with caution.

For example, comparison of the cumulative distribution of tissue residue-based chronic effect concentration with their respective aqueous exposure concentrations (traditional data used to establish water-quality criteria) suggests a ranking of species sensitivities slightly different from a ranking based on aqueous exposure concentrations (Figure 4). Without information on the exposure conditions and time frame of the exposure, the whole-body or tissue residues have little meaning within a toxicological context. Therefore, whole-body residues should be interpreted in relation to the external exposure conditions, including consideration of metal availability, exposure route, and time. The data presented in Figure 3 do not separate route of exposure and therefore do not allow for assessment of this factor.

The potential for metal toxicity in aquatic biota appears to be a function of the metal uptake rate, more so than the absolute concentration in the target tissue (Rainbow 1996; Luoma and Rainbow 2005). Limited data on uptake rates in long-term toxicity studies are available for Cd; however, zebra mussel (*Dreissena polymorpha*) data from Kraak et al. (1992) and bull trout data from Hansen, Welsh, et al. (2002) demonstrate that whole-body concentrations associated with toxicity are a function of exposure time. In other words, whole-body residues resulting in toxicity following exposure to an elevated Cd concentration might not be toxic if they are achieved at a slower rate during exposure to a lower Cd concentration over a longer period of time, which is consistent with the discussions above.

The importance of uptake rate has been reported in a number of laboratory and field investigations (Kraak et al. 1992; Andres et al. 1999; Hook and Fisher 2002). For example, Andres and coworkers deployed *Corbicula* at several stations located along a Cd gradient. None of the clams survived the third sampling at a location where tissue Cd had increased to about 6 μg/g (wet wt) within about 50 d. This is in contrast to the clams at another location, where tissue Cd increased more slowly, to essentially the same concentrations (6 μg/g wet wt) over 150 d, and survival was high. The point to bear in mind from this example is that tissue metal concentrations alone might not always directly indicate effects. Instead, factors such as uptake rate will be important in many circumstances, possibly contributing to some of the variability in results when efforts are made to define CBR relationships for metals. The relationship between bioaccumulation rate and exposure concentration, as well as exposure time, complicates extrapolation of toxicity data from one set of exposure conditions in the laboratory to organisms collected in the field under another set of conditions.

No data are available for evaluating the extent to which varying water-quality characteristics (e.g., ion concentrations) influence the relationship between whole-body Cd concentrations and toxicity during long-term exposures. Although water-quality characteristics can affect tissue metal accumulation (see, e.g., George and Coombs 1977, for Cd), there is considerable uncertainty regarding the broad applicability of such relationships to different organisms and metals. As a result, more work is needed before such interactions can be reliably incorporated into predictive models of metal accumulation.

In a number of toxicity studies, both Cd bioaccumulation and toxicity have been measured in aquatic biota. Because Cd is a nonessential metal and is not well regulated via balancing of uptake and excretion, whole-body Cd concentrations in aquatic biota are generally reflective of concentrations in abiotic media (McGeer et al. 2003; DeForest et al. 2007), which perhaps increases the feasibility of developing Cd tissue-effect residues. However, several uncertainties have to be resolved. Cadmium can be sequestered in detoxified forms (e.g., lysosomal residual bodies) in the tissues of some invertebrates, such that an understanding of the metabolically active metal present in the tissues is required to link internal Cd concentrations to effects. Also, additional studies that evaluate the influence of combined waterborne and dietborne exposures at environmentally relevant concentrations on Cd bioaccumulation and toxicity are needed, along with studies on how varying water-quality characteristics influence possible linkages between internal Cd concentrations and toxicity. Finally, and perhaps most crucially, additional studies are needed on the relationship between Cd bioaccumulation rate (uptake, detoxification, and excretion rates) and toxicity. Reviews of published field studies would help to determine whether whole-body–based toxicity values for individual species are relevant to natural situations.

The SSD concept (e.g., Figure 3) should not be applied to the existing tissue residue-based toxicity data for Cd to identify an ecosystem CBR. The interpretation of this type of data depends on how sensitivity is defined, because the relative sensitivities of individual species to a chemical are usually determined based on their susceptibility to toxicity as a function of the external environmental concentration of the chemical. Furthermore, extrapolation of laboratory-generated tissue-based toxicity information to the field must be considered with extreme caution given all of the factors that must be controlled or integrated, such as uptake and elimination functions, dietary uptake, and other exposure and time issues.

Combining tissue residues and effects observations with measurements of uptake and elimination kinetics provides a robust approach for identification of the species that are truly sensitive in terms of environmental exposure and internal

metal concentrations. A possible approach for selecting appropriate monitoring organisms associated with a defined level of community protection is provided below.

Nickel case study

Nickel accumulation in the freshwater amphipod *Hyalella azteca* provides an example of the application of tissue residue data for quantification of bioavailable metal, identification of the cause of toxicity, estimation of cause–effect based sediment quality guidelines, and interpretation of the spatial and temporal extent of toxic effects. Several studies have demonstrated that the toxicity of metals to *H. azteca* can be predicted much more reliably from bioaccumulated metals than from metals in water or sediment, especially for non- or sparingly essential and nonregulated metals (Borgmann et al. 1991, 1998; Borgman, Néron, et al. 2001). In these studies, concentrations of metals in the bodies of surviving *H. azteca* (referred to as LBC25s) in chronic (4–6-week) exposures at water or sediment concentrations resulting in 25% mortality were much more constant than were concentrations in water or sediment (LC25s). The LBC25s can, therefore, be used to identify metals likely to be responsible for toxic effects. When *H. azteca* were exposed to sediments collected from the Sudbury area (Ontario, Canada), Ni was the only metal accumulated to body concentrations exceeding the LBC25. Hence, Ni was the probable cause of sediment toxicity to *H. azteca* in those sediments (Borgmann, Norwood, et al. 2001).

The bioavailability of Ni in Sudbury area sediments was estimated by reversing the procedure used to derive the LBC25 for Ni. The LBC25 was computed by first calculating the Ni LC25 for *H. azteca* exposed to Ni-spiked sediments (Figure 5A). The LBC25 was then estimated from the linear regression of Ni measured in *H. azteca* against Ni in sediment in the same laboratory experiments (Borgmann, Néron, et al. 2001; Figure 5B). The predicted mean LC25 for field-collected sediments was then computed from the regression of Ni measured in *H. azteca* against Ni in sediment for amphipods exposed to field collected sediments (Figure 6A). The LC25 for Ni-spiked sediments is variable and is not a reliable predictor of effects, because the bioavailability of Ni from the sediments varies with sediment type (Figure 5B). Similarly, Ni leaching from field-collected sediments can be considerably less than Ni leached for sediments spiked with Ni in the laboratory. The predicted mean LC25 for Ni toxicity in field-collected sediments (~900 μg/g) was, in fact, higher than the observed LC25 for any of the spiked sediments (~60–400 μg/g; cf. Figure 5A for spiked sediments and Figure 6A for field sediments). The relationship between Ni in *H. azteca* and Ni in sediments shows much more scatter for field-collected sediments (Figure 6A) than for single Ni-spiked sediments (Figure 5B), which is to be expected insofar as the composition and sediment Ni-binding capacity will vary from site to site. However, this variability can be quantified (e.g., the upper and lower lines representing ±2 SD in Figure 6A).

The predicted LC25 provides a means of quantifying metal-induced toxicity in the field and has been proposed as a method for defining a cause–effect based sediment quality guideline (Borgmann 2003a). This guideline has been used to quantify the spatial extent of toxic impacts in the Sudbury area (Borgmann 2003b). For example, the predicted LC25 of ~900 μg/g in sediment occurs at about 20 km from the Ni contamination source (Ni smelters), suggesting that 50% of the lakes at this distance would demonstrate 25% mortality to *H. azteca* in 4-week exposures (Figure 6B). The lower limit for the LC25 (~100 μg/g) extends to about 60 km, suggesting that about 2.5% of lakes could have toxic sediments at this distance. The lower limit used in this example (mean – 2 SD) was chosen for simplicity, but other criteria (e.g., 90 or 95% CL) could also be used. In addition, consideration of factors that affect Ni bioavailability might also decrease the uncertainty bounds.

The relationship between predicted toxicity and distance from smelters is supported by sediment toxicity tests in the laboratory using both *H. azteca* and mayflies, as well as by the abundance of amphipods, fingernail clams, and tanytarsid midges in sediments from lakes at various distances from the

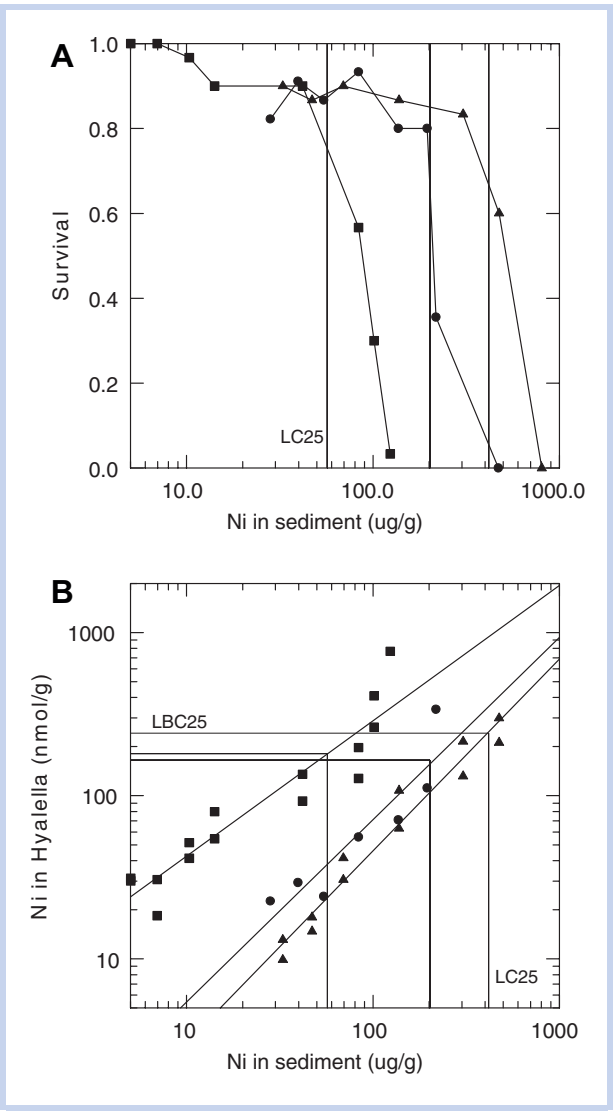

Figure 5. **(A)** Survival of *Hyalella azteca* and Ni bioaccumulation in chronic exposures to 3 different sediments spiked with a range of Ni concentrations in Imhoff settling cones. Vertical lines correspond to concentrations killing 25% of animals, relative to control (LC25), and horizontal lines indicate the equivalent body concentrations (LBC25). **(B)** Ni accumulation in *H. azteca* from the same experiments as a function of sediment Ni with corresponding LBC25. All 3 linear regressions were significant ($p < 0.01$), with r^2 values ranging from 0.83 to 0.94. Slightly modified from Borgmann (2003b); data from Borgmann, Norwood, et al. (2001).

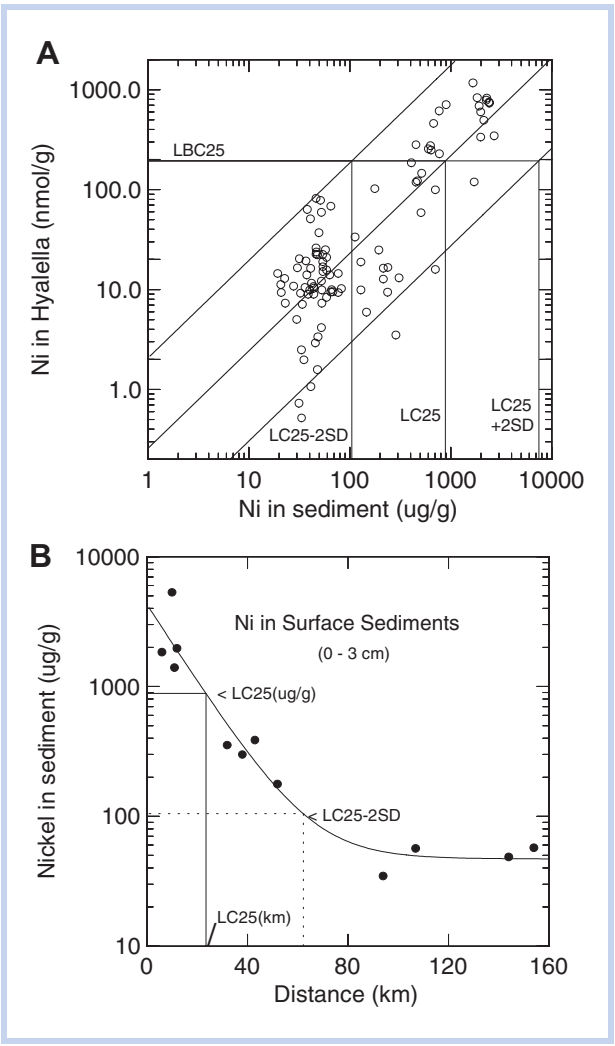

Figure 6. Nickel bioaccumulation by *Hyalella azteca* as a function of Ni in test sediments (**A**) and Ni in surface (0–3 cm) sediments (**B**) as a function of distance from smelters near Sudbury, Ontario, Canada. Vertical and horizontal lines indicate extrapolations from lethal body concentrations (4-week LBC25, nmol/g) to sediment concentration (LC25, μg/g) to distance (LC25, km). Modified from Borgmann (2003b); data from Borgmann, Norwood, et al. (2001).

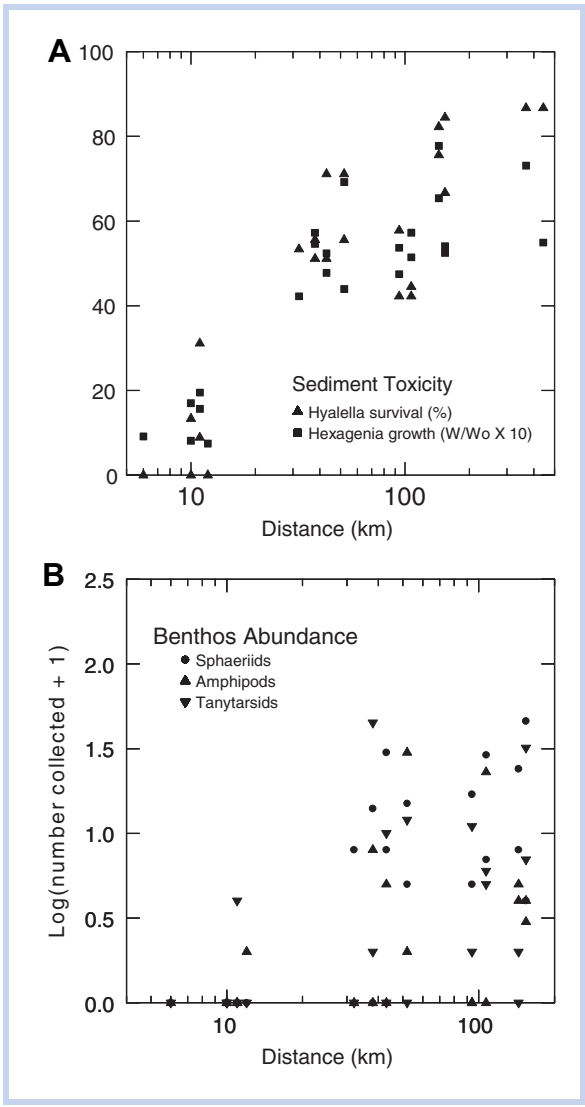

Figure 7. Toxicity of sediments to *Hyalella azteca* (percentage survival) and mayflies (*Hexagenia* sp., growth expressed as final weight [W]/initial weight [Wo] × 10) (**A**) and abundance (total number of animals collected per site) of sphaeriid clams, amphipods, and tanytarsid midges (**B**) as a function of distance from smelters in Sudbury, Ontario, Canada. Data at 440 and 500 km are Lake Erie and Hamilton Harbor control sediments, respectively. Modified from Borgmann, Norwood, et al. (2001).

smelters (Figure 7; Borgmann, Norwood, et al. 2001). This provides some field verification that the prediction of impacts is reasonable.

Two caveats must be mentioned. First, the toxicity of metals in sediments is a function of the chemistry of the overlying water (Nowierski et al. 2005). Overlying water chemistry should, therefore, reflect in situ conditions, or appropriate corrections should be made if water chemistry at the site to be protected differs considerably from that in experiments used to derive the predicted LC25. Second, the relationships shown in Figure 6A and B were obtained using sediments from Canadian Shield lakes in the Sudbury region. If and how much this differs from Ni bioavailability in other freshwater sediments must be determined.

Biomonitors for assessing ecotoxicological effects of metals

A consistent issue raised by the previous case studies is how a tissue residue approach for a specific species (e.g., *H. azteca* for Ni) or taxa (e.g., fish for Se and Cd) might be applied in the larger context of protecting aquatic communities. An approach we consider here would be to use accumulated metal concentrations that are measured in selected biomonitoring species (typically strong accumulators) as surrogate indicators of effects thresholds and metal bioavailabilities in a given habitat. The utility of such an approach would depend on the ability to establish a relationship between the accumulated total metal concentrations in that biomonitor with observed changes in endpoints of ecotoxicological significance in other organisms in that habitat. Bioaccumulation by a biomonitor is a comprehensive measure of exposure across different conditions in that habitat, and the correlated toxicological endpoint in question could be at any level of biological organization from the subcellular to the community. The more specific the nature of the endpoint, such as a

specific response to a specific metal, the closer the correlation approaches an expression of causality.

For example, the abundance of some mayfly species and the number of mayfly taxa decline in the presence of metal contamination in streams (Clements et al. 2000). Some of the mayfly species that disappear have high rates of net bioaccumulation of metals or reduced powers of detoxification of accumulated metal (Cain et al. 2004). Other species, such as caddisfly larvae of the genus *Hydropsyche*, appear to be relatively tolerant of metals and can accumulate significant metal body burdens (Cain et al. 2006). In a recent study on the Clark Fork River drainage and Silverbow Creek (Montana, USA; Luoma et al. 2010), the concentration of Cu accumulated in *Hydropsyche* spp. was strongly correlated with mayfly richness and abundance as well as total macroinvertebrate taxa richness (Figure 8). Thus, the atypically high accumulation of Cu in *Hydropsyche* larvae above a defined threshold is a surrogate measure of ecotoxicologically significant effects on sensitive taxa in the community that are not easily monitored for tissue residues.

Another example of a surrogate organism for which tissue residues could be used as an indicator of toxic effects in more sensitive species is larva of the phantom midge *Chaoborus punctipennis*. This species has been proposed as a biomonitor for Cd in water because of its wide distribution (due in part to its tolerance of high environmental metal concentrations) and easy identification and because the relationship between bioaccumulation and water chemistry is understood. Hare and Tessier (1996) and later Croteau et al. (2002) demonstrated that the accumulated Cd concentration in C. *punctipennis* could be reliably ($r^2 = 0.86$) predicted from

$$[\mathrm{Cd}]_{C.punctipennis} = F \cdot [Cd^{2+}]/([H^+] + K_a), \quad (1)$$

where $[Cd^{2+}]$ is the free ion concentration of Cd in the water, $[H^+]$ is the hydrogen ion concentration, and F and K_a are constants. The $[H^+]$ adjustment explained the increasing body concentrations of Cd observed in C. *punctipennis* with decreasing Cd in water over time in lakes recovering from acidification. C. *punctipennis* is tolerant of high (>2 μg L^{-1}) Cd in water, and its presence alone is not a useful indicator of the health of the ecosystem. In contrast, *H. azteca* is one of the most sensitive species to environmental Cd (Mebane 2006). Unlike the case with C. *punctipennis*, bioaccumulation and toxicity of free Cd ions to *H. azteca* are primarily controlled by Ca^{2+} in the water, rather than pH, under both laboratory and field conditions (Stephenson and Mackie 1988, 1989). According to the BLM, a linear relationship is predicted between LC50s or LC25s, expressed on a free metal ion basis, and competing cation concentrations (e.g., De Schamphelaere and Janssen 2002), giving a relationship of the form

$$\mathrm{LC25}[Cd^{2+}]_{H.azteca} = a + b \cdot [Ca^{2+}]. \quad (2)$$

Replacing $[Cd^{2+}]$ in Equation 1 with the LC25 from Equation 2 gives

$$[\mathrm{Cd}]_{C.punctipennis}\,\mathrm{at\,LC25}_{H.azteca} = F \cdot (a + b \cdot [Ca^{2+}])/([H^+] + K_a). \quad (3)$$

This is the concentration of Cd in C. *punctipennis* that would be expected to predict when Cd toxicity to *H. azteca* equals 25%. This assumes that the previously described linearity between the LC25 and competing cation concentrations is also applicable to chronic toxicity, which remains to be tested.

Sufficiently extensive databases now exist for additional metal biomonitors to allow these species to be used in a similar way to the use of *Hydropsyche* and *Chaoborus* described above. Candidates for coastal habitat biomonitoring include the seaweed *Fucus vesiculosus*, mussels such as *Mytilus* species and *Perna viridis*, oysters (*Crassostrea virginica* and C. *gigas*), tellinid bivalves (*Scrobicularia plana* and *Macoma* species), barnacles (*Balanus amphitrite* and *B. improvisus*), and talitrid amphipods (*Orchestia gammarellus*; Luoma and Rainbow 2008). Use of caged organisms such as bivalves or other mollusks could expand the range of environments and metals that can be monitored. The selection of a suite of biomonitors would allow identification of metal bioavailabilities in different potential sources of metal uptake in the habitat (e.g., water, food, including sediment and suspended matter; Luoma and Rainbow 2008). Biodynamic modeling studies (Luoma and Rainbow 2005) on these tolerant species would allow their future use in modeling the effects of changed metal inputs into a system, for example, as a result of regulatory action. Similar biodynamic modeling and investigation of the subcellular partitioning of accumulated metal in the metal sensitive species would increase our under-

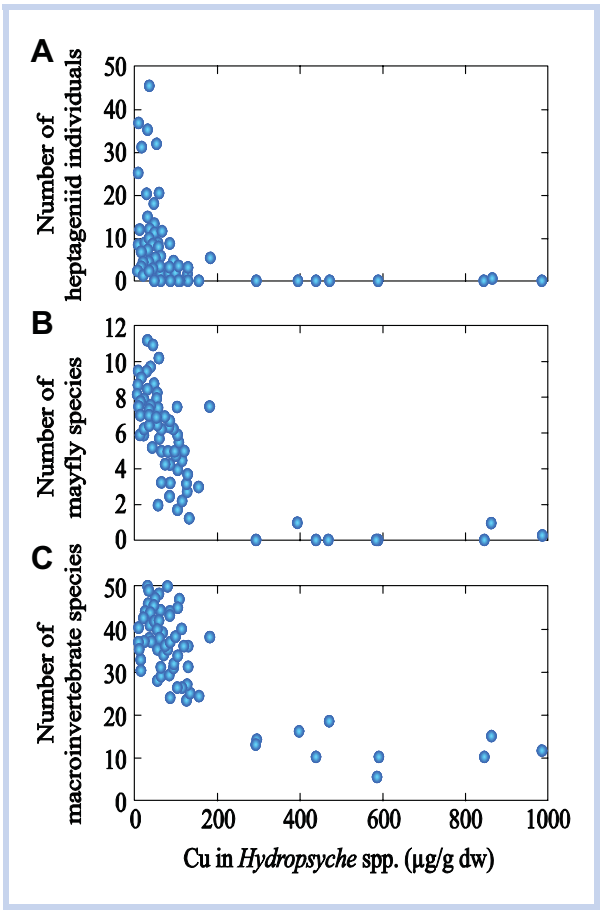

Figure 8. Three measures of ecological community structure from the Clark Fork River, Silverbow Creek, and 3 tributaries of the Clark Fork, Montana, USA, as a function of copper concentration in *Hydropsyche* sp. From Luoma et al. (2010).

standing of the mechanisms underlying their sensitivity to metals (Buchwalter et al. 2007).

The use of accumulated metal concentration as a predictor of community-level effects requires the identification of background and atypically high concentrations in these biomonitor species. To accomplish this, the relationship between the community-level effect of concern and the whole-body or tissue concentration of metal in an MA species that occurs across the range of metal exposure in a specified ecoregion must be plotted. The lower end threshold tissue residue (TR_{low}) is defined as the metal concentration in the biomonitor species at which an unacceptable community-level effect is never observed in the data set, and the upper end threshold tissue residue (TR_{high}) is defined as the metal concentration in the biomonitor species at which an unacceptable community-level effect is always observed in the data set. This leaves an intermediate range of uncertainty, bounded at the top by TR_{high} and at the bottom by TR_{low}, in which unacceptable community-level effects sometimes (but not always) occur at a given tissue metal concentration in the biomonitor species. Reference sites, at which no anthropogenic metal inputs occur, must also be identified. An appropriate reference-tissue residue might be the (upper) 90th percentile of the reference-site tissue residues ($TR_{ref,\ 90\%}$). The following 2 tissue residue ratios (TRRs) can be calculated from measured tissue residues of the biomonitor species at the site in question (TR_{site}):

$$TRR_{low} = (TR_{site} - TR_{ref,90\%})/(TR_{low} - TR_{ref,90\%}), \quad (4)$$

$$TRR_{high} = (TR_{site} - TR_{ref,90\%})/(TR_{high} - TR_{ref,90\%}). \quad (5)$$

A $TRR_{low} < 1$ (including negative values if $TR_{site} < TR_{ref,\ 90\%}$) would indicate no cause for concern; but a $TRR_{high} > 1$ would indicate an unacceptably high tissue residue with high probability of adverse community-level impacts. At sites with $TRR_{low} > 1$ and $TRR_{high} < 1$, further investigation would be warranted (e.g., proceeding to the next step in a tiered assessment; Figure 9). By definition, TR_{low} must be greater than TR_{ref}. Therefore, a major constraint on the percentile chosen for TR_{ref} is that an unacceptable community-level effect cannot occur at that tissue residue in the biomonitor species.

Mathematically, it is important to include $TR_{ref,\ 90\%}$ in Equations 4 and 5, because metal mixtures will often be considered at a contaminated site, and essential metals (e.g., Cu, Zn) accumulate at relative high concentrations in tissues. If $TR_{ref,\ 90\%}$ were not included in these equations, TRR_{low} would equal TR_{site}/TR_{low}. Therefore, at some reference sites and low-concentration contaminated sites, 2 or more essential metals might be expected to have TRR_{low} values greater than or equal to, for example, 0.5, making the sum of their TRR_{low} values >1.0, if $TR_{ref,\ 90\%}$ were not included in Equations 4 and 5.

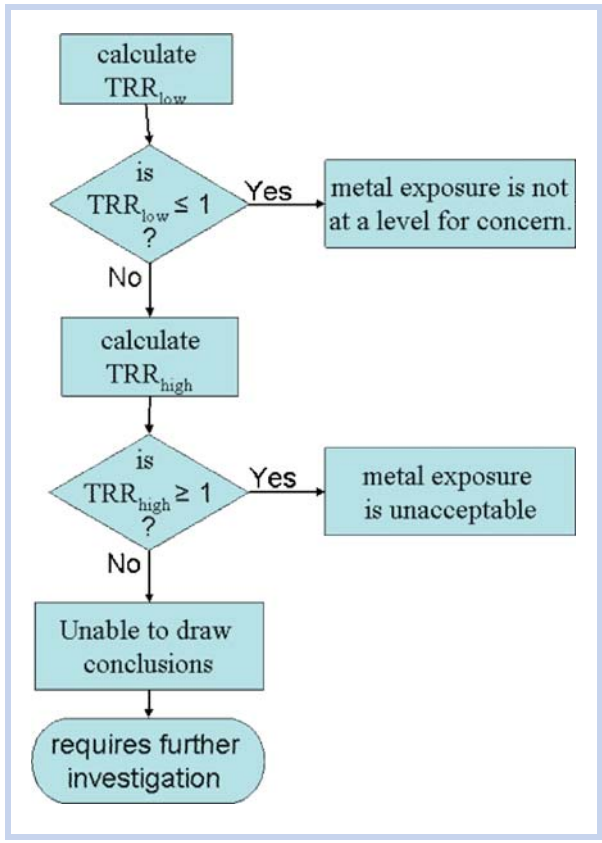

Figure 9. Decision tree for a tier 1 assessment of tissue residues of a metal measured in an MA indicator species collected from a field site. $TRR_{low} = (TR_{site} - TR_{ref,\ 90\%})/(TR_{low} - TR_{ref,\ 90\%})$, and $TRR_{high} = (TR_{site} - TR_{ref,\ 90\%})/(TR_{high} - TR_{ref,\ 90\%})$,; where TRR_{low} = tissue residue ratio low, TRR_{high} = tissue residue ratio high, TR_{site} = tissue residue measured in the indictor species collected at the site, $TR_{ref,\ 90\%}$ = 90th percentile of the tissue residues measured at reference (uncontaminated) sites, TR_{low} = highest tissue residue in the indicator organism at which unacceptable impacts to the community would not be expected to occur, and TR_{high} = lowest tissue residue in the indicator organism at which unacceptable impacts to the community would always be expected to occur.

DEVELOPING MODELS OF METAL ACCUMULATION FOR SENSITIVE AND SURROGATE SPECIES

Models of metal accumulation should be useful for interpreting results in the context of a tissue residue-based assessment. One example described herein illustrates an approach being developed to relate the intracellular fractionation of Cu to the potential for effects in *Mytilus*. A second example illustrates how a regression-based bioaccumulation model could be used in the context of a risk assessment. Another potential use would be to evaluate the change in water or sediment concentration that would be needed for the tissue metal concentration in an indicator species to decrease below a critical threshold, in the event that the threshold had been exceeded. This section describes some alternative bioaccumulation models (or modeling approaches) as examples of the types of models that can be considered. These models, which vary in level of detail, have inherent advantages and disadvantages.

The accumulation of metals and the resulting tissue concentrations are the net effect of metal uptake and elimination by the organism. Metal uptake kinetics largely depend on the concentration and availability of the metals in the environment and the binding properties and transport characteristics of the metal transporters involved in the transfer of the metals across the exchange surface. Metal elimination depends on the internal handling of the metal by the organism and is therefore expected to be considerably less dependent on external conditions. Metal uptake and elimination may be subject to a certain degree of internal regulation, as has been documented for a number of essential metals (Nelson 1999).

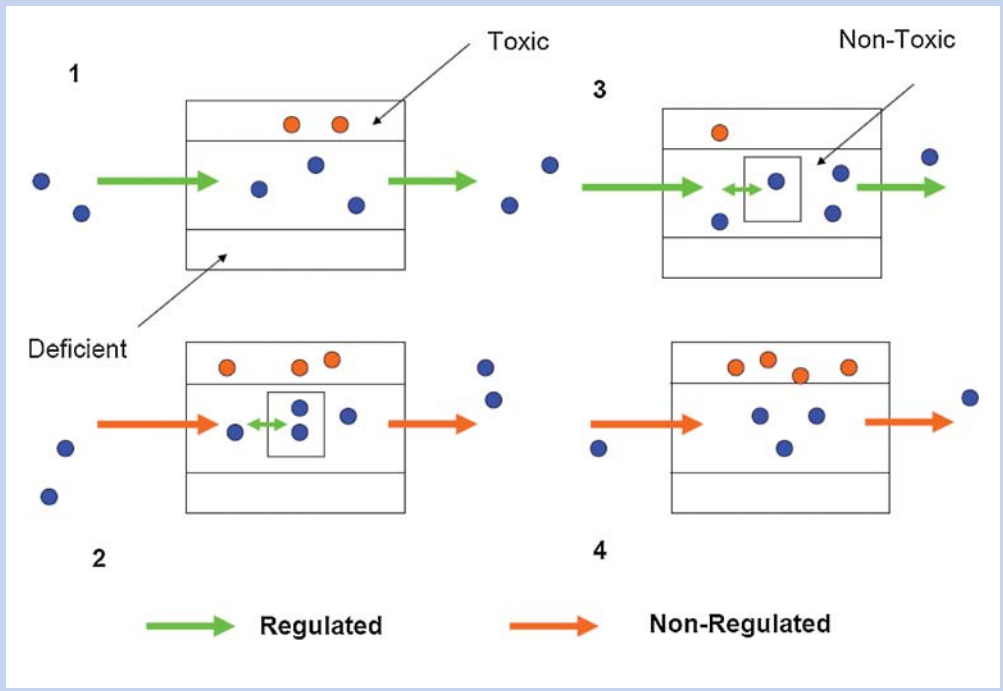

Figure 10. Theoretical metal accumulation and handling strategies found in different types of organisms. Metals may be regulated or nonregulated at the site of uptake or elimination. Organisms may or may not be able to store metals in an internal inert compartment resulting in detoxification.

Metal accumulation can be modeled by a kinetic bioenergetic-based approach in which the organism is seen as a single box or a combination of two or more boxes (Figures 10 and 11). Metal uptake is expressed in terms of uptake rate constants that account for uptake via the water phase and ingestion rate, and assimilation efficiencies that account for uptake via food or sediment. It is also possible to express uptake from food or sediment in terms of an additional uptake rate constant. Likewise, elimination kinetics are described by 1 or more elimination rate constants. Of course, an organism is more complex than a single homogeneous pool, but 1- or 2-compartment models are quite capable of describing metal accumulation kinetics over time (Luoma and Rainbow 2005). In fact, making more complex models does not make them necessarily better in terms of fitting or prediction unless high-resolution data are available, including tissue compartmentalization, which is usually not the case. The appropriate model complexity will also depend on the question to be addressed.

The preceding modeling approach was illustrated by Luoma and Rainbow (2005), who showed that metal accumulation can be predicted accurately using the dynamic

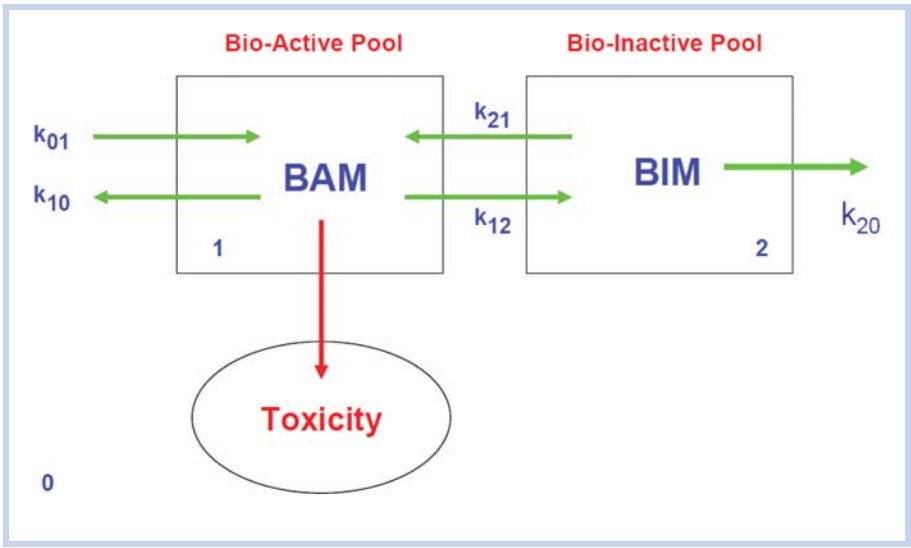

Figure 11. Schematic depiction of the biologically inactive metal (BIM) and biologically active metal (BAM) model. Metals enter the BAM compartment via the water or ingestion. Metals are detoxified by transfer from the BAM to BIM. Metals can be eliminated from the body via the BAM or BIM. The rate constants determine the transfer rate between different compartments, which may differ for water and food. Metal toxicity is a function of metal in the BAM compartment.

bioaccumulation model DYN-BAM, a relatively simple 1-compartment bioenergetics-based metal accumulation model that has been applied to a number of invertebrate and fish species under varying environmental conditions. Models such as DYN-BAM use uptake rate constants to account for uptake via the water phase and ingestion rate, and assimilation efficiencies to account for uptake via food or sediment. The elimination kinetics are described by 1 elimination rate constant. For different metals and species, site-specific uptake rate and other constants have been obtained to make predictions. Predicted metal accumulations with this model are generally within a factor 2 of measured median tissue concentrations over 11 different ecosystems, an excellent result given that the data represent independently observed metal accumulations from the field. An important strength of this model is that considerable effort has been expended to measure the kinetic coefficients that are required as inputs for simulating a variety of aquatic organisms and metals as well as the fact that the model has been widely applied (Reinfelder et al. 1997; Wang and Fisher 1997; Fisher and Wang 1998; Luoma and Rainbow 2005). Although the potential exists for using these same coefficients in other models, it is incumbent upon the analyst to ensure that the uptake and elimination terms are formulated in a consistent manner.

Although Luoma and Rainbow (2005) used site-specific rate constants to model accumulation, it is also worth exploring the extent to which more generic rate constants can be derived, for a given species, and adjusted to a specific situation to make predictions. For example, experimentally derived uptake rate constants expressed on the basis of total metal concentrations in water depend on chemical speciation of the metal and other factors such as competition with major ions. The effect of different conditions on rate constants can be accounted for by using a membrane transport or enzyme kinetics type of submodel in which the rate constants are expressed with respect to the bioavailable metal fraction in the exposure water and the effects of major ion interactions on the metal transporters (Chowdhury and Blust 2001). In this way, the metal bioaccumulation model incorporates the effects of differences in environmental conditions and chemistry and can be used to make site-specific predictions.

In principle, the same approach can be applied to model food web transfer by linking a number of species-specific models to each other. The models can also be used to predict bioconcentration or bioaccumulation factors, simulate how they depend on environmental conditions, and compare the results with real-world data. Thus, the kinetic bioaccumulation models can be used to predict metal accumulation by an aquatic organism under various environmental conditions and exposure scenarios. When the requisite information is available to define the effect of site-specific factors on bioavailability, such refinements to the analysis should help to reduce the uncertainty in predicted metal bioaccumulation. However, the analyst should proceed with care when applying a kinetic model to a particular organism, metal, or site, because the effect of factors such as the concentration of dissolved organic matter on metal bioavailability and accumulation varies in an inconsistent manner and for reasons that are not well understood (George and Coombs 1977; Roditi et al. 2000; Wang and Guo 2000; Guo et al. 2001; Sanchez-Marin et al. 2007).

By defining critical body or tissue concentrations, under either acute or chronic metal exposure regimes for a single species or group of species, the corresponding environmental metal exposure concentrations in water, food, or sediment can then be estimated by reverse modeling. To move from a kinetic bioaccumulation model toward a kinetic toxicity model requires the coupling of information from metal accumulation studies to results from toxicity studies. This can be done by developing whole-body or tissue concentration–effect relationships and incorporating these into the kinetic model, although as discussed throughout this paper there are numerous challenges to developing such relationships. In the case of a time-dependent shift in the shape of the response curve (i.e., the critical whole-body or tissue concentration depends on the rate of metal accumulation), these effects can also be taken into account by the model. Different approaches are possible, and one option could be to include 2 response models; one for acute exposure and one for chronic exposure scenarios, or a more general approach in which the response model is a function of both whole-body or tissue metal concentration and time.

Further detail can be added to the analysis by making use of a multicompartment modeling framework (Blust et al. 2004; Croteau et al. 2004; Crouteau and Luoma 2005). The approach used by Croteau and Luoma is based on the use of stable isotopes as an aid in model parameterization. The multicompartment modeling framework employed by Blust and coworkers simulates the uptake, accumulation, and toxicity of metals by aquatic organisms in relation to environmental conditions and routes of exposure and also attempts to represent metal in metabolically active and inactive compartments (Steen-Redeker and Blust 2004; Steen-Redeker et al. 2004). This kinetic model takes into account exposure-related aspects such as effects of chemical speciation and competition and internal distribution in relation to accumulation strategy, and it relates these factors to toxic responses in a time-resolved manner.

A compartmental model describing the organism as 2 internal compartments that accumulate metals and one intermediate compartment depicts the digestive system to account for uptake via food (Figure 11). The first of the 2 internal compartments contains BAM and the second contains the BIM. Metals inside the BIM compartment are therefore considered in a detoxified form. Metals entering the organism via either water or food enter the BAM compartment, where they can accumulate, transfer to the BIM compartment, or be eliminated from the body. When metals inside the BAM compartment exceed a threshold level, toxicity occurs. How much metal resides in either of these compartments depends on the uptake and elimination kinetics between the organism and its environment and between the 2 internal compartments. The rate constants driving the model are not exactly constant but are a function of external and internal conditions. A Michaelis–Menten type of membrane transport model is used to describe these kinetics. This transport model describes metal uptake as a saturable process and can take into account effects of environmental chemistry and other conditions on transport processes. The effects of chemical speciation can be accounted for by expressing the kinetics in terms of free metal ion activity rather than total metal exposure concentrations, and competitive and other types of inhibitions can be taken into account. For example, the effect of Ca^{2+} on Cd^{2+} uptake is a typical competitive-inhibition effect. Metal toxicity is expressed as a sigmoidal dose–response relationship with the dose being equal to the

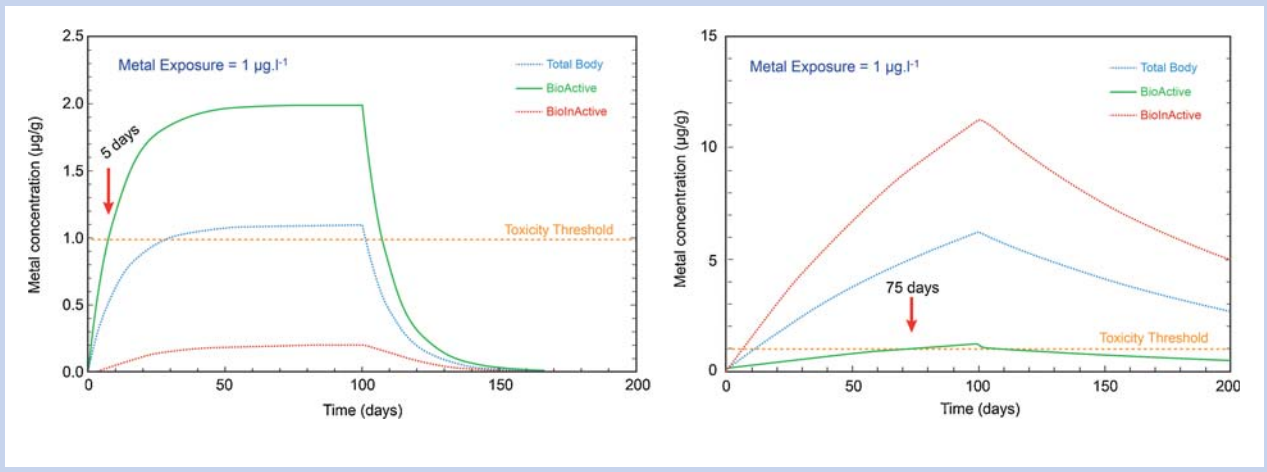

Figure 12. Biologically inactive metal (BIM) and biologically active metal (BAM) model simulation for two hypothetical organisms that differ by a factor of 100 in their rate capacity to shift metal from the BAM to the BIM compartment but are otherwise identical and exposed under the same conditions. In the first organism, metal rapidly builds up in the BAM and the toxicity threshold is reached after 5 days. In the second organism, the toxicity threshold is not reached until 75 days. Note that the total body concentration is considerably lower in the first organism when the toxicity threshold concentration is reached.

concentration in the BAM compartment. The simulations shown in Figure 12 illustrate how different the metal accumulation patterns and time dependence of toxicity can be in different types of organisms, even when the exposure is the same.

As experience is gained with use of models in tissue residue-based evaluations, and as further information regarding internal processes becomes available, the incorporation of additional features into models that are used for assessment purposes should further enhance their utility. For example, the explicit representation of intracellular reactions and transport mechanisms in a bioaccumulation model (e.g., detoxification) will provide a way to answer questions that would otherwise be difficult to address in a quantitative manner (Paquin et al. 2007). However, use of increasingly detailed models will impose an increasing burden on the user to specify a larger number of requisite model inputs. On the positive side, the additional detail that such a modeling framework provides will offer a way to build on an extensive body of information that is already available in the scientific literature and further improve our mechanistic understanding of the processes involved in metal accumulation and toxicity.

MISAPPLICATIONS

Metal bioaccumulation is influenced by exposure conditions and duration, resulting in potential misapplication of critical residue approaches, particularly on a whole-body basis. Through laboratory-based experiments with a given set of exposure conditions and at a fixed time, it is possible to determine a whole-body (or tissue) metal residue associated with a chronic endpoint. However, altering the exposure conditions will change the residue–toxicity relationship for that metal, so application to field situations introduces additional uncertainty (i.e., a laboratory exposure cannot represent all or most field conditions) and must be performed with caution. This means that there is no precise CBR for a metal at which an individual in a population dies or experiences a particular deleterious effect. This general principle has some exceptions, such as Se and some of the organometallic forms of metals. In some specific organism–metal combinations, it is possible to establish whole-body, metal accumulation–toxicity relationships that can be applied beyond the narrow conditions under which they were derived. This is supported by the work of Borgmann, Norwood, et al. (2001) with *Hyalella* sp., in which laboratory studies have been supported by assessment with field-collected sediment samples and field assessments of *Hyalella* populations.

Whole-body residues generally change with exposure because of the dynamic nature of metal uptake (influenced by bioavailability) and elimination as well as internal partitioning of bioaccumulated metal. As discussed previously, the impact of a metal is related to the amount of BAM. The BAM is influenced by the rate of metal uptake and removal from that pool via detoxification and storage (in BIM) as well as elimination. The uptake rate of a metal into an organism depends on the bioavailable concentration (food and water) as influenced by site-specific conditions, biotic and abiotic. Detoxification and elimination are related to the uptake and thus might not occur at fixed rates. Furthermore, for organisms that detoxify and store metal, the whole-body accumulation not only is exposure dependent but also is directly related to the exposure duration. Therefore, if an organism has a significant proportion of the whole-body metal burden in detoxified form, a broadly applicable whole-body metal residue linked to toxic effect cannot be identified. This is the situation for many, but certainly not all, metal–aquatic organism combinations.

In summary, the application of whole-body residues for metals as threshold criteria associated with effects must be approached with caution, rigor, and a mechanistic understanding of the metal–organism relationship. As a general principle it is unlikely that a laboratory-derived, whole-body residue–effect relationship can be applied to evaluate the potential for effects in field populations or that a relationship defined in one field situation will be applicable to another without extensive validation. This is not intended to negate the benefit of monitoring for assessing contaminant trends over time or exposure to a contaminant. There are many benefits to monitoring aside from assessment of effects.

MOVING THE RESEARCH FORWARD

Several research paths in the field, the laboratory, and the modeling arena could be pursued to advance our understanding of mechanistic underpinnings and potential applications of tissue residues of metals in aquatic organisms. Some could reach fruition relatively quickly, whereas others might yield longer term returns.

In the field, surveys of community composition and tissue residues in appropriate indicator organisms are needed to help select decision points for tier 1 assessments of metal contamination. Ideally, these surveys would be conducted in a large number of aquatic ecosystems in a variety of ecoregions, so a large range of community-effect levels could be related to tissue residues in indicator organisms. Indicator organisms should be widely distributed and should not be sensitive to the metal of interest, to ensure that the indicator organisms will be present in heavily contaminated systems; and they should be good accumulators of the metal to ensure that their tissue residues will correlate well with and integrate exposures via water and diet pathways. From those distributions of tissue residues and community-level effects, TR_{ref}, TR_{low}, and TR_{high} could be selected for each metal and each indicator species within a specified type of aquatic ecosystem, within a specified ecoregion, within a country or along a coastline, or across a continent or ocean. However, we expect that, as the range of exposure scenarios in the data set increases (i.e., as the range of combinations of waterborne and diet-borne metal exposures increases), the difference between TR_{low} and TR_{high} will increase (i.e., the range of water bodies in which inconclusive results might be expected will increase). With appropriate modification, the TRR approach could plausibly be extended to predict adverse community-level impacts of exposure to a mixture of metals and organic chemicals. This would constitute a community response indicator tissue residue (CRITR) approach that could be used in tier 1 screening as part of a site-specific risk assessment.

In the laboratory, research into the subcellular partitioning of metals is needed to identify a surrogate measure for the metabolically active pool of accumulated metal that would be more reliable than a whole-body residue. That surrogate measure could help decrease the difference between TR_{low} and TR_{high} in the tier 1 assessment approach discussed above, and, in concept at least, it might even lead to identification of a true critical tissue residue. With the currently most popular fractionation approach (Wallace et al. 2003), the metabolically active pool appears to reside within the organelle, cell debris, or heat-sensitive protein fractions (or some combination of those fractions).

In the modeling arena, dynamic models using, for example, the DYM-BAM or BIM-BAM approaches must be refined to predict tissue residues of metals better in whole body, in individual organs, in the metabolizable pool, or at sites of toxicity using algorithms that predict either explicit or implicit uptake. The DYM-BAM approach should be used for metal-tolerant species, whereas a combination of the DYM-BAM and BIM-BAM approaches will be needed to predict toxicity associated with metal uptake in intermediate- and high-sensitivity species. This will be especially useful for what-if scenarios such as predischarge planning for industry and municipalities and for reclamation of contaminated sites. Thus, a BIM-BAM type of model would have to be parameterized for intermediate- and high-sensitivity species for which DYM-BAM-type models are already parameterized, and either a DYM-BAM-type model or both types of models will have to be parameterized for species for which insufficient data are available. That parameterization would have to include considerable laboratory effort to determine uptake rates from water, assimilation efficiencies from food, generalized elimination rates from organisms, and (for BIM-BAM models) toxicities of metals to a variety of freshwater and marine species. As the mechanistic underpinnings of the relationships between toxicity and tissue residues improve, the understanding of input requirements and appropriate endpoints for these models will improve. Therefore, the quality of the models' outputs should improve, as could be evaluated using field-validation studies.

Because of the limitation of the current fractionation schemes and a lack of data on BAM, we have described an approach whereby biological monitor species are recommended as surrogates for sensitive species. For this approach to be broadly applicable, additional research is needed on the relationship between accumulation by indicator organisms and effects in sensitive organisms. Toxicity–residue relationships have to be developed, and the species have to be identified for a variety of ecosystems.

CONCLUSIONS

The metals workgroup focused its review on the fundamental principles associated with metal accumulation, storage, and regulation as well as pharmacokinetic approaches to assessing metal accumulation and effects. Case examples in which tissue residue approaches have been developed for metals were reviewed and summarized. These include, the BLM approach to relate the accumulation of metals to effects, use of metal residues in *Hyalella* to assess effects, use of a bivalve accumulation model to assess effects, the USEPA Se tissue residue data set, and the development of a data set on Cd tissue residues versus effects. Key conclusions include the following.

1. The use of a tissue residue-based approach for deriving a threshold for effects in aquatic organisms appears to be possible for Se and methylmercury. This approach might work for other organometals as well, but this was not explored.
2. Available information suggests that it is not possible to develop universally applicable whole-body CBRs for metals (except as noted above). Aquatic organisms differentially handle accumulated metals with respect to storage, detoxification, and excretion. As a result, measuring total metal in an organism provides limited information on the metal concentration associated with the biologically active pool. However, the benefits of monitoring for contaminant trend and exposure assessment are acknowledged.
3. The rapid development of BLMs (acute and chronic) for several metals provides evidence that the tissue residue-based approach can work for aquatic organisms when the target organ and receptors have been identified and the amount of metal required to produce toxicity has been measured or calculated.
4. Kinetic modeling approaches provide a unique opportunity to assess uptake and elimination rate constants as a function of exposure route and water chemistry or metal bioavailability. Recent publications offer promise for predicting field bioaccumulation using laboratory exposure

systems. Efforts to assess bioavailability and water chemistry using these models are in an early stage of development.

5. There is the potential to develop specific organism–metal–ecosystem combinations for which it will be possible to establish a whole-body metal accumulation–toxicity relationship that can be applied beyond the narrow conditions under which they were derived. This is supported by the work of Borgmann, Norwood, et al. (2001) with a sensitive amphipod (*H. azteca*), in which laboratory toxicity–residue studies have been supported by assessment with extensive field-collected sediment samples and field assessments of *Hyalella* populations.
6. An approach for using metal tissue residues was presented based on knowledge about the organism–metal metabolic strategy of specific organisms. We recommend that insensitive species such as *Hydropsche* sp., barnacles, and some bivalves be used as biomonitors of metal accumulation through comparison against their normal background concentrations. These biomonitors can then be used to assess potential impact on sensitive species that may exist in the same ecosystem or retrospectively to provide evidence on the absence of any expected sensitive species. This requires identification of appropriate biomonitor species and development of relationships between metal accumulation in the relatively insensitive biomonitor species and toxicity in the sensitive indicator species.

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