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Metal Bioavailability Modeling: Critical Review

Metal Bioavailability Models: Current Status, Lessons Learned, Considerations for Regulatory Use, and the Path Forward

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Abstract: Since the early 2000s, biotic ligand models and related constructs have been a dominant paradigm for risk assessment of aqueous metals in the environment. We critically review 1) the evidence for the mechanistic approach underlying metal bioavailability models; 2) considerations for the use and refinement of bioavailability-based toxicity models; 3) considerations for the incorporation of metal bioavailability models into environmental quality standards; and 4) some consensus recommendations for developing or applying metal bioavailability models. We note that models developed to date have been particularly challenged to accurately incorporate pH effects because they are unique with multiple possible mechanisms. As such, we doubt it is ever appropriate to lump algae/plant and animal bioavailability models; however, it is often reasonable to lump bioavailability models for animals, although aquatic insects may be an exception. Other recommendations include that data generated for model development should consider equilibrium conditions in exposure designs, including food items in combined waterborne–dietary matched chronic exposures. Some potentially important toxicity-modifying factors are currently not represented in bioavailability models and have received insufficient attention in toxicity testing. Temperature is probably of foremost importance; phosphate is likely important in plant and algae models. Acclimation may result in predictions that err on the side of protection. Striking a balance between comprehensive, mechanistically sound models and simplified approaches is a challenge. If empirical bioavailability tools such as multiple-linear regression models and look-up tables are employed in criteria, they should always be informed qualitatively and quantitatively by mechanistic models. If bioavailability models are to be used in environmental regulation, ongoing support and availability for use of the models in the public domain are essential. *Environ Toxicol Chem* 2020;39:60–84. © 2019 SETAC

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EVIDENCE FOR THE MECHANISTIC APPROACH

In 1996, the Society of Environmental Toxicology and Chemistry (SETAC) held a workshop to evaluate how regulatory criteria for protecting aquatic life could better reflect the science of metal bioavailability and toxicology (Bergman and Dorward-King 1997). This was followed by an irruption of publications on biotic ligand models (BLMs), related mechanistic or quasi-mechanistic models, and simpler empirical approaches such as multiple linear regression (MLR) models. Although some of these bioavailability models have been incorporated into regulatory frameworks, many jurisdictions retain 1980s vintage criteria. In December 2017, SETAC sponsored a follow-up workshop titled Bioavailability-Based Aquatic Toxicity Models for Metals in Pensacola, Florida, USA. The purpose of the workshop was to consider the status of different modeling approaches for predicting the bioavailability and toxicity of metals in freshwaters and their incorporation into regulatory water quality criteria. This is one of 5 articles that evaluated the performance of the

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models and sought to identify best practices in the use of these models for developing and applying bioavailability-based criteria, benchmarks, or guidelines for metals that are intended to protect aquatic life (Adams et al. 2020; Brix et al. 2020; Garman et al. 2020; Van Genderen et al. 2020).

The concept of mechanistic models incorporating metal bioavailability as a key factor governing toxicity (Paquin et al. 2002; Niyogi and Wood 2004) can be traced back to early experimental studies. These studies established that toxicity could vary considerably according to the water chemistry, reflecting influences of factors such as salinity, pH, hardness (i.e., Ca + Mg concentration), alkalinity, and dissolved organic matter (DOM; Jones 1938; Holm-Jensen 1948; Lloyd and Herbert 1962; Zitko et al. 1973). Today we recognize that these factors reflect competition by naturally occurring cations (e.g., Ca^{2+}, Mg^{2+}, Na^{+}, H^{+}) for the binding of free metal cations (Me^{n+}) to ligands on target organisms and complexation of these Me^{n+} ions by waterborne anions (e.g., HCO_3^{-}, Cl^{-}, and most importantly DOM). In both cases, the binding of the metal to ligands on the organism is decreased, thereby offering protection. The first regulatory tools incorporating this bioavailability concept proposed different ambient water quality criteria (AWQC) for freshwater and sea water and, in freshwater, applied hardness as the key factor modifying metal toxicity (e.g., Alabaster and Lloyd 1980; US Environmental Protection Agency 1986; Canadian Council of Ministers of the Environment 2007). These approaches were empirically based and either proposed different AWQC for waters in different hardness ranges or else used equations to adjust AWQC for hardness (US Environmental Protection Agency 1986). The latter were forerunners to the current MLR models that incorporate multiple toxicity-modifying factors (Brix et al. 2017a, 2020). In hindsight, it is now clear that hardness was often a surrogate for other water chemistry variables (e.g., alkalinity, specific ions, pH), which may have been equally or more important in the regression data sets that were used to derive these AWQCs and that another crucial water chemistry variable (DOM) was completely overlooked.

Pagenkopf (1983) presented the first mechanistic model, the gill surface interaction model (GSIM). This recognized that metals could bind to biological ligands on the respiratory surfaces of target organisms, thereby causing toxicity. The GSIM postulated that toxicity was attributable to free metal ions (Me^{n+}) and used trace metal speciation, gill surface interaction, and competitive inhibition to explain the protective effect of water hardness. The model also recognized that pH and alkalinity influenced metal speciation and that inorganic anions (the role of DOM was curiously discounted) could complex metals, decreasing their bioavailability. These reactions, including those at the gills, were assigned conditional equilibrium constant (log *K*) values, and steady-state conditions were assumed, allowing prediction of toxicity through equilibrium modeling. Almost simultaneously, Morel (1983) formulated the free ion activity model (FIAM), which focused on algae and made very similar assumptions to the GSIM but in addition recognized the importance of DOM in complexation reactions. Again, a similar geochemical modeling framework was used, and chemical equilibrium was assumed. In both the GSIM and the FIAM, the degree of toxic response was related to the fraction of sites to which Me^{n+} was bound, a concept that became a key component of future models. The GSIM and the FIAM can be considered the parents of modern bioavailability models such as the BLM. In this same era, the advent of geochemical modeling programs (e.g., MINEQL+, MINTEQA2) facilitated further progress. Subsequently, the development of the Windermere Humic Aqueous Model (WHAM) (Tipping 1994) incorporated multisite binding to deal with metal interactions with DOM, an important breakthrough.

Pagenkopf (1983) had proposed that the cause of lethality when Me^{n+} bound to critical sites on the gill surface was respiratory toxicity. However, many studies over the next 2 decades demonstrated that the proximate cause of lethality was interference with the active branchial uptake of either Na (Cu, Ag) or Ca (Zn, Cd, Co, Pb) from the water, at least for fish at metal levels causing acute toxicity (Paquin et al. 2002; Niyogi and Wood 2004). These were associated with inhibition of basolateral Na^{+}, K^{+}-ATPase (for Na uptake) and Ca^{2+}-ATPase (for Ca uptake), as well as blockade of apical Na^{+} and Ca^{2+} channels, and were compounded by increased diffusive losses of these major nutrient ions at higher metal concentrations. Thus, different metals targeted different specific sites (transport proteins) on the gills, and measurements of net Na or Ca loss rates to the water or net decreases in plasma or whole-body concentrations provided physiological evidence for this mechanism of toxicity.

Subsequently, Playle and colleagues made a major conceptual breakthrough based on experiments with fathead minnow and trout exposed to Cu or Ag (targeting Na transport sites) and Cd (targeting Ca transport sites) in ion-poor synthetic soft water of defined composition (Playle et al. 1993a, 1993b; Janes and Playle 1995). Natural DOM decreased the binding of metals to the gills. Through the analysis of gill metal burdens, the use of competitive waterborne ligands with known log *K* values, Langmuir isotherm analysis, and a geochemical modeling program (MINEQL+), they were able to estimate distinct log *K* (affinity) and B_{max} (site density = capacity) values for these metals at the gills in short-term exposures (2–3 h). Calculated metal accumulation on gills correlated well with measured gill metal concentrations and adverse physiological effects (e.g., Na loss attributable to Ag) in a number of different field-collected waters. These gill accumulation experiments likely reflect correlates to the true accumulation on the "biotic ligand," for there is a wide range of possible binding sites on a gill surface (including excreted mucus), with a wide spectrum of affinities for different metals. Although it is incorrect to think that by "titrating" a gill surface one can expect to probe and characterize the "biotic ligand," the strong relationships between short-term gill metal accumulation and toxicity gave operational support to gill-binding modeling.

The gill-binding model for Ag (Janes and Playle 1995) was later transformed into a physiologically based BLM by relating the gill Ag burden on trout to the fractional inhibition of gill Na^{+}, K^{+}-ATPase activity associated with 96-h mortality (McGeer et al. 2000). This work provided the mechanistic step from

short-term gill metal accumulation to the proximate cause of acute toxicity. Similar studies by MacRae et al. (1999) with trout more rigorously demonstrated that short-term gill metal accumulation (in this case Cu at 24 h of exposure) was a constant predictor of acute toxicity (in this case percentage of mortality at 120 h) among a range of test media containing different copper-binding ligands. In fathead minnow, Meyer et al. (1999) demonstrated elegantly that the gill burden of Ni at 24 h associated with 50% mortality at 96 h was constant over a range of water qualities, even though the concentration of the free Ni^{2+} ion associated with 50% mortality was not. From these studies arose the concept of the LA50, the short-term accumulation at the biotic ligand that is predictive of 50% mortality at a later time. This is a key component of all BLMs, now more commonly known as the intrinsic sensitivity parameter, which can be varied in model fitting to compensate for differences in sensitivity among species, strains, and clones. The gill log *K*, B_{max}, and LA50 concepts still underpin all modern BLMs. The terminology varies, with the gill accumulation log *K* values often referred to as biotic ligand log *K* values because gills are not the sole site of ion exchange in small animals such as cladocerans, and plants obviously do not have gills. Likewise, LA50s can be related to fractional effects other than 50% or to sublethal endpoints, and thus the LA50 term is often replaced by the more general term for a critical accumulation associated with *x*% effects (CA*x*). But despite the varied (and sometimes confusing) terminology, the underlying concepts are fundamentally similar.

Building on these early results, subsequent investigations have successfully correlated short-term gill metal accumulation with toxic effects in longer-term exposures for a variety of metals (Table 1). For example, Figure 1 shows how measured gill Ag accumulation (first bar in each pair) can be predicted in 2 forms (Ag^+ and AgCl; second bar) as a function of water chemistry (dissolved organic carbon [DOC], Cl, Na, Ca, pH) using a chemical equilibrium model (Paquin and Di Toro 2008). Toxicity data were then used to evaluate the expected LA50, and the derived BLM could then be used in combination with the LA50 to predict dissolved Ag median lethal concentrations (LC50s) over a wide range of water quality characteristics (Figure 2).

Moving from models based on physiological mechanisms and gill metal burdens to models based on toxicity only

A SETAC Pellston Workshop in Pensacola in 1996, and the subsequent book that arose from it (Bergman and Dorward-King 1997), greatly accelerated the pace of BLM development. Thereafter, landmark publications by Paquin et al. (2000), Di Toro et al. (2001), and Santore et al. (2001) laid out the formal technical framework for the BLM and demonstrated its utility in predicting acute toxicity of Cu and Ag to fish (e.g., Figure 2) and invertebrates in a range of natural waters. Although these papers were firmly rooted in the concept that the short-term metal burden on the biotic ligand was the key factor causing longer-term toxicity, they showed that this quantity did not have to be measured but rather could be back-calculated (if required) from toxicity data for the purpose of model generation. The extensive results of Erickson et al. (1996) on acute Cu toxicity to fathead minnow where single water chemistry parameters were varied, one at a time, provided the key data used to illustrate this principle.

Relative to the number of BLMs developed since that time, there are relatively few studies where the physiological mechanisms (e.g., gill enzyme inhibition, ion loss) and/or surrogates for the actual metal burden at the biotic ligand (e.g., gill metal

TABLE 1: Summary of studies in which physiological mechanisms (e.g., gill enzyme inhibition or body ion loss) and/or the actual metal burden at the biotic ligand (e.g., gill metal concentration) have been measured

Metal	Organism	Physiological endpoints	Toxicity endpoints	Sources
Ag	*Daphnia magna*	1-h whole-body accumulation, gill enzyme inhibition	Mortality not measured	(Bianchini and Wood 2003)
Ag	Rainbow trout	2- to 3 h accumulations, gill enzyme inhibition	Accumulation and 96-h mortality data related across different studies	(Janes and Playle 1995; McGeer et al. 2000)
Ag	Rainbow trout	3- and 24-h gill accumulation	Mortality at 96 h	(Morgan and Wood 2004)
Al	Atlantic salmon	140-h accumulation	Mortality at 140 h	(Santore et al. 2018)
Cd	Rainbow trout	3-h gill accumulation	Mortality at 96 h	(Niyogi et al. 2008)
Cd, Cu	Fathead minnow	2- to 3-h gill accumulations	Mortality at 96 h	(Playle et al. 1993a, 1993b)
Cd, Cu, Pb, Zn	Rainbow trout	0.75- to 24-h gill accumulation	Accumulation and 96-h mortality data related across different studies	(Balistrieri and Mebane 2014)
Cd, Pb	Rainbow trout	3- and 24-h gill accumulation; Ca^{2+} and Na^+ influx	Mortality at 96 h	(Birceanu et al. 2008)
Cu	Rainbow trout	24-h gill accumulations	Mortality at 120 h	(MacRae et al. 1999)
Cu	Rainbow trout	24-h gill accumulations	Mortality at 96 h and 30 d	(Ng et al. 2010)
Cu	Rainbow trout	24-h gill accumulations from previous work	Mortality at 96 h and 30 d	(Crémazy et al. 2017)
Ni, Cu	Fathead minnow	2- to 3-h gill accumulation	Mortality at 96 h	(Meyer et al. 1999)
Pb	Rainbow trout	3-h gill accumulation	Time to mortality of a single concentration in different waters	(Macdonald et al. 2002)
Pb	Rainbow trout	0- to 96-h Pb accumulation, enzyme inhibition, ion flux rates	Mortality not measured	(Rogers et al. 2005; Rogers and Wood 2004)
Zn	Rainbow trout	0.5- to 72-h gill accumulations	Mortality at 96 h	(Alsop and Wood 2000)
Zn	Rainbow trout	0.75- and 3-h gill accumulations	Mortality at 96 h	(Todd et al. 2009)

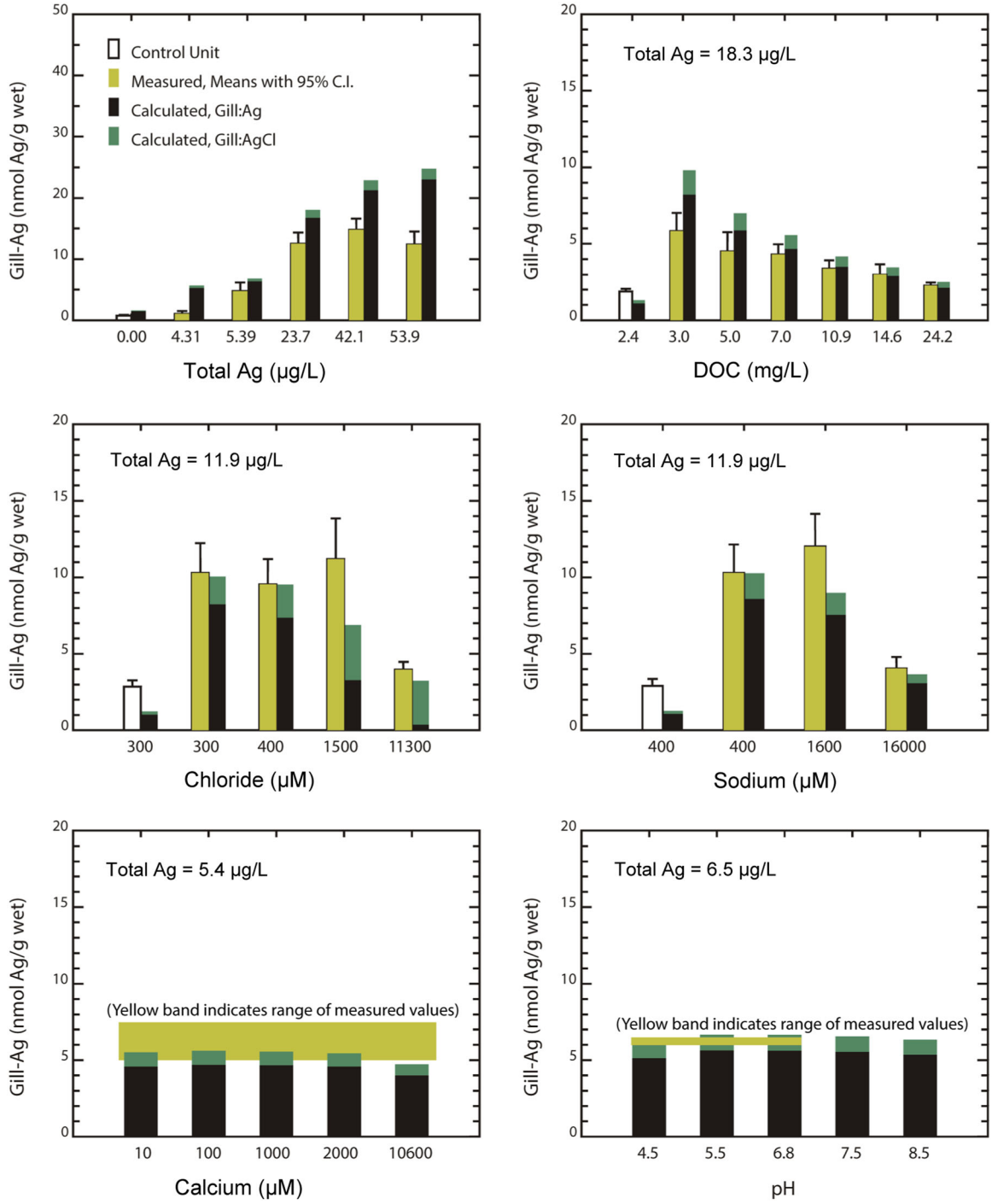

FIGURE 1: An example of a biotic ligand model calibrated to measure rainbow trout gill Ag accumulation data, over a variety of water chemistry conditions. In most cases, the patterns of measured and calculated accumulations matched well (redrawn from Janes and Playle 1995).

concentration) have been measured (see summary in Table 1). This move to model fitting to toxicity data only maintains the conceptual mechanistic framework (that a theoretical critical metal burden at the biotic ligand causes a critical level of toxicity); it has arisen partly as a matter of convenience and partly as a matter of necessity. The former reflects the time-consuming, technically demanding, and costly nature of the measurements, whereas the latter reflects the fact that in many cases the measurements simply cannot be done. Even when metal concentrations in a tissue or an organ are measured, the concentration of metal at the site of toxic action most likely is not the only accumulation being measured. Instead, it is usually assumed that the measured concentration in a tissue or organ is proportional to the currently unmeasurable concentration at the site of action. With Ni, the mechanism of chronic toxicity remains unresolved (Brix et al. 2017b); and indeed for most metals, the mechanism(s) of chronic toxicity, and therefore the target biotic ligands, remains poorly understood. Furthermore, the most sensitive organisms which "drive" AWQC are usually very

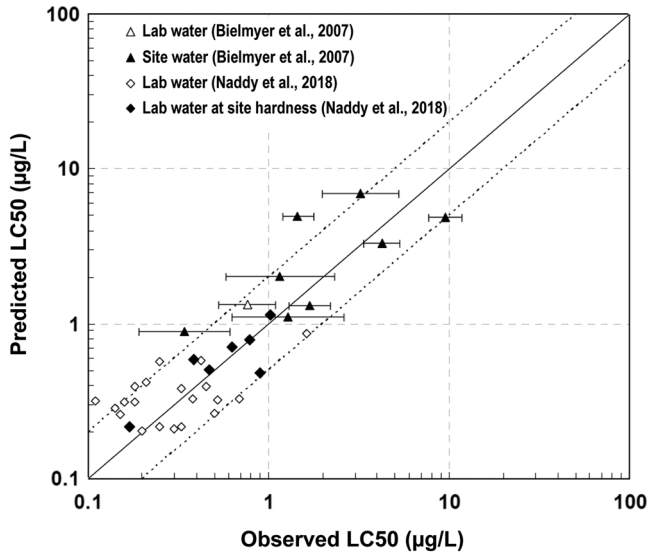

FIGURE 2: The biotic ligand model (BLM) used to predict gill accumulation in Figure 1 also predicts toxicity well. Median lethal concentrations of Ag experimentally obtained with *Ceriodaphnia dubia* from laboratory (open symbols) or natural (closed symbols) water exposures compare well with calculated BLM predictions. Solid diagonal is line of perfect agreement; dashed lines denote a factor of ±2 deviations from the 1:1 line. (Bielmyer et al. 2007; Paquin and Di Toro 2008; Naddy et al. 2018). LC50 = median lethal concentration.

small (e.g., daphnids, snails, algae), in which the biotic ligands are unknown and the metal burdens difficult to measure. However in daphnids, acute Ag toxicity, active Na uptake inhibition, whole-body Na decrement, whole-body Ag burden, and whole-body Na^+, K^+-ATPase inhibition were well correlated (Bianchini and Wood 2003); and chronic Ag toxicity seemed to result from a failure of Na regulation in both fish (Naddy et al. 2007) and daphnids (Bianchini and Wood 2002). These data provide some confidence that the conceptual framework remains valid.

In approximately 2001, the rate of BLM development was greatly accelerated by the shift from modeling based on gill metal burden/physiological effects to modeling based on toxicity data alone. De Schamphelaere, Janssen, and colleagues have exploited this approach to the greatest extent, particularly for chronic BLMs, which have been in high demand for European regulations (De Schamphelaere and Janssen 2002, 2004a; De Schamphelaere et al. 2005a, 2005b). The chemical submodel remains identical and mechanistic, whereas on the biological/ toxicity side of the model, the implicit mechanistic assumption is that toxicity results faithfully reflect an imaginary metal burden at an imaginary biotic ligand. Studies on chronic (30-d) toxicity of Cu to trout represent one of the rare cases where this assumption has been tested (Ng et al. 2010; Crémazy et al. 2017). These studies concluded that the 24-h gill LA50 predictive of 30-d mortality remained constant from pH 6.0 to 8.0 but not at extreme pH values (5.5, 8.5). However, De Schamphelaere et al. (2005b) reported that both surface-bound Cu and internal Cu concentrations were relatively good predictors of chronic toxicity (48–72 h) in algal growth tests across a slightly smaller pH range (5.9–8.5). Possible scenarios for such anomalous effects at extreme pH values are explored in the section *Dealing with extreme waters*. Regardless, if the model is calibrated with data in the water chemistry range of interest (and hybrid model strategies for doing so are outlined in the section *Complexity versus simplicity: Use of mechanistic and hybrid models to inform development of simpler models*), the model predictions should be reliable.

Incorporating dietary metal exposure into bioavailability models: The importance of equilibration

The question of whether chronic toxicity of metals in aquatic environments is the result of waterborne, dietary, or combined exposures has generated much study and extensive reviews (Clearwater et al. 2002; Meyer et al. 2005; DeForest and Meyer 2015). In nature, ingestion may be a significant route of metal uptake, and for nutrient metals (e.g., Cu, Fe, Zn) it is undoubtedly the major route. Acute models do not take this into account because testing protocols dictate that the organisms must be fasted during the exposure. Because it is almost impossible to envisage a natural situation where dietary metal would cause acute toxicity to aquatic organisms, this is not an issue of concern. However, during chronic exposures, dietary metal may contribute to toxicity or acclimation, and this may occur by both direct (metal poisoning) and indirect (metals affecting the nutritional quality of the diet or causing food aversion) routes (e.g., Irving et al. 2003; Niyogi and Wood 2003; Besser et al. 2005; De Schamphelaere et al. 2007; Golding et al. 2013; Tomczyk et al. 2018). Hook and Fisher (2001, 2002) reported extremely low waterborne effect levels for reproductive impairment when metal–exposed algae (Ag, Hg, Cd, Mn, and Zn) were fed to zooplankton; for Ag, the threshold was below the chronic AWQC. These notable results stimulated subsequent investigations, which confirmed that, in some settings, algae could accumulate metals to harmful levels from low waterborne concentrations (Bielmyer et al. 2006) but, in other settings, metal bioaccumulation occurred without obvious adverse effects (Kolts et al. 2009). Similarly, discordant results were obtained with mayflies fed Cd-exposed algae in repeated experiments (Xie et al. 2010). The reasons for the differing responses are unclear. Further, the literature is not consistent on whether metals incorporated into natural diets by chronic waterborne exposure of the prey organisms are more or less bioavailable than metal salts or prey dipped in metals (DeForest and Meyer 2015).

In the real world, we expect that a natural diet will be in some sort of dynamic equilibrium with the metal in the water column. Direct evidence for this is sparse, and metal accumulation studies have shown that time to reach constant tissue burdens ranges from hours for algae to >28 d for predatory insects and oligochaete worms (Timmermans et al. 1992; Stephenson and Turner 1993; Roy and Hare 1999; Meylan et al. 2003). Nevertheless, DeForest and Meyer (2015) argued that "exposure of test organisms to matched water-borne and diet-borne metal concentrations is perhaps the most relevant for evaluating the protectiveness of water-borne metal guidelines." In this

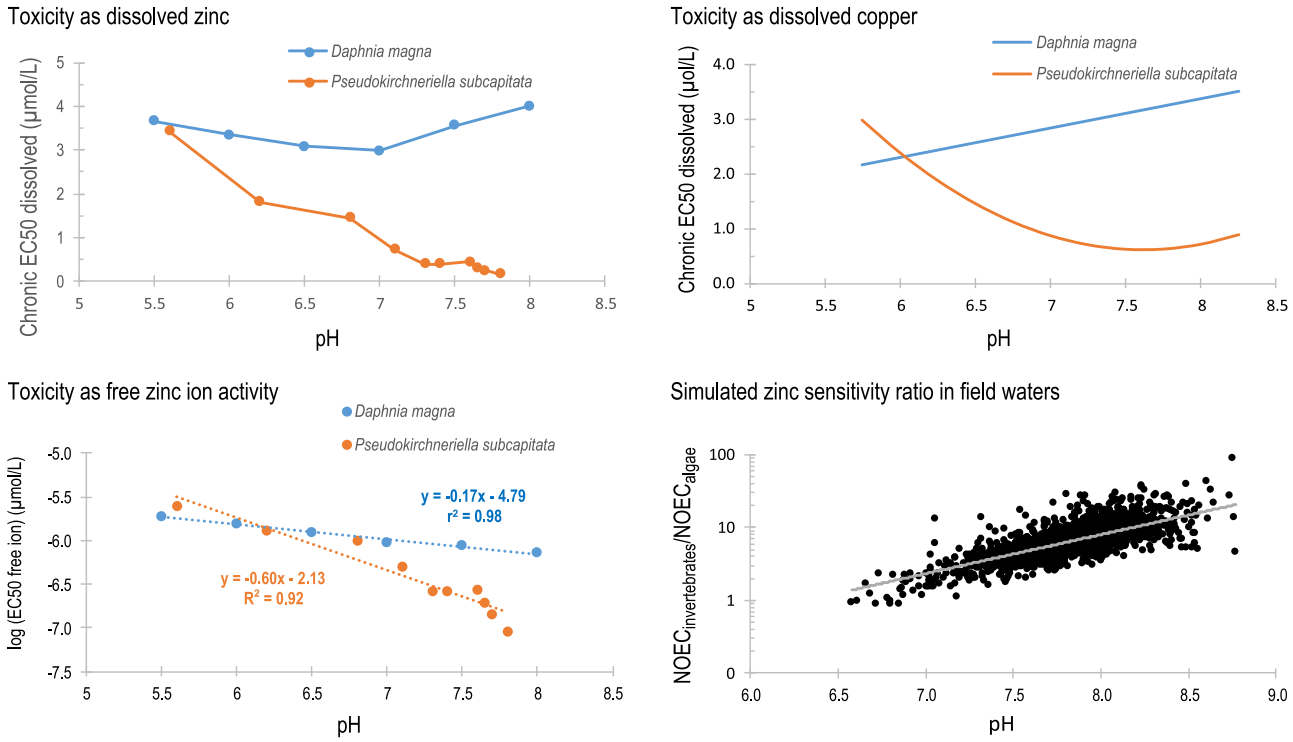

FIGURE 3: Effect of pH on dissolved zinc (upper left) and copper toxicity (upper right) to *Daphnia magna* and *Pseudokirchneriella*. The figure for zinc shows the originally reported 72-h median effect concentration (EC50) for algae biomass (Heijerick et al. 2002) and the 21-d reproductive EC50 (Heijerick et al. 2005). The figure for copper shows simulated toxicity data for the same endpoints, using multiple linear regression models fitted to data from a multivariate test design, as reported for algae (De Schamphelaere et al. 2003) and *D. magna* (De Schamphelaere and Janssen 2004b). The 2 upper panels show a clearly distinct effect trend of pH on dissolved metal toxicity and form clear examples that strongly suggest that merging algae and animal bioavailability models into a single model is not appropriate. The lower left panel shows that, when expressed on a free ion activity basis (data also from Heijerick et al. 2002, 2005), the direction of the effect of pH on zinc toxicity is the same for algae and *Daphnia*, but the magnitude of the effect is clearly stronger for algae than for daphnids. The strong effect on free zinc ion toxicity for algae dominates over the speciation effect of pH, overall resulting in increased toxicity at higher pH for algae. In contrast, at higher pH, the speciation effect dominates for *Daphnia*, explaining the decreasing toxicity toward higher pH levels. A similar reasoning applies to copper (not shown). Data in the lower left panel have been used to construct and apply separate bioavailability models for algae and invertebrates in normalizing toxicity data for criteria and predicted-no-effect concentration derivation in Europe. The lower right panel shows possible regulatory implications of the different bioavailability relationships with pH (data taken from Supplementary 5 in Van Sprang et al. [2009]). The sensitivity ratio of algae versus invertebrates (geometric mean of normalized no-observed-effect concentration of all invertebrates/algae) is simulated as a function of pH for 2250 water samples. This panel shows that, although on average algae show similar sensitivity as invertebrates at low pH (~6.5), algae become by far more sensitive with increasing pH, up to approximately 10-fold and more at pH 8 and above. Algae also become increasingly more sensitive than invertebrates at higher pH for copper and lead (not shown). NOEC = no-observed-effect concentration.

context, "matched" means that the test organisms were exposed to the same waterborne-metal concentration to which its food was exposed. This was also a key recommendation of the 2002 SETAC Pellston workshop on this topic (Meyer et al. 2005). However, this has rarely been done. There are 2 aspects to this equilibration: 1) physicochemical equilibration reflecting the slow kinetics of diffusion into and sorption onto the food item, which is mainly a concern when dead organisms or artificial food (e.g., trout pellets) are used as the diet—in this case, the same kinetic constraints as for equilibration of metal with DOM will likely apply, a process that can take 24 h or more (see section *Equilibrium issues*), and 2) biodynamic equilibration (achievement of constant concentrations) with a live diet, where the prey organisms may concentrate the metal many fold above that in the water column, which may take days to weeks.

Two recent studies assessed dietary impacts of Pb with matched waterborne and diet-borne metal exposure concentrations. These examined the interactive effects of waterborne and dietary Pb exposure in daphnids (*Ceriodaphnia dubia*; Nys et al. 2013) and rainbow trout (Alsop et al. 2016) and concluded that dietary Pb exposure to these freshwater organisms may not be of concern under the scenarios tested.

At present, there is insufficient evidence to conclude that chronic bioavailability models would be underprotective if based on waterborne-only exposures or on combined exposures with insufficient equilibration. Therefore, this should not be a reason for rejecting the large amount of otherwise high-quality data available for use in model generation or for current models that exist based on such data. However, we recommend, for best practice in the future, that during chronic tests combined waterborne and dietary matched exposures should be performed. These should be based on natural live diets that have undergone full biological equilibration with the waterborne metal through pre-exposure. If it becomes apparent during such tests that whole-body and target organ–specific metal concentrations are not at equilibrium with ambient metal concentrations

in water and food, a biodynamic modeling framework that incorporates uptake (via water and food) and elimination kinetics may be needed.

Incorporating behavioral endpoints (e.g., olfaction, mechanoreception) into bioavailability models

In most jurisdictions, mortality, growth inhibition, and reproductive inhibition are the only toxicity endpoints that can be used in a regulatory framework. Nevertheless, there is increasing evidence that disruptions of behavior caused by metal exposure may be equally or more sensitive endpoints and that these disruptions are mediated by disturbances in olfaction and/or mechanoreception (reviewed for many metals in Wood et al. 2012a, 2012b). If organisms cannot navigate properly, sense predators or prey, maintain social hierarchies, or find mates, population impacts will likely occur. Furthermore, the limited information available suggests that mechanisms governing olfactory toxicity are rather different from those governing toxicity for other endpoints. For example, for waterborne Cu, inhibitory effects are almost immediate, Ca provides little protection, the log *K* value for Cu at the olfactory rosette is lower than at the gill, and there is evidence of recovery/acclimation from olfactory inhibition during chronic exposure (e.g., McIntyre et al. 2008; Mirza et al. 2009; Green et al. 2010; Dew et al. 2012). Furthermore, there are different viewpoints (e.g., Green et al. 2010 and Dew et al. 2012 vs Meyer and Adams 2010, DeForest et al. 2011, and Meyer and DeForest 2018) on whether or not mechanistic bioavailability models based on concepts of ionoregulatory disturbance, such as the BLM-based US Cu criterion (US Environmental Protection Agency 2007), are protective against olfactory effects such as behavioral disturbance. If they are protective, it would appear that this is because highly sensitive taxa are included (e.g., cladocerans) for criteria derivation, not because the bioavailability models are mechanistically correct for behavioral endpoints (i.e., the comparison is of apples vs oranges). The matter remains unresolved, but moving forward, as argued by Pyle and Wood (2007), we recommend that mechanistically based bioavailability models for behavioral toxicity should be developed. These should be built from the ground up using behavioral endpoints, rather than by adjusting the intrinsic sensitivity parameter in existing BLMs. The areas of agreement and disagreement with models built on traditional endpoints will then be highly informative, and there will be a stronger foundation for deciding whether models based on behavioral endpoints should be used in environmental regulation.

CONSIDERATIONS FOR THE USE AND REFINEMENT OF BIOAVAILABILITY-BASED TOXICITY MODELS

Types of bioavailability-based models currently available

Table 2 presents a representative summary of available models, but a thorough listing of all models would be beyond the scope of this article. The models include classic BLMs that predict acute toxicity based on measured accumulations (Table 1); models fitted to acute and chronic toxicity data; models predicting toxicity using humic acid or surfaces as surrogates for biotic ligands; and "generalized bioavailability models," which may be as simple as a single-variable regression such as pH against free metal ion toxicity (Table 2). Metal bioavailability models directly fitted to acute or chronic toxicity data such as those in Table 2 are often used to normalize single-species toxicity data to a target water chemistry prior to inputting such data into species-sensitivity distributions for guideline development. The problem of relying on acute models to predict chronic effects is further explored in the Supplemental Data. Many recent models have also extended single-metal approaches to mixtures (Table 2).

Some themes become apparent from inspecting different models. All include chemical speciation calculations, which require as inputs at least major ion chemistry (e.g., Ca, Mg, Na, K, Cl, SO_4, and alkalinity or dissolved inorganic carbon), DOC, pH, and temperature. For shorthand, we refer to these as the "BLM" inputs. Some models, in addition, include Al and Fe. Thus, even the generalized bioavailability models (gBAMs), which predict free metal ion toxicity as a function of pH and/or free major cation activities, require the full BLM water chemistry to compute free ion activities. Further, pH is consistently incorporated as an important toxicity-modifying factor, but the direction of responses (i.e., whether an increase or a decrease in pH would increase or decrease the effect concentrations of dissolved metal) often differ between plant and animal models (Figure 4). In addition to affecting speciation, pH affects the bioavailability and toxicity of metals to plants by changing membrane permeability (Boullemant et al. 2009; Lavoie et al. 2012). These are key reasons why it will likely never be feasible to combine plant and animal bioavailability models into a single model. The important but complex role of pH in bioavailability models is discussed in the section *Complexity versus simplicity: Use of mechanistic and hybrid models to inform development of simpler models*.

Alternatives to the conventional single-site unidentate BLM

When the BLM was first formulated (Di Toro et al. 2001), the biotic ligand was considered a single unidentate binding site, for reasons of simplicity and lack of support for a more complicated formulation. It was later shown that this formulation dictates a linear relationship between the chemical activity of a competing cation and the *x*% effect concentration (EC*x*) of the toxic metal expressed as free metal ion activity (i.e., EC*x* Me^{n+}) and that stability constants of competitive cations could be estimated directly from a linear regression (De Schamphelaere and Janssen 2002). This prompted an explosion of studies (both acute and chronic) that estimated log K_{BL} values for various organisms and metals directly from toxicity data, using univariate test designs.

However, observed relationships often deviated from perfect linearity. Various potential mechanistic hypotheses have

TABLE 2: Selected examples of available freshwater bioavailability-based modes of metal toxicity

Metal	Model type or name	Reference	Organisms used in development	Organisms presumed applicable to	Necessary inputs	Example applications	Notes
Al	Acute and chronic BLM	(Santore et al. 2018)	Various fish, invertebrates and green algae	All freshwater aquatic life	"BLM"	Model supported a simplified MLR model which became federal water quality guidance for United States (US Environmental Protection Agency 2017)	Toxicity is considered a function of both dissolved and precipitated Al, caused by either ionoregulatory or respiratory disturbance depending on pH
Cd	Acute BLM	(Niyogi et al. 2008)	Rainbow trout		"BLM"		Classic BLM with binding coefficients derived from measured gill accumulation
Cd	Acute BLM	(Clifford and McGeer 2010)	*Daphnia pulex*		BLM		Binding coefficients calculated from toxicity values from a wide variety of water types
Cu	Acute BLM	(Di Toro et al. 2001; Santore et al. 2001; US Environmental Protection Agency 2007)	Fish, *Daphnia*	All freshwater animals	"BLM"	Acute model extrapolated to chronic criterion by ACR; adopted as federal water quality guidance in United States; some adoption by states	Me-BL log *K* values from fish gill accumulation tests, toxicity data used to adjust CA values for *Daphnia* and others
Cu	Generalized bioavailability model (gBAM)	(De Schamphelaere et al. 2003; Van Sprang et al. 2008)	Green algae	All primary producers	pH ("BLM" set needed for FIA calculation)	ERA for REACH, EQS (Europe)	pH only factor affecting algal growth when expressed as FIA; full "BLM" chemistry needed for FIA
Cu	Chronic BLM	(De Schamphelaere and Janssen 2004a)	*Daphnia magna*	All invertebrates	"BLM"	ERA for REACH, EQS (Europe)	Only H and Na considered to compete with Cu; $CuOH^+$ and $CuCO_3$ also bind to BL and significantly contribute to toxicity
Cu	Chronic gBAM	(Van Regenmortel et al. 2015)	*Daphnia magna*	All invertebrates	"BLM"		Toxicity of FIA predicted as a function of pH, Na, Ca, Mg
Cu	Chronic BLM	(Crémazy et al. 2017)	Rainbow trout	Fish	"BLM"		Toxicity decreased (higher effect concentrations) with increasing pH, but pattern reversed at high pH (>8); even in long-term exposures, Cu was an acute toxicant
Ni	Hybrid BLM	(Deleebeeck et al. 2007)	Rainbow trout, fathead minnow	Fish	"BLM"		Nonlinear pH response could not be modeled as a single-site BLM; model predicted toxicity well, but the exact mechanisms by which Ca, Mg, and pH modify Ni toxicity were undetermined
Ni	Hybrid BLM	(Deleebeeck et al. 2008)	*Daphnia magna*	All invertebrates	"BLM"		Stronger effect of pH on chronic toxicity than acute; similar issues and resolution with nonlinear pH response as their fish model

(*Continued*)

TABLE 2: (*Continued*)

Metal	Model type or name	Reference	Organisms used in development	Organisms presumed applicable to	Necessary inputs	Example applications	Notes
Ni	Hybrid BLM	(Deleebeeck et al. 2009)	Green algae	All primary producers	"BLM"		Predictions from classic BLM limited to a narrow pH range; incorporating a nonlinear pH function expanded prediction ranges
Ni	Chronic BLMs	(Schlekat et al. 2010)	Fish, daphnids, duckweed, snails, rotifers, insects, *Chironomus dilutus*	All freshwater aquatic life	"BLM"	ERA for REACH, EQS (Europe)	Tested existing BLMs; BLMs developed with cladocerans outperformed BLMs developed for more taxonomically similar taxa
Pb	Chronic BLM	(Nys et al. 2014)	*Ceriodaphnia dubia*	Freshwater invertebrates	"BLM"	ERA for REACH, EQS (Europe)	pH had a strong influence on toxicity but not Ca; H^+ (as log $K_{BL\text{-}H}$) was the only competitive constant needed
Pb	gBAM	(Van Sprang et al. 2016)	Algae and fish	All aquatic organisms	"BLM"	ERA for REACH, EQS (Europe)	Toxicity of FIA for algae is a function of pH and DOC only; for *Ceriodaphnia*, pH; and for fish, pH and Ca
Pb	Acute and chronic BLMs	(DeForest et al. 2017)	Daphnids, mayflies, fathead minnow, rotifer, snails	All aquatic animals	"BLM"		Various animal toxicity data sets were fit by adjusting critical accumulation values with a single set of unidentate log *K* values; includes $PbOH^+$ toxicity
Zn	Chronic BLM	(Heijerick et al. 2005)	*Daphnia magna*	*Daphnia magna*	"BLM"	RA for REACH, EQS (Europe)	Toxicity of FIA increased (lower effect concentrations) with increasing pH
Zn	gBAM	(De Schamphelaere et al. 2005a)	Green algae	All primary producers	pH ("BLM" set needed for FIA calculation)	RA for REACH, EQS (Europe)	Toxicity of FIA and dissolved Zn increased (higher effect concentrations) with increasing pH; pH was the only factor affecting Zn suppression of algal growth when expressed as FIA; full "BLM" chemistry needed for FIA
Zn	Chronic BLM	(De Schamphelaere et al. 2005a)	*Daphnia magna* and rainbow trout	All invertebrates and fish	"BLM"	ERA for REACH, EQS (Europe)	Similar model structure and log *K* values between *Daphnia* and trout
Zn	Acute BLM	(Clifford and McGeer 2009)	*Daphnia pulex*		BLM		Binding coefficients calculated from toxicity values from a wide variety of water types
Zn	Acute and chronic BLMs	(DeForest and Van Genderen 2012)	Various aquatic animals and rotifers, *Brachionus calyciflorus*	All aquatic animals	"BLM"	ERA for REACH, EQS (Europe)	Multiple BLM log *K* values from previous models were averaged to produce a "unified BLM which performed well predicting toxicity to a phylogenetically diverse group of species, including daphnids, rotifers, snails, and fish"

(*Continued*)

TABLE 2: (*Continued*)

Metal	Model type or name	Reference	Organisms used in development	Organisms presumed applicable to	Necessary inputs	Example applications	Notes
Al, Cu, Pb, Zn	"F-Tox" humic acid model	(Stockdale et al. 2010)	Field surveys of stream aquatic insect communities	Macroinvertebrate communities	"BLM"		Humic acid used as proxy for bioaccumulation on insect biotic ligands; fitted potency factors were used to relate modeled accumulation of metals to species richness
24 metals	"F-Tox" humic acid model	(Tipping and Lofts 2013, 2014; Tipping et al. 2019)	Fish, cladocerans, lettuce	Various aquatic species	"BLM"		F-Tox humic acid model expanded to predict effects in classic single-species toxicity tests; fitted potency factors were used to relate modeled accumulation of metals to various endpoints
Al, Cd, Cu, Ni, Pb, Zn	"Tox" (generalization of the F-Tox humic acid model)	(Balistrieri and Mebane 2014; Balistrieri et al. 2015; Mebane et al. 2017)	Trout in single-species toxicity tests; stream and lake aquatic invertebrate communities	Natural aquatic communities	"BLM"		Similar to F-Tox but generalized to use various bioaccumulation models, including but not limited to humic acid
Cd, Cu, Zn	2-pK_a bidentate model with Tox addition	(Farley and Meyer 2015; Mebane et al. 2017)	Trout, cladocerans, stream insect communities	Freshwater animals	"BLM"		"Streamlined" mixture models used bidentate, single-site accumulation models with Tox addition to predict effects; bidendate structure increased flexibility for handling nonlinear pH responses
Ag, Cd, Cu, Zn	BLM and biodynamic hybrid model	(Veltman et al. 2010)	Various freshwater fish	Freshwater fish	"BLM" chemistry; dietary and gill uptake, assimilation, and elimination rates; organism size		The covalent index, reflecting metal affinity for proteins, was used to estimate metal adsorption efficiencies
Pb	Surface complexation	(Antunes and Kreager 2014)	Duckweed (*Lemna minor*)	Vascular plants	"BLM"		Used the oxide surface complexation model within WHAM VII
Cationic metals	Plasma membrane	(Kinraide 2006)	Wheat (*Triticum aestivum*)	Vascular and single-cell plants	I "BLM set"		Most successful when calculated with free ion activities. Concept is broadly applicable to aquatic organisms

"BLM set" of model inputs are temperature, pH, dissolved organic carbon, Ca, Mg, Na, K, Cl^-, SO_4, and alkalinity or dissolved inorganic carbon.
ACR = acute to chronic effects ratio; BLM = biotic ligand model; CA = critical accumulation values on the biotic ligand associated with a level of effect; EQS = environmental quality standard; ERA = environmental risk assessment; FIA = free ion activity; log K = stability constant for cations to biotic ligands; MLR = multiple linear regression; RA = response addition; REACH = Registration, Evaluation, Authorisation and Restriction of Chemicals; WHAM VII = Windermere humic aqueous model VII.

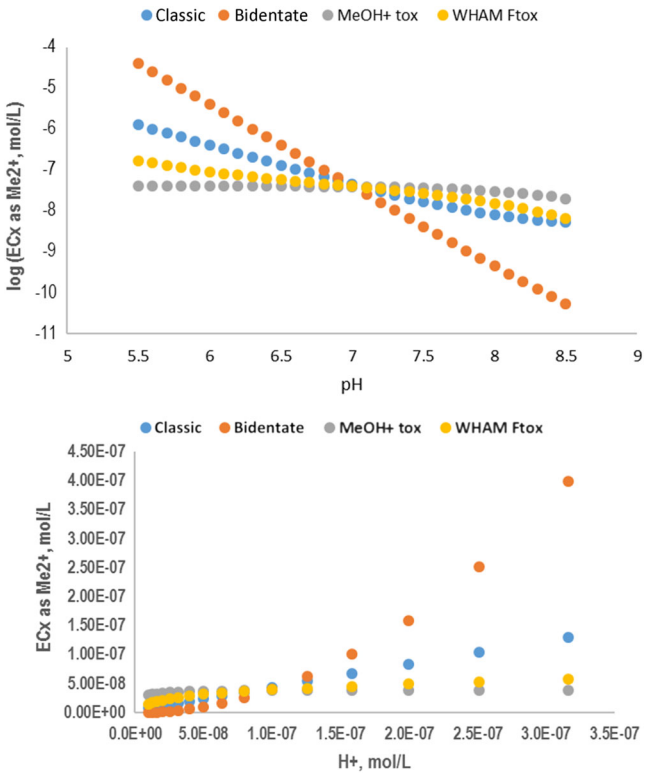

FIGURE 4: Simulated relationships between toxicity of the free metal ion and pH, presented on a log-scale versus pH (upper panel) or on a linear scale versus H^+ ion activity, according to various assumed mechanisms, that is, the classic biotic ligand model (BLM) with a unidentate binding site (blue), a BLM with a bidentate binding site (orange), contribution of the hydroxide complex to toxicity (gray), and assuming that humic acid is a good multiple-site surrogate for the biotic ligand. This figure shows that, even if the emerging relationships are not perfectly linear (except for the classic BLM in the lower panel and the bidentate model in the upper panel), reasonably good linear fits can be obtained. ECx = x% effect concentration; WHAM = Windermere humic aqueous model.

been put forward to explain these deviations, including binding of metal species (e.g., $MeOH^+$, $MeCO_3$) other than the free metal ion (De Schamphelaere et al. 2004), differences between pH in bulk solutions and the organism microenvironment (Playle and Wood 1989), multiple biotic ligand sites relating to multiple uptake sites or multiple simultaneous mechanisms of toxicity (Peters et al. 2011), bidentate biotic ligand sites (Farley and Meyer 2015), and the influence of plasma membrane potential on free metal ion activity at the biotic ligand (Kinraide 2006). In quite a few cases, researchers (including some authors of the present study) have generated models providing good fits and predictive capacities but at the expense of unrealistic parameter estimates (e.g., assuming that $MeOH^+$ species are almost equally as bioavailable as the free metal ion [De Schamphelaere and Janssen 2004b; De Schamphelaere et al. 2004]).

It is always possible to perform a linear regression and derive classical log *K* values, often with still reasonably accurate representation of observed bioavailability relations. Yet, an increasing number of observations have shown such strong deviations from linearity that alternative modeling approaches have been pursued. Indeed, some of them have already been implemented in European Union regulations, whereas others have not been implemented. Deviations from linearity appear to be greatest for H^+ ions (i.e., pH), with some very obvious examples where log-linear relationships describe the observations much better than linear regressions (e.g., algae–Cu [De Schamphelaere and Janssen 2006]; *Daphnia*–Ni [Deleebeeck et al. 2008]; *Daphnia*–Cu [Van Regenmortel et al. 2015]). These observations have led to the formulation of "hybrid models," or gBAMs, that combine a log-linear pH effect (e.g., Figure 5) with the classic competition for other cations. Although this practice can be perceived as less mechanistic than the classic unidentate single-site BLM, it should be emphasized that 1) the BLM is also just a fitted regression when the link between accumulation at a critical site and toxicity is not made (e.g., Figure 3) and 2) various alternative mechanisms may lead to log-linear pH effects on free metal ion toxicity (e.g., Figure 3).

The overall message of Figure 3 is that different mechanistic theories and model formulations can generate either approximately linear or log-linear relations. In addition, assuming, for instance, a bidentate binding model, the pK_a values can generate a wide range of pH–EC*x* Me^{n+} slopes (on a log scale) In the absence of mechanistic evidence, such relationships are equivalent to MLRs. Nevertheless, hybrid models which incorporate such regressions into a BLM framework are useful for regulatory purposes, as long as they accurately predict toxicity over a wide range of conditions.

A consequence of approaching the BLM as just equations to be solved by using optimization routines to find the best values for unknown parameters is that the fitted solutions may be disconnected from the BLMs' mechanistic foundations. Prominently, a fundamental BLM tenet is that toxicity follows accumulation on the biotic ligand (Table 1). In model construction, if the speciation model and log *K* values used successfully reproduce measured accumulation values, then the model is grounded in reality. However, if this step is bypassed, such as when appropriate accumulation data are not available, it is possible to successfully fit BLMs to toxicity values using binding constants or LA50 values that appear to be chemically or biologically unrealistic.

With Cu and Na, the apparent protective effect of Na^+ against Cu toxicity has been incorporated into BLMs as a competition between Na^+ and Cu^{2+} for binding to the biotic ligand. The binding constant for Na^+ that can be extracted from toxicity experiments tends to produce log *K* (biotic ligand–Na) values of 2.5 to 3.5 in various BLMs. This agrees well with the common observation that the binding affinity (K_m) values for unidirectional active Na^+ uptake values in most freshwater organisms are in the range of 10^{-3} M. It is curious therefore that, in contrast, log *K* (organic acid–Na) stability constants for various organic acids and Na are mostly between 0.7 and 1.9 (Stumm and Morgan 1996). This illustrates that organismal biology is more complicated than simple chemistry. Figure 5 illustrates a further example, comparing measured and predicted Cd LC50s with rainbow trout from diverse studies using 2 BLM constructs. In Figure 5, model A, the log *K*

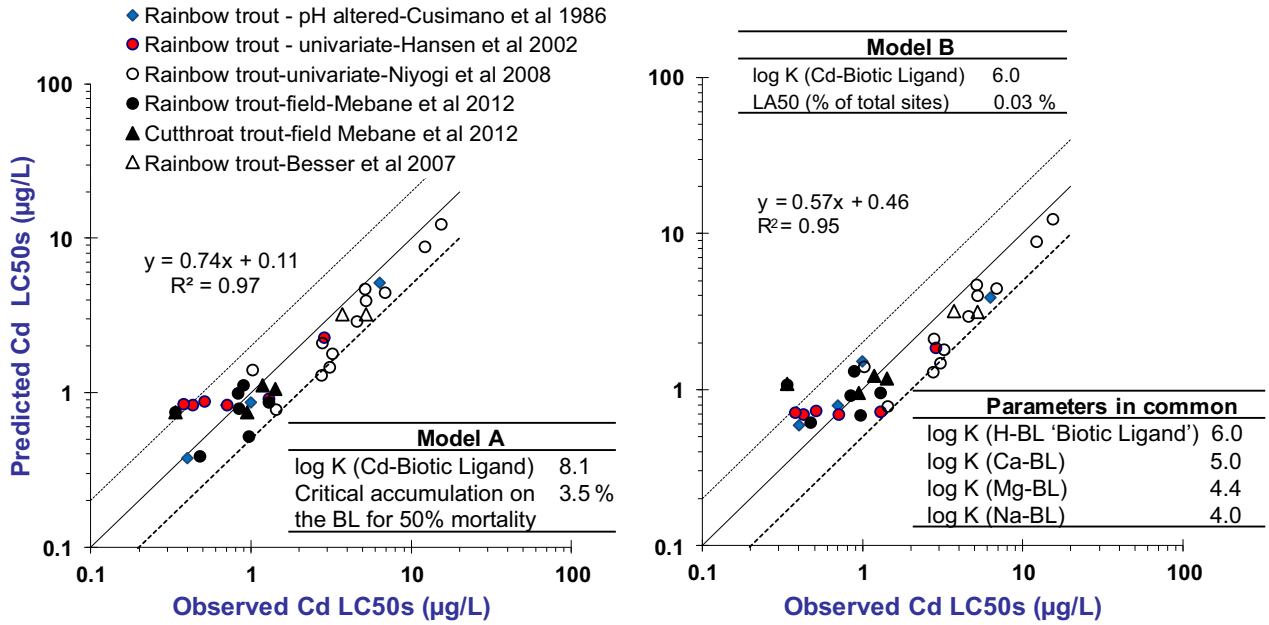

FIGURE 5: An example of nonunique solutions possible when deriving biotic ligand models by fitting toxicity test data without regard to actual critical accumulations (LA50). The parameters for model A are grounded in experimental findings. Model B fits the toxicity data just as well, even though the large reciprocal changes to the log *K* and critical accumulation fractions make the latter physiologically implausible (see online Supplemental Data SI-2 in Farley et al. 2015). The solid diagonal line is the line of 1:1 agreement; the dotted and dashed diagonal lines are a factor of ±2 deviations from the 1:1 line. BL = biotic ligand; LA50 = short-term accumulation at the biotic ligand that is predictive of 50% mortality at a later time; LC50 = median lethal concentration.

Cd–biotic ligand binding affinity coefficient of 8.1 is similar to those derived from gill accumulation experiments (e.g., 8.6 [Playle et al. 1993a]; 8.0 [Niyogi et al. 2008]). Yet in Figure 5, model B, even when the Cd–biotic ligand binding affinity is decreased 2 full orders of magnitude (2 log units), the fit of the toxicity data is just as good, so long as the LA50 is reciprocally lowered 2 orders of magnitude to 0.03% (see online Supplemental Data SI-2 in Farley et al. 2015). However, fit aside, both of these LA50 values are lower than experimentally determined values, and an LA50 of 0.03% seems implausible, assuming that binding to the site of toxic action is proportional to binding sites on the gill. An LA50 of 0.03% implies that Ca regulation would be fatally compromised with channels that are 99.97% intact, which does not seem physiologically plausible. For comparison, experimentally derived LA50 values for Cd in rainbow trout studies have been approximately 10 to 30% of the strong binding sites on the gill (Birceanu et al. 2008; Niyogi et al. 2008).

The reason for these modeling manipulations was to try to find a combination of parameters that would mimic tests that showed that Cd toxicity was reduced by adding Cu, implying that Cu may have a higher affinity than Cd to the biotic ligand. Although ungrounded from accumulation, such manipulations have shown practical success across complex and varied exposure conditions (Farley et al. 2015). The capacity of the BLM structure to use widely available toxicity data to predict responses over a wide combination of waters, metals, and organisms is a major strength of the approach. However, too much flexibility from many adjustable parameters can lead to unconstrained models that would more accurately be described as "mechanistically inspired" models, rather than "mechanistic" models.

Complexity versus simplicity: Use of mechanistic and hybrid models to inform development of simpler models

The broad influence of metal bioavailability models within science communities has not always translated to their broad adoption by regulatory authorities (e.g., Wood et al. 2012a, 2012b). The necessity of determining the full composition of each water sample and the complexity underlying bioavailability–based models has proven to be a limitation for their wide application. To broaden access to potential users, various functional interfaces and simplified versions have been developed. In the early 2000s, Robert Santore and colleagues developed and freely shared an intuitive, spreadsheet-style software interface that executed the BLM structure developed by Di Toro et al. (2001) and Santore et al. (2001). The software provided users a flexible platform to explore toxicity data and water chemistry and to expand on the work of the developers with new models. The influence and utility of this modeling platform are evidenced by approximately 300 literature citations to the software to date and by its incorporation into the US Environmental Protection Agency's national recommended aquatic life criteria for copper (US Environmental Protection Agency 2007). At the time of writing, the software was on its fourth major version. In the European Union, environmental quality standards under the Water Framework

Directive and risk assessments under the policy Registration, Evaluation, Authorisation and Restriction of Chemicals employ computationally intensive applications of multiple BLMs developed to protect algae/plants, invertebrates, and fish (see Nys et al. (2016) for Ni; Van Regenmortel et al. (2017) for Cu and Zn; and Van Sprang et al. (2016) for Pb). Various simplified proxies have been developed including Bio-Met, which employs look-up tables that approximate BLM calculations for Cu, Ni, Zn, or Pb with over 20 000 values covering a wide range of environmentally relevant water chemistries, using fewer parameters (Ca, pH, and DOC) than the full BLMs. Similar algorithm-based approaches that simplify inputs and user calculations include the Metals Bioavailability Tool and PNEC-Pro (Peters et al. 2016; Verschoor et al. 2017). For Pb, a separate tool simplifying speciation and toxicity predictions is also available (Van Sprang et al. 2016). All these resources can readily be found through internet searches.

Because mechanistic and hybrid models typically integrate the effects of water chemistry on geochemical speciation as well as interactions of toxic metal species (mostly Me^{n+}) with the organism, obvious roles of these models are to populate look-up tables and to inform the development of MLRs (Brix et al. 2017a; DeForest et al. 2018). For example, a statistical approach to developing MLRs using stepwise automated routines to maximize partial regression coefficients may yield a good fit between effects and predictor variables. Yet, variables that are only important in a subset of the data or that have subtle effects may be missed, and a statistical approach alone cannot distinguish between causative and correlative variables. In the case of MLRs for Cu and Al, the choice of potential toxicity predictor variables (DOC, pH, and water hardness or Ca) was informed by associated BLMs (e.g., Santore et al. 2001, 2018), not by statistical explorations.

To further illustrate how mechanistic bioavailability models can inform simpler approaches, we have used a chronic gBAM for fish (De Schamphelaere 2018) to predict how the 30-d LC20(dissolved) of Cu in soft water (hardness ~10 mg/L) for fish varies as a function of the DOC, species sensitivity, and pH (Figure 6). This gBAM accounts for geochemical speciation effects, competition of Cu^{2+} with Ca^{2+} and Mg^{2+}, and effects of pH on Cu^{2+} ion toxicity. The simulations provide relationships between ECx and DOC that "emerge" from the joint effects of these 3 processes in the hybrid gBAM model. These simulations illustrate 3 important points: 1) the relation between ECx and DOC is nearly perfectly linear, on both linear and logarithmic scales; 2) on a linear scale, the slope of the ECx versus DOC relation is higher for less sensitive organisms and at higher pH; and 3) on a logarithmic scale, slopes appear nearly independent of species sensitivity or pH. This indicates that an MLR on a linear scale should contain not only a linear DOC term but also an interaction term between pH and DOC and furthermore that an MLR slope derived on the basis of an insensitive species should not be extrapolated to a more sensitive species. Similar simulations can be performed for other

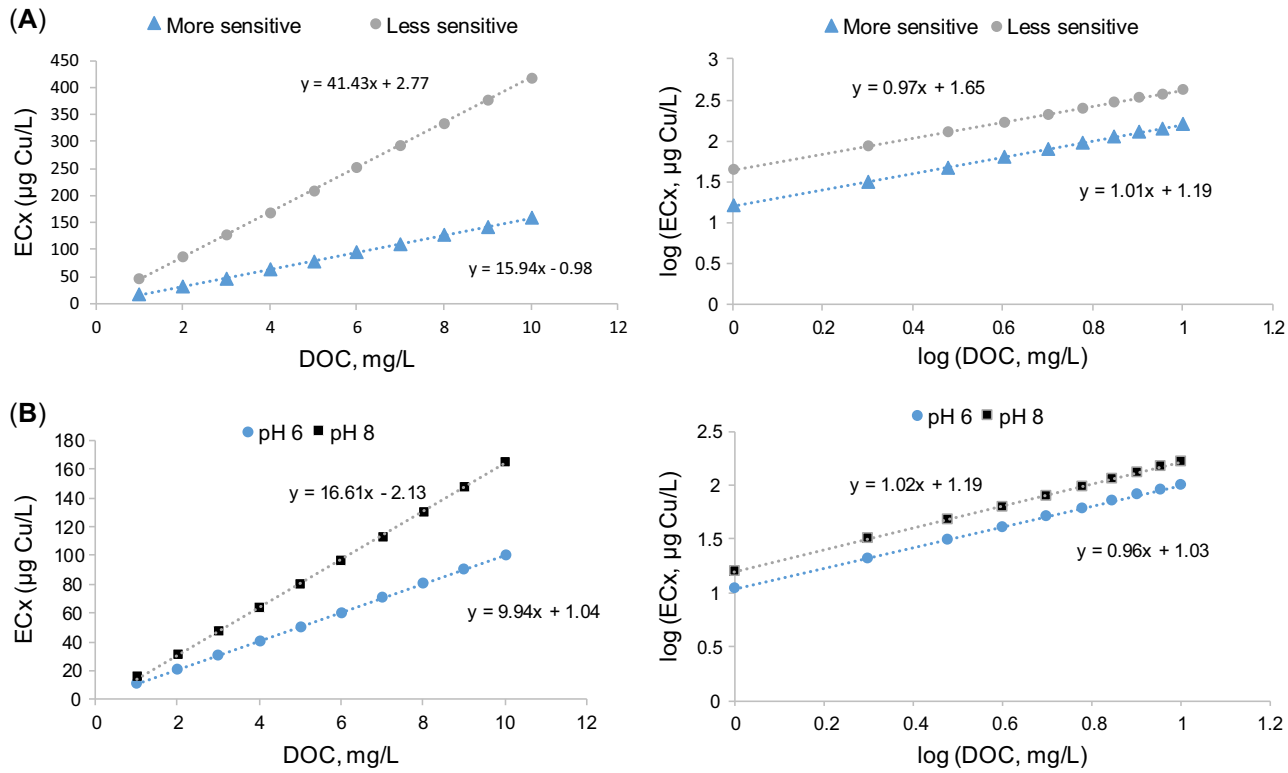

FIGURE 6: (**A**) Modeled 30-d 20% lethal concentrations (LC20s) as a function of dissolved organic carbon (DOC; at pH 7) for rainbow trout (more sensitive) and a 10 times less sensitive hypothetical fish species, presented on linear and log scale, and (**B**) 30-d LC20s as a function of DOC for rainbow trout at pH 6 and 8, presented on linear and log scale. ECx = x% effect concentration.

species, water quality variables, and metals, resulting in specific recommendations for MLR construction.

Equilibrium issues

From both chemical and biological perspectives, BLMs and related constructs all assume equilibrium conditions. If this is not the case, the predictive abilities of the model may be compromised. Instances where equilibrium may not occur include laboratory exposures with incompletely equilibrated diet and/or DOC and environmental exposures such as pulse exposures from stormwater or meltwater runoff (Kayhanian et al. 2008; Nimick et al. 2011; Balistrieri et al. 2012; Figure 7) and exposures within mixing zones downstream of point-source releases (Vandenberg et al. 2005). Nonequilibrium conditions can cause metals which equilibrium-based speciation calculations assign to complexes to actually be bioavailable, whereas equilibrium models such as BLMs generally assume that complexed metals are not bioavailable (Zhao et al. 2016).

Reactions of metal ions with dissolved inorganic ligands typically reach equilibrium in seconds to minutes. However, equilibration of metals with DOC can take hours to days. This issue was initially manifested in fathead minnow Cu toxicity data reported by Erickson et al. (1996). In nonrenewal static trials, the 96-h test duration was sufficient for any disequilibrium associated with Cu–DOC complexation to be effectively eliminated during the early part of the exposure. However, in flow-through tests, a Cu stock solution was mixed with unamended Lake Superior water in the diluter head tank just prior to entering the 45-min residence time test chamber. In this case free Cu was continuously elevated relative to equilibrium conditions, and toxicity was increased relative to static tests at the same dissolved Cu concentration. Model-predicted LC50s were almost identical in the 2 flow regimes, but in the flow-through tests, they were consistently higher than observed values (i.e., toxicity was underestimated by the model), whereas in the static tests they were consistently lower than observed values (i.e., toxicity was overestimated), under otherwise similar conditions (Figure 8A,B; Santore et al. 2001). This interpretation is consistent with tests that found that *Ceriodaphnia dubia* Cu LC50s were directly related to equilibration time with DOC (Kim et al. 1999; Ma et al. 1999). A caution, however, is that DOC tends to increase over time during static tests, as a result of accretion of organic carbon from the test organisms. For Ag and Cu, metals with strong affinities for DOC, small increases in DOC in the range of 0.2 to 0.5 mg/L can produce noticeable changes in modeled or measured toxicity (Erickson et al. 1998; Welsh et al. 2008). Thus, the pattern of greater toxicity of Cu in flow-through tests than static tests could be influenced by both incomplete equilibration in the former and increasing DOC over the course of the latter tests.

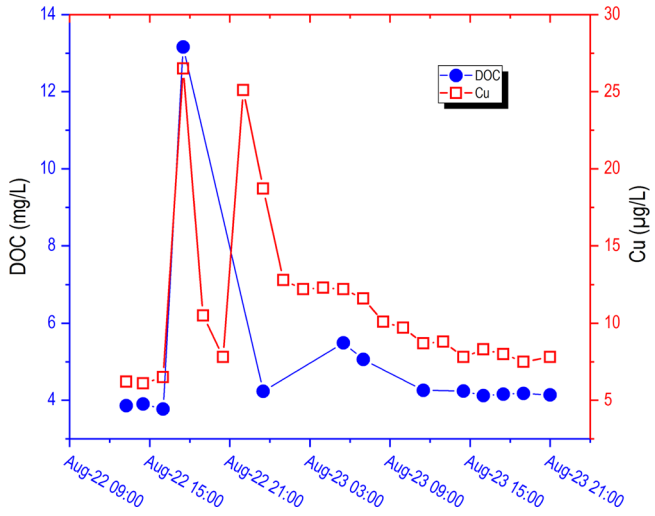

FIGURE 7: Biotic ligand models (BLMs) assume equilibrium conditions, which may not be true with metal–dissolved organic carbon (DOC) binding that can take up to 24 h to reach equilibrium in some tests. The data shown here for Cu and DOC sampled in a stream during a rainstorm show that concentrations can change rapidly and not necessarily in synchrony. Copper is expected to have greater bioavailability and toxicity in nonequilibrium conditions than would be predicted by equilibrium-based BLMs. Data from Balistrieri et al. (2012).

At high humic acid (>5 mg/L DOC) and Cu (>1 µM) concentrations, up to 30 h were required to reach equilibrium (Ma et al. 1999). Under more dilute conditions with DOC <1 mg/L, Cu more rapidly equilibrated in 0.1 to 4 h (Louis et al. 2009; Meyer and Adams 2010). As an example, the kinetic data of Ma et al. (1999) were used to calculate that at the start of a static exposure the free Cu (Cu^{2+}) concentrations might be elevated as much as 10-fold relative to the concentration at equilibrium (Figure 9A). After the first 24 h, the deviation from equilibrium is small; and after 30 h, equilibrium is achieved (Figure 9B). Similarly, in static-renewal tests, the solution is refreshed at regular intervals, resulting in elevated free Cu with each renewal unless pre-equilibration is used (Figure 9C). Most seriously, in flow-through tests with a short hydraulic residence, free Cu will be elevated throughout the test (Figure 9D). Similarly, Meyer and DeForest (2018) invoked the Ma et al. (1999) kinetic model to argue that a lack of adequate equilibration could explain the apparently very low threshold effect concentrations (<2 µg Cu/L) reported by Dew et al. (2012) for olfactory impairment in fathead minnows exposed to Cu for short durations (1–24 h), as an alternative to the damage-repair hypothesis proposed by Dew et al. (2012) for explaining the decrease of olfactory impairment as exposure time increased.

Reports on the importance of pre-equilibration as a factor modifying metal toxicity have been inconsistent. For instance, although Glover et al. (2005) found that Ag was more toxic to *Daphnia magna* (lower 24-h LC50s) in tests initiated after 3-h metal–DOM contact time than in tests initiated after 24 h contact time (Figure 10), Erickson et al. (1998) found little effect on Ag toxicity from aging solutions for 72 h before testing. Further, in contrast to the Ma et al. (1999) results, Wang et al. (2011) found little differences in Cu toxicity between tests initiated with freshly mixed exposures versus solutions that had been aged for 24 h.

Equilibrium models are a reasonable simplification of real-world systems in many cases, such that simple relationships

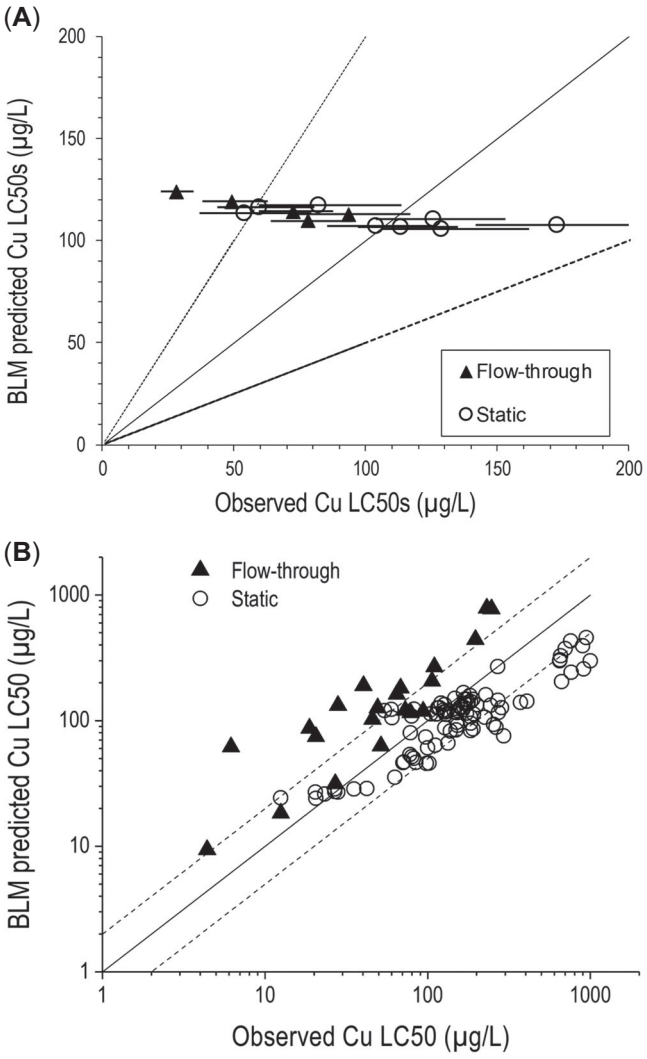

FIGURE 8: Comparisons of biotic ligand model (BLM)–predicted and measured fathead minnow median lethal concentrations (LC50s) in flow-through and static test designs. (**A**) Repeated tests of same-age fish from the same broodstock in constant exposure water (unamended Lake Superior reference water) varied by a factor of approximately ±2, which is the origin of the "factor-of-2" rule of thumb for evaluating BLM performance. (**B**) Measured and BLM-predicted LC50s from tests amending Lake Superior water with added major ions or dissolved organic carbon. Both comparisons show that Cu tended to be more toxic in flow-through than static test designs, which is likely at least partially related to nonequilibrium conditions in the flow-through tests. Original data from Erickson et al. (1996) plotted after Santore et al. (2001) but using the US Environmental Protection Agency's (2007) updated fathead minnow species mean critical accumulation value of 2.97 nmol/g wet weight. The solid diagonal line is the line of 1:1 agreement; the dotted and dashed diagonal lines are a factor of ±2 deviations from the 1:1 line.

have been obtained that relate metal speciation to biological effects (e.g., the present review; Zhao et al. 2016). This simplification is probably necessary when using bioavailability models, whether mechanistic or empirical, to inform regulatory applications. Nevertheless, the controversies over equilibrium assumptions and contrasting results preclude conclusive generalizations to resolve the apparent equilibration dilemma. Still, unless time-varying conditions are the focus of testing, we suggest a 24-h pre-equilibration period in experimental designs to allay concerns of nonequilibrium.

Toxicity test design considerations for developing mechanistic and hybrid bioavailability models

Historically, mechanistic and hybrid metal bioavailability models have been developed from 1) multiple univariate toxicity experiments, where each factor (e.g., Ca, Na, DOC, pH) is varied alone; 2) full-factorial test designs (which can be considered a series of univariate experiments at various conditions); 3) multivariate toxicity experiments, where factors are varied in various combinations; or 4) a combination of 1) and 2). Regardless of the design, well-informed selection of the water chemistry variables is crucial. This selection should be based on prior knowledge of suspected influential variables, using information on other organisms, other metals, previous metal uptake and toxicity data, and physiological understanding.

Multivariate experiments are particularly useful as a first step to discriminate the more from the less important toxicity-modifying variables. However, on their own, they are not necessarily the most useful for mechanistic model development, as illustrated in the following example. De Schamphelaere and Janssen (2004b) performed chronic *D. magna* toxicity experiments with Cu in a multivariate design with pH, DOC, and hardness as the factors. They found that DOC and pH were significant factors but that hardness was not. They subsequently used the data to calibrate a chronic Cu-BLM for *Daphnia*, in which Ca or Mg competition was not included. Also, Na was included in the model because of evidence from a parallel univariate experiment with Na that showed a correlation with Cu toxicity. Later, Rodriguez et al. (2012) performed univariate experiments and did find protective effects of Ca and Mg. Van Regenmortel et al. (2015) developed an optimized chronic Cu bioavailability model by reformulating it as a gBAM and including biotic ligand constants for Ca and Mg. This optimized model accurately predicted the toxicity observed in both data sets.

This example indicates that non-full-factorial multivariate test designs can "miss" toxicologically significant modifying factors, especially when the design is run over multiple test series where between-batch variability may play a role (see Supplemental Data). On the positive side, the example illustrates that, over time, existing models can be improved if new data become available and that data sets from different sources can be used jointly for model calibration. It also suggests that univariate test designs are best for calibration of individual model parameters (e.g., biotic ligand stability constants for competing cations in BLMs or pH slopes in gBAMs). Furthermore, the mathematical method to estimate log *K* values is well known and relatively straightforward (De Schamphelaere et al. 2002). Full-factorial designs (the special case of multiple univariate experiments) have been rarely used in generating bioavailability models because they are the most costly and labor-intensive, but they are particularly useful to detect interactive effects. Using this design, Deleebeeck et al. (2009) were able to show no interactions between pH and Mg on chronic Ni toxicity to algae, allowing them to formulate a model that was

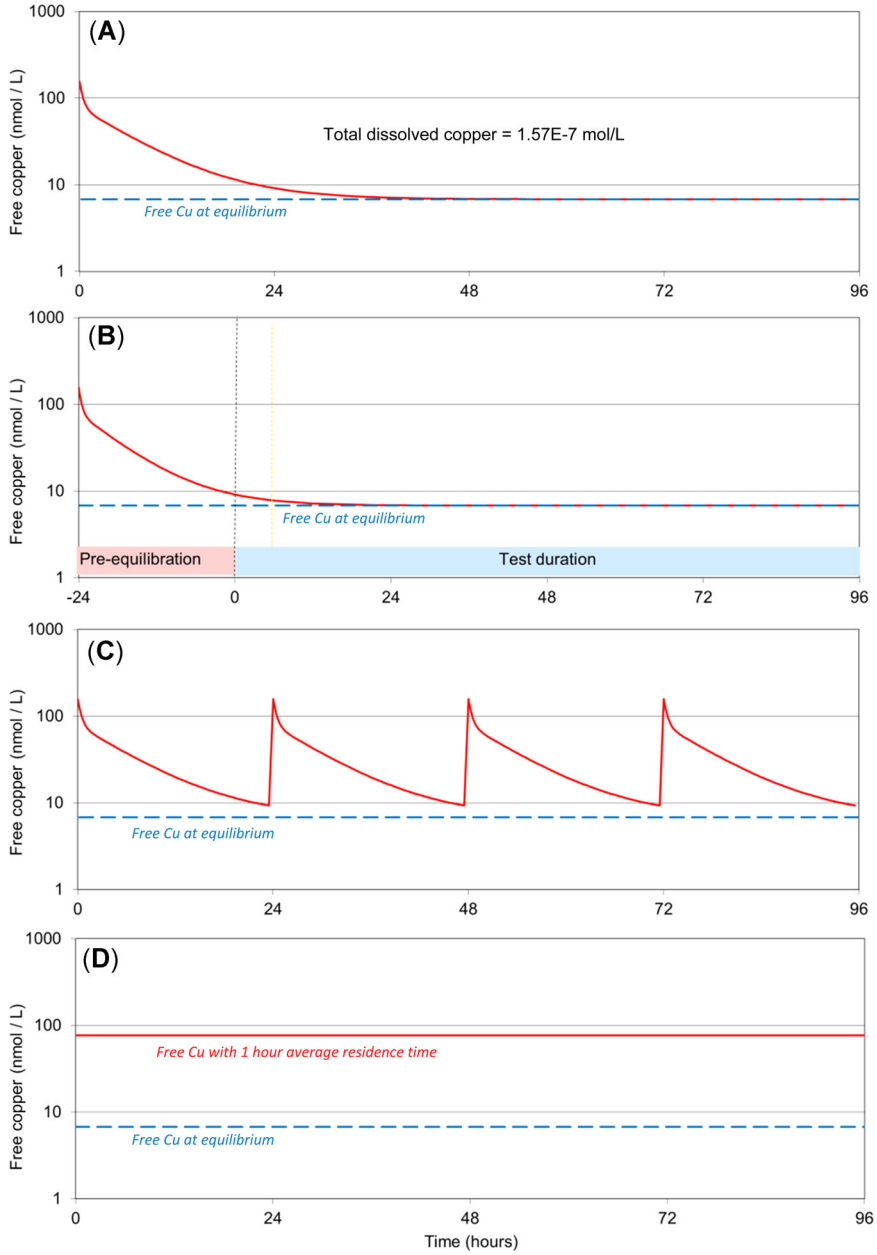

FIGURE 9: Kinetic patterns of free Cu concentrations over the course of a toxicity test in the presence of 5 mg/L dissolved organic carbon in 4 different hypothetical experimental conditions. In each simulation, the dissolved Cu concentration is 10 µg/L. In each panel, the free Cu concentration at equilibrium is shown as a horizontal dashed blue line, and the simulated free Cu in the experiment as a function of time is shown as a red line. For a static exposure with no pre-equilibration (**A**), the free Cu (Cu^{2+}) at the start of the test is elevated but decreases over time until it is near equilibrium at approximately 30 h. For a static exposure with a 24-h pre-equilibration period (**B**), the free Cu is close to equilibrium for the entire test duration. For a static test with daily renewals (**C**), the free Cu is elevated at the beginning of each renewal and then decreases but never reaches equilibrium. In a flow-through test (**D**) with a 1-h residence time, the free Cu is constant but far from equilibrium.

successful at predicting Ni toxicity in a range of spiked field waters. In summary, all sorts of designs can help in initial model development, but univariate or full-factorial designs are the best for calibration of individual model parameters.

Consideration of other toxicity-modifying factors

Although pH, DOC, and major ions have been incorporated into most bioavailability models (Table 2), other factors have received much less attention by bioavailability model developers. We are not aware of any current regulatory framework that explicitly incorporates effects of other metal toxicity factors such as acclimation to prior metal exposure, temperature, nutrients, suspended solids, or iron hydroxides in the assessment of metal toxicity. Yet we now know that such factors may strongly affect metal toxicity, and in the case of nutrients and temperature, for example, a wealth of empirical data is available.

Acclimation to chronic metal exposures can have major effects on responses to subsequent acute exposures, through

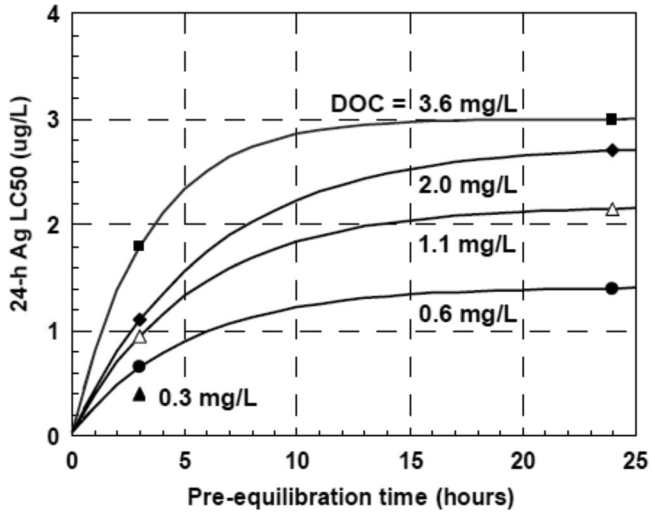

FIGURE 10: Laboratory toxicity testing of metals with increased dissolved organic matter (DOM) should consider metal–DOM equilibrium time. Silver was more toxic to *Daphnia magna* (lower 24-h LC50s) in tests initiated after 3-h metal–DOM contact time than in tests initiated after 24-h contact time (Glover et al. 2005). DOC = dissolved organic carbon; LC50 = median lethal concentration.

acquired tolerance. During long-term, low-level exposures, gill metal burdens may increase above the concentrations usually associated with acute toxicity (reviewed by Niyogi and Wood 2003). This has been explained by the "damage-repair hypothesis," whereby the repair processes increase the tolerance of the organism to a particular metal burden (McDonald and Wood 1993). Likewise, the log K and B_{max} conditional binding constants of the metal biotic ligand and of the various cation–biotic ligand complexes will be markedly changed following acquired tolerance, yet these constants are considered to be unchangeable with the changes in environmental conditions in BLMs (Niyogi and Wood 2003). Although acquired tolerance from exposure to elevated metals has long been recognized (Chapman 1985), acclimation to soft or hard water, or differences in the composition of the diet (e.g., high calcium or sodium content) may also have profound effects on metal tolerance and modeling (e.g., Niyogi and Wood 2003; Franklin et al. 2005; Todd et al. 2009; Mebane et al. 2010). In criteria development, acclimation is considered a confounding, false protection to be guarded against because acquired protections may not be persistent and, furthermore, the energetic costs of tolerance may lead to other adverse effects (Stephan et al. 1985; Brinkman and Woodling 2014). However, the implications of variations in binding constants depending on earlier metal exposure, water hardness, diet, and other environmental or physiological conditions seem to be underrecognized in the development and application of metal bioavailability models (Niyogi and Wood 2003).

Most studies on temperature effects have investigated acute toxicity. These have reported both increasing metal toxicity with increasing temperature (Rao and Khan 2000; Heugens et al. 2003) and increasing toxicity with decreasing temperature (Hodson and Sprague 1975; Hansen et al. 2002). Much more limited data are available on chronic metal toxicity, but a recent study showed that chronic metal toxicity to *D. magna* varied by approximately 2-fold, with a clear pattern of higher toxicity at lower temperature, opposite to what is most commonly expected for acute toxicity (Pereira et al. 2017). Interestingly, for Cu, the response pattern could be explained by the computed effect of temperature on speciation but not for Ni and Zn. Until now, most BLMs and hybrid models do include temperature as an input parameter, but they only compute the effect of temperature on inorganic speciation, whereas DOC interactions, which rely on WHAM V, VI, or VII, are not temperature-adjusted. Most importantly, any temperature effects at the biological receptor are not incorporated. More empirical and mechanistic research is clearly needed to better integrate temperature into bioavailability models and AWQC.

Effects of nutrient concentrations, especially phosphate, on metal toxicity have been studied extensively and can be relatively strong but are inconsistent across various studies. Notably, phosphate concentrations in natural freshwaters (European annual mean in rivers as of 2012 was 65 μg PO_4-P L^{-1} [European Environment Agency 2018]) are typically considerably lower than those applied in standard algae toxicity test protocols (310–1550 μg PO_4-P L^{-1} [Organisation for Economic Co-operation and Development 2011; US Environmental Protection Agency 2002]). Thus, a better mechanistic understanding of nutrient effects on metal toxicity, and associated incorporation into models, is urgently needed to improve laboratory-to-field extrapolation. Gao et al. (2016), for example, developed a mechanistic dynamic model that integrated effects of external phosphate, algal cell P content, and Zn on instantaneous algal growth rate. Their model was able to explain why different exposure scenarios (duration, phosphate supply, and initial P content of algae) can lead to opposite apparent effects of phosphate on Zn-induced declines of algal biomass in standard toxicity tests. As bioavailability modeling expands into metalloids and plants, we expect nutrients will have an important role as a modifying factor, especially with pairs such as phosphate and arsenate (Zhao et al. 2016).

The role of organic ligands that bind metals and form lipophilic complexes has received little attention in metal bioavailability modeling. Similarly, the potential role of low–molecular weight metabolites such as thiosulfate or citrate (which can bind metals and then transport them across epithelial surfaces as the intact metal–ligand complex via anion transporters) has received little attention (Zhao et al. 2016). Although BLMs and related constructs usually treat complexed metals as nontoxic, attributing toxicity only to the free ion metals, we now know that complexed metals can be bioavailable and toxic under some circumstances (Erickson et al. 1996; Zhao et al. 2016).

Other trace metals, such as Fe and Al, may also influence metal toxicity, notably via 1) competition with the toxic metal ion for binding on DOC, and 2) by providing an adsorption phase when in the colloidal hydroxide-precipitated form (Cain et al. 2016). It would be worthwhile to explore how effects of these and other bioavailability-modifying factors could be added to metal bioavailability models.

The choice of speciation modeling platforms for BLMs

Given the importance of DOM (natural organic matter, DOC) in metal speciation, the speciation modeling component of a mechanistic bioavailability model must be capable of accurately predicting metal–DOM complexation. Several mechanistic models exist for computing metal binding to humic and fulvic acid, which are the dominant components of DOM in freshwaters. These include the humic ion–binding family (Models V, VI, and VII; Tipping and Hurley 1992; Tipping 1998; Tipping et al. 2011), the nonideal competitive absorption (NICA)-Donnan model (Kinniburgh et al. 1996) and the Stockholm humic model (Gustafsson 2001). These are combined with inorganic speciation codes in tools such as WHAM, Visual MINTEQ, and the Hydroqual/Windward BLM software. These inorganic speciation models differ somewhat in the binding affinity values used and even the presence of equilibrium constants in their code. Some speciation models do not account for precipitation/dissolution reactions. The absence of the relevant equilibrium constants can lead to the use of unstable metal exposure regimes in experiments, where the metal's solubility limit is exceeded.

A number of speciation models have been used in BLM development. Di Toro et al. (2001) implemented WHAM V within the CHESS framework, and many subsequent studies (e.g., De Schamphelaere and Janssen 2002; Heijerick et al. 2005) also used WHAM V. Others have used WHAM VI (e.g., Deleebeeck et al. 2008; Peters et al. 2011), WHAM VII (Vukov et al. 2016), or NICA-Donnan (Van Sprang et al. 2016). The choice of speciation model platform may be informed by a number of factors, particularly the availability of binding constants for the metal of interest. Derived BLM binding constants (i.e., log *K* values) are conditional on the choice of speciation model, and different BLM binding constants can be obtained from the same data set using different speciation models. This has implications for metal-mixture BLMs—here, a single speciation model should be used for all metals to account for intermetal competition on DOM binding sites, and the provenance of any binding constants used should be carefully evaluated. We did not reach consensus whether any single speciation model for either DOM binding or inorganic speciation could be considered optimal. The choice of models may be informed by a number of factors, particularly the availability of binding constants for the metal of interest.

Specification of DOM (DOC) in models

All of the DOM-binding models listed require concentrations of humic and/or fulvic acid to be specified. This requires the use of "activity factors"—the ratio of measured DOC concentration to the concentrations of humic and/or fulvic acid that reproduce the metal-binding properties of that DOC. The activity factor is composed of 3 components, each of which may be assumed or estimated: the carbon content of DOM, the metal-binding properties of that DOM, and the attribution of the binding properties of that DOM to humic and/or fulvic acid. Development of a BLM has usually used a global activity factor, sometimes derived from metal–DOM binding studies (Dwane and Tipping 1998; Bryan et al. 2002). Early developments (e.g., Di Toro et al. 2001) assumed DOM to be 50% carbon, with 100% activity and comprising 10% humic and 90% fulvic acid. Because the activity factor is in reality water-specific, some have researched whether the activity correlates to measurable DOM properties. De Schamphelaere et al. (2004) found that optimizing water-specific BLM predictions of acute Cu toxicity to *D. magna* produced activities (as fulvic acid only) that correlated significantly with the specific absorbances of the DOM samples at 350 nm (SAC_{350}). Al-Reasi et al. (2012) found significant correlations between acute Cu toxicity to *D. magna* and a number of optical and physicochemical properties of DOM, with SAC_{340} being the most significant predictor of protective ability. They suggested that the predictive capability of BLMs could be improved by the use of SAC_{340} to adjust the activity factor on a water-by-water basis. In general, larger, more lipophilic, more aromatic DOMs of terrigenous origin, with higher humic acid-like content, and therefore higher SAC values, appear to be more protective against Cu toxicity. Despite these findings, however, many BLM studies still use a global activity factor in model development because this has the advantage of requiring only measurement of absolute DOC concentration for application.

Evaluation of speciation models

The ability of models to predict metal speciation, for the chemical conditions relevant to BLM development and application, needs to be fully evaluated. Because BLM parameterization employs predicted free metal ion activity, testing should ideally be done on measurements of the free activity. Speciation measurement is complex, and many methods remain under active development rather than in routine use. Relatively well-established methods (e.g., ion-selective electrodes [ISEs]) are challenging to apply at the dissolved metal and DOC concentrations encountered in natural waters because of issues such as membrane dissolution and fouling (Eriksen et al. 1999), although they can be highly useful in toxicity tests, particularly acute toxicity studies (e.g., Al-Reasi et al. 2012; Crémazy et al. 2016). Some have used DOC preconcentration (Ahmed et al. 2013) or continuous-flow (Tait et al. 2016) systems to enable measurement at the metal:DOM ratios encountered in natural waters. Competitive ligand exchange voltammetry (Xue and Sigg 1999; Cao et al. 2006) has also been used, though its validity has been criticized (van Leeuwen and Town 2005; Lofts and Tipping 2011). Nonetheless, continued research in this area is essential, alongside critical assessment of speciation models against such data. Of particular note is a pair of landmark studies comparing the reliability and performance of different trace metal speciation analytical methods and comparative model performance to modeling of metal speciation (Sigg et al. 2006; Unsworth et al. 2006). Examples of model predictions compared against recent measurements using ISEs (for Cu) and an ion-exchange technique (for Co, Ni, Zn, and Cd) are shown in Supplemental Data, Figures S1 through S6.

CONSIDERATIONS FOR THE INCORPORATION OF METAL BIOAVAILABILITY MODELS INTO ENVIRONMENTAL QUALITY STANDARDS

Dealing with extreme waters

The occurrence of extreme natural water conditions (e.g., unusual pH, hardness, DOC levels, or combinations thereof) is a common reality for almost all geographical regions. These situations have been recognized in working with BLMs (Van Genderen et al. 2005; Natale et al. 2007; Hoppe et al. 2015a, 2015b) and pose a common challenge in terms of bioavailability model development and application for regulatory criteria. There are various reasons for this. First, the taxa typically used for model development (i.e., organisms commonly used in all laboratory testing) may not be tolerant of extreme water conditions. All of the water conditions that modify metal toxicity are themselves environmental characteristics that limit habitat suitability in ways that have nothing to do with metal toxicity. For example, DOM has been called an ecological driving force for aquatic ecosystems with well-documented effects on the pH and primary productivity of natural waters (Steinberg et al. 2006). Furthermore, there is evidence that DOC can bind to the gills (Campbell et al. 1997) and alter the basic physiology of ion transport in a way which can beneficially mitigate the damaging effects of metals and low pH (Galvez et al. 2008; Wood et al. 2011; Duarte et al. 2016). These actions of DOC are separate from their ability to reduce the bioavailability of metals by complexation. Some crustaceans and snails will not thrive in culture waters of very low hardness or pH (i.e., control performance will not be acceptable). Equally, they will not be present in natural waters of low hardness (Lodge et al. 1987; Hooper et al. 2008; Cairns and Yan 2009). Second, the available models are not generally validated for extreme water conditions, and thus, the predictions can be erroneous and/or generate uncertainty. And third, the extreme water types often have specific ecological assemblages with organisms of different physiological characteristics, which may or may not show similar sensitivities and/or metal–bioavailability relationships (e.g., different log *K* values or pH slopes) to organisms typically used for model development.

The main target for model development should be the central distribution of data, such as the 5th to 95th percentile of the distribution of water chemistry parameters, rather than undue focus on exceptions and extreme values. The following options can be considered in deciding the derivation of a new model or use of existing model for ecosystems with extreme water parameters.

Extending the boundaries of existing models. This includes recalibration of an existing bioavailability model with the testing of local waters and organisms to extend the physicochemical boundaries of the model. A series of papers has been published with methods describing how the validation boundaries of BLMs or hybrid models for Cu, Ni, Zn, and Pb can be extended to also accurately predict metal toxicity under more extreme pH and hardness (Van Genderen et al. 2005; Deleebeeck et al. 2007; Nys et al. 2016, 2017; Van Regenmortel et al. 2017).

Developing new bioavailability models. In general, bioavailability models should be developed and tested in media that resemble surface water conditions that are within the natural limit of the test organism. Testing organisms outside their usual physiological range of tolerance is inadvisable. Site-specific models should be developed using toxicity testing that employs site waters and native organisms from the extreme sites.

Metal mixtures

Our discussions so far have treated metals as if they occur one by one in the environment. Likewise, regulatory criteria are developed as if individual metals occurred in isolation, with no interactive toxicities. Both are, of course, complete fiction. In the real world, metals always occur in mixtures that are a function of the mineral composition of the watershed. Anthropogenic inputs will invariably produce mixtures of metals, and some generalities about mixture occurrences in ambient waters can be made. The most predictable metal combination is probably Cd and Zn, which seem to naturally occur at close to a 1:200 mass ratio around the world (Mebane et al. 2017). Nickel and Co commonly occur in association with Cu, and Pb is commonly associated with Zn; but the ratios and particular combinations may be highly variable across geological domains (Salminen 2005). Empirical models are not well suited to such variable scenarios, whereas mechanistic bioavailability and toxicity models do provide a flexible approach to handle these combinations.

It has long been recognized that the single-metal framework for BLMs could logically be extended to metal mixtures (Di Toro et al. 2001; Playle 2004), and much recent progress has been made in this area (e.g., Farley et al. 2015; Meyer et al. 2015; Nys et al. 2018). These metal mixture modeling tools may be highly useful in risk-assessment scenarios. However, because of the overwhelming diversity of possible combinations, we expect that regulatory criteria to protect aquatic environments will continue to be developed for individual substances for the foreseeable future. Toxic unit models assume that potency-normalized concentrations of metals can be added together to predict the toxicity of a metal mixture; these provide a simple approach to estimate mixture toxicity risks from single-metal toxicity models (concentration addition or toxic unit models). Alternatively, predicted toxic responses from single-substance toxicity models can be added (response addition or, more appropriately stated, independent action models). The concentration addition approach tends to be more conservative than the response addition approach; that is, concentration addition may predict greater effects than observed (Van Regenmortel et al. 2017; Crémazy et al. 2018). Both approaches implicitly assume that chemicals in the mixture do not physically, chemically, or biologically interact and thereby overlook competition for metal binding sites on DOC

and on the target biotic ligand, which could make metal mixtures more or less toxic than if there were no interactions. Recent studies at acutely toxic metal levels indicate that such binding interactions can occur at biotic ligands (Niyogi et al. 2015; Brix et al. 2016, 2017c). However, interactions between metals for biotic ligand or DOC binding sites are not predicted to be important at mixture concentrations at the low µg/L levels relevant to most chronic regulatory criteria (Balistrieri and Mebane 2014). We believe that mixture toxicity models are ultimately needed for the application of metal criteria, and as mentioned earlier, they should be developed using a common DOM speciation platform. However, the development of bioavailability-based criteria on a single-metal basis remains a reasonable approach. Concerns over how to apply criteria in the ubiquitous settings with metal mixtures present should not hold back the development and application of single-metal bioavailability-based criteria.

Ownership and maintenance of bioavailability models

Setting up the reaction equations for bioavailability models, writing code to execute them, developing software to provide a functional user interface and interpretive output display, documenting the construction and performance of the package, and preparing detailed documentation for users is no trivial undertaking. The expectations are particularly onerous when bioavailability models are used to set regulatory water criteria sufficient to protect diverse communities in diverse environments with legally enforceable limits that drive costs for engineering design, capital construction, operating, and monitoring. Furthermore, the use of models in public policy settings requires sustaining commitments by sponsors over the long term.

It takes no ongoing effort to maintain regulatory criteria that are expressed as simple mathematical functions of toxicity-modifying factors. For instance, some of the criteria values based on hardness equations published by the US Environmental Protection Agency (1986) are still in use 3 decades on. In contrast, for criteria calculated with the aid of custom software, that software needs ongoing maintenance to upgrade to new operating systems, to fix bugs, and to modify the model capabilities following advances in the underlying science. These maintenance needs pose a challenge to regulatory authorities. They must not just provide one-time support for a model to be used in criteria and then move on; an ongoing commitment to maintain the model is needed. Further, the opportunistic use of model software that is not fully in the public domain to set environmental regulations raises intellectual property ownership questions. Institutions supporting chemical speciation models may sell licenses to partially offset their development and maintenance costs and allow the developers to keep advancing their models. Regulatory authorities may hesitate to rely on a software application that is not in the public domain and that cannot be guaranteed to be functional indefinitely, and they may be unwilling or unable to commit to ongoing support of model applications on behalf of their affected dischargers. Until these practical model support and public domain issues are addressed, the pragmatic path forward is to use bioavailability models as research tools to inform simpler, lower-maintenance translational tools for regulatory adoption, such as MLRs and look-up tables (see section *Complexity versus simplicity: Use of mechanistic and hybrid models to inform development of simpler models*).

CONSENSUS RECOMMENDATIONS FOR DEVELOPING OR APPLYING METAL BIOAVAILABILITY MODELS

Our workgroup reached consensus on the following points. It would be prudent for scientists and environmental managers to consider these when developing or applying metal bioavailability models for environmental quality standards or risk assessment.

1) Empirical bioavailability tools such as MLRs and look-up tables should always be informed qualitatively and quantitatively by mechanistic models.
2) Going forward, equilibrium speciation should be considered in the design of experiments for bioavailability models. We recommend a 24-h pre-equilibration period in experimental designs to allay concerns of nonequilibrium test conditions. Care should be used to include speciation models that encompass precipitation reactions so as to ensure that the solubility limits are respected for the metal(s) of interest and that no loss of solubility occurs.
3) The chemical speciation model used should be tested independently of its ability to predict toxicity.
4) Data obtained from tests conducted with organisms outside the chemistry boundaries from where they live should not be used. The main target for model development should be the central distribution of data, such as the 5th to 95th percentile of the distribution of water chemistry parameters, rather than undue focus on exceptions and extreme values.
5) Some potentially important toxicity-modifying factors are currently not represented in bioavailability models and have received insufficient attention in toxicity testing. Temperature is probably of foremost importance; P is likely important in plant and algae models.
6) Plant and animal bioavailability models should not be combined because of the divergent influences of pH.
7) pH is a unique toxicity-modifying factor, with multiple possible mechanisms. These effects are currently best captured by hybrid models, which can inform improved mechanistic understanding and foster better mechanistic models of pH effects. Failures of mechanistic models to explain experimental data can advance our understanding of actual mechanisms.
8) To develop models for mixture toxicity, a common chemical speciation platform should be used. This is particularly important for DOM.

9) To estimate bioavailability model parameters, univariate or full-factorial designs are most useful because multivariate designs may miss responses for variables with limited effect. In univariate test designs, nonsimultaneous testing can introduce confounding variability. In real laboratories in the real world, this may be unavoidable, but repeating treatments between studies is important.
10) For best practice during chronic tests, combined waterborne and dietary matched exposures should be performed. These should be based on natural live diets equilibrated with the associated waterborne metal concentration. However, the absence of such designs should not be a criterion for rejecting currently available chronic data.
11) There is a need to develop mechanistically based bioavailability models for behavioral toxicity and to consider these for future regulatory application. Such models should be built from the ground up using behavioral endpoints, rather than by adjusting the sensitivity parameter in existing BLMs.
12) If bioavailability models are to be used in environmental regulation, ongoing support and availability for use of the models in the public domain are essential. Until this can be guaranteed, simpler, lower-maintenance translational tools based on bioavailability models, such as MLRs and look-up tables, may be preferable.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.4560.

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Data Availability Statement—Contact the authors for data availability (cmebane@usgs.gov or cmebane1@gmail.com).

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