Hazard/Risk Assessment

BIOTIC LIGAND MODEL OF THE ACUTE TOXICITY OF METALS.
1. TECHNICAL BASIS

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Abstract—The biotic ligand model (BLM) of acute metal toxicity to aquatic organisms is based on the idea that mortality occurs when the metal–biotic ligand complex reaches a critical concentration. For fish, the biotic ligand is either known or suspected to be the sodium or calcium channel proteins in the gill surface that regulate the ionic composition of the blood. For other organisms, it is hypothesized that a biotic ligand exists and that mortality can be modeled in a similar way. The biotic ligand interacts with the metal cations in solution. The amount of metal that binds is determined by a competition for metal ions between the biotic ligand and the other aqueous ligands, particularly dissolved organic matter (DOM), and the competition for the biotic ligand between the toxic metal ion and the other metal cations in solution, for example, calcium. The model is a generalization of the free ion activity model that relates toxicity to the concentration of the divalent metal cation. The difference is the presence of competitive binding at the biotic ligand, which models the protective effects of other metal cations, and the direct influence of pH. The model is implemented using the Windermere humic aqueous model (WHAM) model of metal–DOM complexation. It is applied to copper and silver using gill complexation constants reported by R. Playle and coworkers. Initial application is made to the fathead minnow data set reported by R. Erickson and a water effects ratio data set by J. Diamond. The use of the BLM for determining total maximum daily loadings (TMDLs) and for regional risk assessments is discussed within a probabilistic framework. At first glance, it appears that a large amount of data are required for a successful application. However, the use of lognormal probability distributions reduces the required data to a manageable amount.

Keywords—Bioavailability  Metal toxicity  Metal complexation  Risk assessment

INTRODUCTION

The importance of explicitly considering bioavailability in the development of water and sediment quality criteria for metals has been recognized for some time [1–3]. Criteria that incorporate this concept are being recommended to, and are being considered for application by, regulatory authorities [4,5]. A long history of experiments demonstrates the importance of water chemistry on the degree of toxicity of metals. What has been missing is a practical modeling implementation that can predict these variations in toxicity with some degree of generality and reliability.

This is the first in a series of three papers that present the biotic ligand model (BLM) of acute metal toxicity to aquatic organisms. This paper will focus on the structure of the model, the logic behind the choices made for the partitioning model, and some of the initial data analyses. The succeeding papers present more complete applications to the toxicity of copper [6] and silver [7] to fish and Daphnia.

The BLM is a synthesis of ideas that have a long history of development. Our contribution is to combine these pieces into an operational model of metal toxicity and to analyze enough data to demonstrate its strengths and weaknesses.

DESCRIPTION OF THE MODEL

Toxicity model

The conceptual framework for the BLM is an adaptation of the gill surface interaction model, originally proposed by Pagenkopf [8,9] and more recently utilized by Playle and coworkers [10–17], and the free ion activity model of toxicity; see Morel [18,19] and Campbell [20] for reviews. The general framework is illustrated in Figure 1. The model is based on the hypothesis that toxicity is not simply related to total aqueous metal concentration but that both metal–ligand complexation and metal interaction with competing cations at the site of action of toxicity need to be considered [9,21]. Mortality occurs when the concentration of metal bound to the biotic ligand exceeds a threshold concentration.

The BLM simply replaces the fish gill as the site of action with a more generally characterized site, the biotic ligand. The reason for this replacement is to emphasize that this model should be applicable to other aquatic organisms, for example, to crustaceans, as shown in the following, for which the site of action is not readily accessible to direct measurement. In fact, it is likely that these principles should apply to any organism for which the site of action is directly in contact with the external aqueous environment.

The role of metal complexation is critical because formation of organic and inorganic metal complexes renders a significant fraction of the total metal nonbioavailable. In fact, this modeling framework defines bioavailability of metals. As shown in Figure 1, dissolved metal exists in solution partially as free metal ion. This species is hypothesized to be the bioavailable species in more simplified versions of the free ion activity model of toxicity. The rest of the metal exists as nonbioavailable metal complexes that result from reactions of the metal...
with organic and inorganic ligands, as shown in the following. Of course, no way exists to verify directly which chemical species are bioavailable. All one can do is to examine whether the consequences of these hypotheses agree with observations. It is for this reason that we concentrate on comparing predicted and observed toxicity as the test of the model’s utility.

The toxicity of metals to organisms is assumed to occur as the result of free metal ion reacting with the physiologically active binding sites at the site of action. This is represented as the formation of a metal–biotic ligand complex. For fish, the biotic ligand appears to be sites on the surface membrane of the gill [22]. For modeling purposes, the site of action is treated as a biotic ligand—the biological counterpart of chemical ligands—to which metals can bind (Fig. 1, right side). For fish, the metal binding results in the disruption of ionoregulatory processes of the organism (e.g., sodium transfer across the gill is restricted), which leads to mortality [22].

The principal feature that distinguishes the BLM from considering only the free metal ion as the toxic species is that in the BLM, the free metal ion competes with other cations (e.g., Ca\(^{2+}\) and H\(^+\)) for binding at the biotic ligand as shown in Figure 1. As a result, the presence of these cations in solution can mitigate toxicity, with the degree of mitigation depending on their concentrations and the strength of their binding to the biotic ligand.

Model equations

The BLM is based on the hypothesis that the metal–biotic ligand interaction can be represented in the same way as any other reaction of a metal species with an organic or inorganic ligand. Consider a ligand \(L_0\), in this case the biotic ligand, and a divalent metal cation \(M_2^{2+}\). The charge on the biotic ligand is unknown. We assign a negative charge to be definite since it binds positive cations. However, this choice has no practical significance. The concentration of the metal-ligand complex \(ML_0\) is determined by the mass action equation

\[
[ML_0] = K_{ML_0}[M_2^{2+}][L_0]
\]

where \(K_{ML_0}\) is the stability constant for the metal-ligand complex and the square brackets denote molar concentration. It is assumed that protonation can also occur with the formation of a proton biotic ligand complex \(HL_0\) with concentration \([HL_0]\) and stability constant \(K_{HL_0}\):

\[
[HL_0] = K_{HL_0}[H^+][L_0]
\]

The mass balance equation associated with the biotic ligand \(L_0\) is

\[
[L_0] = [L_0]^f + [HL_0] + \sum_{i} N_{L0i} [ML_i]
\]

where \([L_0]^f\) is the total binding site density of the biotic ligand (e.g., mmol of available sites/g of tissue), \([HL_0]\) is the concentration of protonated sites, and \(N_{L0i}\) is the number of metal complexes \(ML_i\), e.g., CuL_0, CaL_0, etc., that form with the biotic ligand \(L_0\).

The analogous equations for the metal cation \(M_2^{2+}\) and the other aqueous ligands \(L_j\) that form metal-ligand complexes are

\[
[M_iL_j] = K_{ML_i}[M_2^{2+}][L_j]
\]

\[
[HL_i] = K_{HL_i}[H^+][L_j]
\]

\[
[L_j] = [L_j]^f + [HL_i] + \sum_{i} N_{Lji} [ML_i]
\]

The analogous equations for the proton and metal biotic ligand complexes (Eqns. 1–2) can be solved as discussed below. Then the biotic ligand equations (Eqns. 3–8) can be evaluated. This convenient approach is adopted below.

The parameters required that are specific to the BLM are the conditional stability constants, \(K_{HL_i}\) and \(K_{ML_i}\) \(i = 1, \ldots, N_L\), for the proton and metal biotic ligand complexes (Eqns. 1–2), and the total site density \([L_0]^f\) (Eqn. 3). For fish, the site densities and stability constants are determined on the basis of experimental fish gill measurements. These are currently available for a number of metals, including copper, cadmium [11,23] and silver [12]. For other organisms, the values are obtained by fitting the model to observed mortality data, as discussed below.

The model is based on the idea that mortality (or other toxic effect) occurs if the concentration of metal on the biotic ligand reaches a critical concentration \(C_{ML}^b\):

\[
C_{ML}^b = [ML_0]
\]

This critical concentration for mortality can only be determined from toxicity experiments that establish the LC50 or EC50...
concentrations—which are the concentrations causing mortality (LC50) or another effect (EC50) to fifty percent of the test organisms—for a variety of toxic metal and competing cation concentrations. Once the site densities and stability constants are known, the critical concentration can be determined by computing the biotic ligand concentration corresponding to the aqueous LC50 concentration. The validity of the BLM can be established only if the critical concentration $C_0$ is the same over the entire range of water chemistry tested. Examples of this analysis will be given below.

**Chemical model**

The chemical speciation computations are standard and may be performed with any of several models that exist, for example, MINEQL [24], MINTEQA2 [25,26], or the program used for the computations presented here and in the accompanying papers, CHESS (Chemical Equilibria in Soils and Solutions) [27]. The inorganic speciation is the straightforward part of the computation because the ligands are well characterized, for the most part, and their binding constants are known [28]. The difficult part is modeling the complexation of metal cations by organic matter. Although Pagenkopf [9] recognized the ameliorating effect of organic matter on toxicity, the effect of organic matter was neglected in his original model formulation. It was applied to test results using laboratory waters with low organic matter content. However, dissolved organic matter is known to be an important ligand for most metals in most natural waters.

**Dissolved organic matter complexation**

Many models have been proposed for modeling the complexation of metals to dissolved and particulate organic matter, those of Van Riemsdijk and colleagues [29,30] being a recent example of a comprehensive modeling framework. For many of these models, the parameters that have been estimated apply to a specific experimental data set. What is required is a model that has been calibrated to multiple data sets and for as many metals as possible.

Since this is our most important criterion, we have chosen WHAM Version 5, the Windermere humic aqueous model, by Tipping and coworkers [31,32]. The model is fully described and the computer code available [33]. It contains a detailed model of proton binding. This is then expanded to include metal cation binding. The idea is that the proton and the metal cations are competing for the same sites, so a detailed model of proton binding is the essential first step.

This description follows Tipping et al. [31,32]. Protons bind to carboxyl (type A) and phenolic (type B) sites. A uniform distribution of $pK$s is specified for each type of site where $pK = -\log_{10}K$ and $K$ is the stability constant for the proton-site binding reaction. The site distributions are parameterized by the median $pK_a$ and $pK_h$ and their ranges $\Delta pK_a$ and $\Delta pK_h$. Each uniform distribution is approximated using four discrete $pK$s for each type: $pK_1$, ..., $pK_4$, and $pK_2$, ..., $pK_8$, respectively, where

$$pK_{h1} = pK_a - \frac{\Delta pK_a}{2}$$

$$pK_{h2} = pK_a - \frac{\Delta pK_a}{6}$$

$$pK_{h4} = pK_a + \frac{\Delta pK_a}{6}$$

$$pK_{h8} = pK_a + \frac{\Delta pK_a}{2}$$

and the analogous equations for $pK_{h5}$, ..., $pK_{h14}$. The site density of type A sites is $n_a$. Humic and fulvic acids have fewer B sites and their site density is assumed to be $n_b = n_a/2$. The electrostatic interactions are modeled using an empirical formulation with one adjustable parameter, $P$, which is related to the surface complexation model formulations [34,35]. Tipping and co-workers have fit this model to various sets of acid-base titrations of organic matter to determine the six model parameters: $n_a$, the $pK$s and $\Delta pK$s, and the electrostatic parameter $P$.

The stability constants for metal binding are parameterized by only two additional constants. This is a remarkably parsimonious construct. The idea is to specify the metal binding constants using the proton binding $pK$s. The metal stability constants, $K_{Mi}$, $i = 1, \ldots, 8$ are defined relative to the proton binding constants $K_i$ via two parameters: $K_{MA}$ and $K_{MB}$:

$$K_{M_i} = \frac{K_{MA}^i}{K_{MA}}$$

where the $K_{M_i}$ are the discrete metal stability constants analogous to the $K_{h}$ proton binding stability constants. In addition to binding to the proton sites individually, binding to two sites at once (bidentate sites) is also allowed. The binding constants for these sites $K_{M_i}$ are computed from the product of the metal monodentate binding constants:

$$K_{M_i} = K_{hA}K_{hB}$$

For the sake of simplicity only 11 of the possible pairs are used. From data fitting, Tipping and Hurley [31] noted that there was a correlation between $K_{MA}$ and $K_{MB}$:

$$pK_{MB} = 1.38pK_{MA} + 2.57$$

which reduces the number of metal specific parameters to one. This is the most parsimonious parameterization possible. For each metal, only one parameter $K_{MA}$ is required. The rest follow from the equations given previously (Eqs. 14 to 17).

Figure 2 presents a selection of the titration data used to determine the parameters. The plots, redrawn from Tipping and Hurley [31], utilize the following convention. The $y$-axis variable $v$ is the concentration of metal bound to dissolved organic carbon (mol/g DOC), and the $x$-axis variable is the free metal concentration (mol/L). Both are plotted as

$$\log_{10}$$

Figure 2A to C present the data for copper. Figure 2A illustrates the effect of increasing ionic strength ($I = 0.001$–0.01 M). Increasing the ionic strength slightly reduces the amount of copper complexed to DOC. Figure 2B illustrates the effect of calcium competition ($[Ca] = 0.001$, and 0.01 M) on copper binding. Increasing the calcium concentration decreases the quantity of complexed copper since Ca$^{2+}$ competes with Cu$^{2+}$ for the same binding sites. Since calcium also competes with copper at the biotic ligand, the proper modeling of this competition for DOC sites is important. Figure 2C illustrates the effect of pH = 5.14, 7.00, and 8.44. As the pH decreases, the increasing concentration of H$^+$ competes with Cu$^{2+}$ for the binding sites, and less copper is complexed to the DOC. The deviations at the higher complexed copper con-
centrations are of less concern in this application since they are usually above toxic concentrations ([Cu] ~ 10^{-9} M). It is the low concentrations that are important. Figure 2D to F present the effect of pH on the complexation of calcium, cadmium, and lead. As with copper, less metal binds to DOC at lower pHs. The careful and complete calibration of the WHAM model, illustrated in Figure 2, was the principal reason for its choice in this application.

Figure 3 presents the WHAM parameters needed to fit the data presented in Figure 2 and the rest of the calibration data set [31,32]. They are presented as variations from the mean. The numerical values are presented in Table 1. Figure 3A presents the proton binding constants. The site density \( n_A \) is remarkably constant, as are the \( pK_A \)s. Only the \( \Delta pK_B \)s appear to vary by more than an order of magnitude. This variability is due to the finite range of pHs that comprise any one data set. To identify the full range of \( pK \)s precisely would require

<table>
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<th>( pK_B )</th>
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<th>( \Delta pK_B )</th>
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\(^a\) WHAM = Windermere humic aqueous model.
\(^b\) Arithmetic mean of the values.
experimental data spanning a large pH range. Figure 3B presents the variation in pK<sub>MA</sub> found from fitting various data sets. The variation for all metals except calcium is less than 0.5 pK units. Thus, it appears that WHAM provides a robust model for the most difficult portion of the chemical speciation calculation: the complexation of metals to organic matter. Nevertheless, it is likely that further refinements will be necessary, particularly at lower metal concentrations, where even stronger sites may need to be included. This is almost certainly the case for silver at low concentrations, where binding to sulfur-containing ligands has been suggested as being important [36].

**RELATIONSHIP OF METAL ACCUMULATION TO COPPER ACUTE TOXICITY**

The BLM is used here to describe the toxicity of copper to fathead minnows (*Pimephales promelas*) and rainbow trout (*Oncorhynchus mykiss*). These species have comparable sensitivities to copper [37] and have been studied extensively. For acute toxicity, the biotic ligand for fish is the gill. Hence, it is necessary to predict metal accumulation at the surface of the fish gill in order to predict metal toxicity to fish. Several studies have shown that when juvenile fathead minnows and rainbow trout are exposed to copper, a relatively rapid increase occurs above background levels of copper bound to the gill. This rapid initial increase takes place over a time scale of a few hours to a day [10]. Similar data for juvenile rainbow trout indicates that this rapid initial increase in gill copper is followed by a more gradual, longer-term increase [38]. It is believed that the rapid initial increase in gill copper reflects binding to physiologically active receptor sites at the gill surface, the sites that control the ionoregulatory processes of the fish.

To predict toxicity, it is necessary to demonstrate a relationship between gill accumulation and mortality. This relationship is illustrated in Figure 4, which shows the dose response for rainbow trout mortality after 120 h of exposure as a function of the gill copper accumulation after just 24 h of exposure [38]. These experiments were performed using a constant total dissolved copper concentration (10 µg/L). The gill Cu concentration was regulated by adding different organic ligands with varying affinities for copper to the test water. As shown in Figure 4, the gill copper LC50—the copper concentration on the gill that causes 50% mortality—is estimated to be 22 nmol/g wet weight (nmol/gw). The background gill copper concentration—the concentration associated with no mortality—is approximately 12 nmol/gw. This compares well with the fathead minnow background gill copper level, approximately 12 nmol/gw, that was measured by Playle [10] in the absence of added copper. Based on these results, the gill copper LC50 should be approximately 10 nmol/gw—the gill Cu LC50 of 22 nmol/gw minus the background level of 12 nmol/gw.

**MODEL APPLICATION**

The first application of the BLM will be made using a very complete and well-characterized set of experiments by Erickson [39] investigating copper toxicity to larval fathead minnow. In these experiments, fish were exposed to increasing concentrations of copper, and the LC50s were determined. Systematic variations of important water quality characteristics were employed to produce LC50s as a function of concentration. Three sets of experimental results will be used to illustrate the effects of DOC: hardness and pH and the model performance. The remainder of the data are presented in a companion paper [6]. The thermodynamic constants used for WHAM, the gill computations, and the base water chemistry are listed elsewhere (tables 1 to 3 in Santore et al. [6]). The chemical species concentrations for the experiments discussed here are listed in Table 2.

**Effect of dissolved organic carbon on copper toxicity**

The effect on copper LC50 of variations in DOC concentration is shown in Figure 5. The pH and hardness were held approximately constant for these experiments. The added DOC is Alrich humic acid. Figure 5A demonstrates that the measured total Cu LC50 (filled data points and associated trend line) increases with increasing DOC concentration, as is often observed. This is rationalized in the model by assuming that the copper that forms a complex with DOC is not bioavailable. As a result, as DOC increases, more copper is needed to exert the same degree of toxicity. Figure 5B shows the corresponding free copper (Cu<sup>2+</sup>) LC50s. These LC50s were calculated using WHAM and the reported chemistry and the total copper LC50s in Figure 5A. The results are somewhat variable, but they are approximately independent of DOC concentration. This is consistent with the free ion activity model of toxicity, where toxicity is directly related to the concentration (actually the activity) of free copper.

Figure 5C presents the calculated gill copper concentrations associated with the measured total copper LC50 data. The gill copper concentration were calculated using WHAM to compute the metal–humic acid complexes and CHESS to compute the gill accumulation. The Cu<sup>2+</sup>-gill, Cu<sup>2+</sup>-gill, and H<sup>+</sup>-gill conditional stability constants and gill site density estimated by Playle [11] from measured copper gill concentrations were used. The exchangeable gill copper LC50 averages slightly less than 5 nmol/gw. This level of fathead minnow gill copper accumulation is a factor of two lower than the measured exchangeable gill copper LC50 for rainbow trout of 10 nmol/gw (Fig. 4), which is quite encouraging. The fact that the gill copper LC50 is approximately constant across the DOC range tested indicates that, as was the case with free copper, gill copper concentration can also be used to predict acute mortality when DOC is varied.
Table 2. Water chemistry employed in the WHAM calculations for the experimental data presented in the listed figures*

<table>
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<th>Figure</th>
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* HA is the humic acid percentage in the dissolved organic carbon (DOC). The rest is fulvic acid. TIC is the total inorganic carbon concentration = [CO3][HCO3][CO2]. It is calculated from the reported pH and alkalinity data. The sulfide concentrations are [S2] = 0 for Figures 5 to 7 and [S2] = 10 mM for Figures 9 and 10. WHAM = Windermere humic aqueous model.

** L = lab water; E = effluent.

Effect of hardness on copper toxicity

The effect of variations in calcium concentration on copper LC50 is shown in Figure 6. The experiments were performed with DOC and pH held constant. Over the range of experimental conditions of 0.5 to 2.5 meq Ca/L, the ratio of Ca:Mg was approximately 2:1, and the corresponding hardness range was 75 to 375 mg CaCO3/L. The data are displayed as before except that the LC50 results are plotted versus calcium. As was the case with increasing DOC, the measured total Cu LC50 increases with increasing Ca concentration. This increase in LC50 with hardness is qualitatively consistent with the current water quality criteria (WQC) for copper, which increases as a function of hardness as well [37]. However, as discussed in the following, the magnitude is significantly less than expected.

Free copper LC50s corresponding to the total copper LC50s are shown in Figure 6B. Like the total copper LC50s, the free copper LC50s varied over the range of calcium levels tested. Therefore, a single free copper concentration is no longer uniquely associated with 50% mortality. This is not unexpected because copper, a cation, does not form complexes with calcium, another cation. Therefore, except for a relatively minor ionic strength effect (see Fig. 2A), no interaction of calcium with copper occurs in the water. Note that, in combination with the results from the DOC experiments shown in Figure 5B, these results indicate a more than 5-fold range in variation of free copper concentration, from less than 0.015 to 0.075 μmol/L, associated with the fathead minnow free copper LC50. These results indicate that considering only the free ion as the bioavailable ligand cannot be used to rationalize these data.

In contrast to the free copper results, the calculated gill Cu concentrations shown in Figure 6C indicate a relatively consistent concentration for the range of calcium concentrations tested. The average fathead minnow gill copper concentration, 12 nmol/gw, is in reasonably good agreement with the gill copper concentration of slightly less than 5 nmol/gw calculated for the DOC experiments (Fig. 5C) and with the gill Cu LC50 of about 10 nmol/gw of Figure 4. The reason that the gill copper tends to be relatively constant, even as the free copper concentration increases, is that the calcium competes with the free copper for binding at the biotic ligand on the gill. Hence, a higher free copper concentration is required to achieve the same gill Cu concentration associated with mortality.

It is interesting to note that for the calcium experiments, the observed increase in total copper LC50—slightly more than a factor of two over the range of conditions tested—is half as much as is expected from the hardness correction incorporated in the current copper WQC—4.5-fold, for hardness increasing from 75 to 375 mg CaCO3/L [37]. The BLM framework provides an explanation for this difference [21]. The experiments by Erickson [39] with varying hardness were conducted with constant alkalinity by adding calcium in the form...
Fig. 5. Relationship of copper LC50s to variations in dissolved organic carbon (DOC) concentration. The lines are drawn by eye to represent the data. (A) LC50 expressed as the concentration of total dissolved copper. (B) LC50 expressed as the concentration of the free ion activity of copper. (C) LC50 expressed as the concentration of the copper sorbed to the gill. The LC50s in (B) and (C) are calculated using the Windermere humic aqueous model (WHAM) and the biotic ligand model from the concentrations of total copper and the other relevant aqueous species (Table 1). Data from Erickson [39].

Effect of pH on copper toxicity

The effect of changes in pH on total copper LC50 is shown in Figure 7A. The total copper LC50 increases from about 0.1 to 2 μmol/L as the pH increases from 6.5 to 8.8. In addition to the total Cu concentrations, the free copper ion activity was also measured with a selective ion electrode. It is fortunate that Erickson [39] made these measurements. When the concentration of total Cu is used to predict the free copper in solution using WHAM, the computed results for Cu activity were significantly less than the measured data. The apparent difficulty, which is limited to this set of data only, and its solution are discussed in the companion paper [6]. However, the measured free copper activity, shown in Figure 7B, can be used to compute gill copper concentrations, which are shown in Figure 7C. The variation in predicted gill copper concentrations reflects the variation in the cupric ion activity measurements. The average gill copper concentration of approximately 7 nmol/gw is within the range of the results for the two previous sets of experiments in which DOC and calcium were varied.

In the context of the BLM framework, pH affects copper toxicity in several ways. First, the model predicts that toxicity will decrease with increasing pH as a result of the effect of pH on speciation and complexation of copper. As pH increases, the fraction of Cu that exists as copper carbonate complexes increases, thereby reducing toxicity. Further, the deprotonation of DOC at higher pH levels increases the degree to which the copper–DOC complex forms, which reduces bioavailability as well. These effects on toxicity are offset to some degree by the competition between the H+ and Cu2+ ions binding to the biotic ligand, a factor that by itself would result in an increase in toxicity as pH increases (as the H+ concentration decreases).
The ability of the BLM to reproduce the effect of variations in water chemistry on copper toxicity to fish is, in itself, of interest. However, of more practical significance is its potential use in setting permit limits and defining site-specific WQC. One component of this procedure is to conduct bioassays to develop water effect ratios (WERs) [2,40]. The WER is defined as the ratio of the LC50 in the receiving water to the LC50 in laboratory water for the species being tested. The WQC is then multiplied by the WER to define a site-specific WQC.

An example of this procedure is the WER study by Diamond [41] for copper in a stream in Pennsylvania, USA. The water quality characteristics of the laboratory, upstream, effluent, and mixtures of the effluent and receiving water are summarized in Figure 8. The thermodynamic constants used for WHAM, the gill computations, and the base water chemistry are listed elsewhere (tables 1 to 3 in Santore et al. [6]). The chemical species concentrations are listed in Table 2. Total organic carbon (TOC) varied from approximately 1 mg/L in the lab water, 3 mg/L in the upstream receiving water, and 12 mg/L in the effluent. Alkalinity (70–100 mg CaCO3/L) and hardness (75–160 mg CaCO3/L) varied to a lesser degree, while the pH was relatively constant in these waters (pH ~ 8). The water quality characteristics of the mixtures of upstream and effluent water were generally consistent with what would be predicted from mass balances with the exception of the 75% effluent sample for alkalinity and hardness.

The procedure used to predict the WER on fathead minnows is as follows. The water quality characteristics for a test sample are used in WHAM and CHESS to compute the gill copper concentration as described previously. A numerical titration is then performed in which the dissolved copper is varied over a range of concentrations and the computed gill copper concentration determined for each concentration of copper added. Representitive results are illustrated for an effluent sample and a laboratory sample in Figure 9. For the effluent sample, the gill copper concentration initially increases hardly at all as copper is added to the system, reflecting the complexation of the copper by DOC high-affinity binding sites. Once the high-affinity binding sites are saturated, the gill copper concentration begins to increase more rapidly as copper is added. The dissolved copper concentration corresponding to a gill copper concentration of 6.3 nmol/gw, the gill copper LC50, is 1,440 µg/L. This dissolved copper concentration is used as the predicted LC50 for copper in the effluent.

In contrast, the predicted gill copper concentration in laboratory water increases much more rapidly as copper is added. The more rapid increase occurs because of the lower concentration of DOC in laboratory water. Hence, less Cu–DOC complex forms. This, together with lower alkalinity that produces a lower concentration of copper carbonate complexes, and lower hardness so that less calcium competition occurs, results in a more rapid increase in gill copper concentration as dissolved copper concentration increases. The result is that the predicted gill copper concentration reaches the gill Cu LC50 of 6.3 nmol/gw at a much lower dissolved copper concentration of 220 µg/L. This concentration is the predicted LC50 for copper in the laboratory water. The ratio of the predicted effluent to laboratory water LC50s is the predicted WER.

A summary of the observed and predicted results of the WER analysis for the January test data is shown in Figure 10. The dissolved copper LC50s for the lab water, upstream, water mixtures of upstream and effluent (53 and 75% effluent), and 100% effluent are compared in Figure 10A. The general trend of increasing LC50 with increasing effluent is reproduced for both the observed results and model predictions, with the ex-
Fig. 8. The aqueous concentrations of total organic carbon (TOC), taken to be equivalent to dissolved organic carbon in the application of biotic ligand model, alkalinity, hardness, and pH, in laboratory water (LAB), in upstream water (U/S), and at the indicated percentages of effluent dilutions in upstream water (Table 1). Data from Diamond [41].

Fig. 9. Method of calculating the LC50 using the biotic ligand model. Relationship of copper concentration sorbed on the gill and dissolved copper as computed using the biotic ligand model (BLM) for laboratory water and 75% effluent.

Fig. 10. Comparison of measured and calculated LC50s and the water effect ratios in laboratory water (LAB), in upstream water, (U/S), and at the indicated percentages of effluent dilutions in upstream water (Table 1). Data from Diamond [41].
ception of the 75% effluent sample, for which the measurements of alkalinity and hardness are inconsistent with the mixing of the end members (Fig. 8). Analogous results for the predicted WERs are shown in Figure 10B. Since both the predicted and measured lab water LC50s are used to compute the WER, the lab water WERs equal one by definition. The predicted LC50 and WER results are generally within a factor of two of the measured results.

**OBSERVATIONS AND SPECULATIONS**

The BLM provides a basis for understanding a number of observed features of metal toxicity and for suggesting experiments designed to test hypotheses derived from the model.

**Other organisms**

The main reason for using the description biotic ligand model rather than the gill model is the expectation that the same framework can be applied to organisms other than fish. For some organisms (e.g., small crustacea), the metal concentration on the respiratory organs, where ionoregulation presumably occurs, either is too small a mass or is otherwise not conveniently measured. For other organisms, the site of action may be elsewhere. However, a model that incorporates competitive interaction at the site of action and also computes metal complexation with other aqueous ligands is likely to be either the whole or part of the answer to the problem of determining the influence of varying water chemistry on toxic response.

The toxicity of copper and silver to crustacea, specifically *Daphnia*, are examined elsewhere [6,7]. Since no biotic ligand metal concentrations are available, no direct way exists to determine the critical biotic ligand concentration associated with 50% mortality. For these cases, the BLM is fit to this organism by determining the critical biotic ligand concentration for copper and silver that reproduces the observed LC50s.

It is not intuitively apparent that this is the proper procedure since it assumes that the binding constants for a particulate metal are the same for each species to which it is applied. If the biotic ligand is a respiratory surface, then the assumption seems reasonable. Of course, the only test is a comparison to LC50 data over a wide range of water chemistry conditions.

Summary plots comparing predicted and observed LC50s for copper [6] and silver [7] to fathead minnows, rainbow trout, and *Daphnia* are shown in Figure 11. The dotted lines delineate factors of two uncertainty limits. For each of the metals, the only difference between the fish and *Daphnia* model is the critical biotic ligand concentration for copper and silver that reproduces the observed LC50s.

**Comparative toxicity of metals**

The toxicity of three metals, Cu, Ag, and to a lesser extent Ni [42], have been analyzed within the context of the BLM. A comparison of the binding constants, site densities, and critical biotic ligand concentrations is presented in Table 3. For Cu and Ag, both fathead minnows and crustacea have been analyzed.

Consider, first, the differences among the organisms. The *Ceriodaphnia* and *Daphnia magna* biotic ligand binding constants and site densities are assumed to be the same as for the fathead minnow, as described previously. However, the critical biotic ligand concentrations for nickel (239 nmol/gw) are much lower than for fathead minnow, except for copper (fathead minnow = 10 nmol/gw, *Ceriodaphnia* = 0.19 nmol/gw). Thus, organism sensitivity is modeled by changing the critical biotic ligand concentration. It is assumed that the chemical properties of the crustacea biotic ligand—biotic ligand binding constants for copper and silver are remarkably similar, as are the biotic ligand binding constant and site density for copper and silver (10 nmol/gw) and silver (17 nmol/gw)—suggests that metal toxicity is due to the absolute number of sites occupied by the metal cation, independent of the identity of the metal cation. However, the fathead minnow critical biotic ligand concentration for nickel is much lower than for either copper (10 nmol/gw) or silver (17 nmol/gw). Thus, it appear that metal toxicity is not simply due to the absolute number of sites occupied by the metal cation, but is also influenced by the identity of the metal cation: nickel versus copper or silver. Copper and silver are more toxic than nickel at the same biotic ligand concentrations. This observation has direct implications for the toxicity of metal mixtures, as discussed next.
Metal mixtures

The BLM can be used to formulate the possible types of behavior for mixtures of metals that exert toxicity at the same biotic ligand. The criterion in Equation 9 relates the LC50 to a critical concentration of metal at the biotic ligand. If the identity of the metal is not relevant but only the fact that it is binding to the biotic ligand and thereby disrupting ionoregulation, then any toxic metal would exhibit a similar effect. This would correspond to equal toxicity at the site of action. Hence, the sum of the toxic metals bound to the biotic ligand would be compared to the same critical concentration that is, 

\[ C_{b}' = [M_1L_b] + [M_2L_b] \]  

where \( M_1^+ \) and \( M_2^+ \) are the two metals in the mixture. The critical body burden for narcotic chemicals behaves in this way. However, if ligands bound to the biotic ligand are of varying toxicity, then the usual relative toxicity would need to be taken into account.

The terms in the right-hand side of Equation (18) are additive. Note that copper and silver exhibit the stronger form of additivity (Egn. 18) since the critical ligand LC50 concentrations are essentially equal (Table 3). For nickel and either copper or silver, the additivity would be relative to the individual LC50s (Egn. 19) since the critical concentrations are different (see Table 3) indicating differing potencies for ionoregulatory disruption.

The additivity of LC50s when expressed as aqueous concentrations is well known to be inaccurate. The most straightforward use of the BLM is to compute the LC50 for a particular set of metal and aqueous ligand concentrations. Since exposure conditions vary considerably, it is useful to make this computation for every set of concentrations to which the aquatic organisms would be exposed. This allows the use of probabilistic risk assessment procedures, which evaluate the probability of an impact for the species of interest.

Table 3. Parameter values for copper, silver, and nickel binding to the biotic ligand

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where \( [M^2+] \) is a competing nontoxic cation, e.g., Ca\(^2+\). The concentration of free biotic ligand \([L_b]\) can be found and substituted into Equation 20:

\[ [M_iL_b] = \frac{[K_{M_iL_b} [M_i^+] [L_b]]}{(1 + K_{M_iL_b} [H^+] + [M_iL_b] [M_i^+] + K_{M_iL_b} [M_i^2+] + K_{M_iL_b} [M_i^3+])} \]  

This equation relates the biotic ligand concentration of \( M_i \), \([M_iL_b]\), to the aqueous concentrations of \( M_i \) and \( M_{i-} \), as well as to pH \([H^+]\) and calcium \([M_{Ca}^2+]\) concentrations. If the biotic ligand concentration \([M_iL_b]\) were only a function of the concentration \([M_{Ca}^2+]\), then an additive equation analogous to Equation 19 could be written for \([M_i^2+]\) and \([M_i^3+]\). Note that the biotic ligand concentration \([M_iL_b]\) of \( M_i^+ \) depends on the concentration of \( M_{Ca}^2+ \) via the denominator of Equation 22. Additivity occurs if the interaction term \( K_{M_iL_b} [M_i^2+] \) in the denominator of Equation 22 is small and

\[ 1 + K_{M_iL_b} [H^+] + K_{M_iL_b} [M_i^+] + K_{M_iL_b} [M_i^2+] + K_{M_iL_b} [M_i^3+] \]

In this case Equation 22 is dependent only on the concentration of \( M_{Ca}^2+ \) and the rest of the water chemistry:

\[ [M_iL_b] = \frac{[M_i^2+] [L_b]}{1 + K_{M_iL_b} [H^+] + K_{M_iL_b} [M_i^+] + K_{M_iL_b} [M_i^2+] + K_{M_iL_b} [M_i^3+] + K_{M_{Ca}L_b} [M_{Ca}^2+] + K_{M_iL_{Ca2}} [M_{Ca}^2+] \]  

APPLICATIONS TO RISK ASSESSMENT

The use of total metal concentration to evaluate risk to aquatic organisms is well known to be inaccurate. The BLM can be quite useful in this context since it can be used to convert total metal concentrations to the appropriate bioavailable fraction. The most straightforward use of the BLM is to compute the LC50 for a particular set of metal and aqueous ligand concentrations. Since exposure conditions vary considerably, it is useful to make this computation for every set of concentrations to which the aquatic organisms would be exposed. This allows the use of probabilistic risk assessment procedures, which evaluate the probability of an impact for the species of interest.

Figure 12 illustrates an application to a stream receiving a metals discharge. It is assumed that the time series of data are...
The probability of exceedence is determined. The ratio of the metal concentration to the LC50 is compared to one (or the log [metal/LC50] is compared to zero) and the LC50. The ratio of the metal concentration to the LC50 is computed using the BLM, from which the frequency of violation can be determined. The only problem that remains is the presence of cross-correlation between the variables. Our own practical experience has demonstrated that unless these cross-correlations are large ($R \geq 0.7$ so that $R^2 \geq 0.5$), they do not significantly influence the computation. Thus only the strongest cross-correlations need to be considered. In the absence of the necessary data, a sensitivity analysis can be conducted to bracket the probable effect of cross-correlations. A computer program is available that implements a simplified version of the BLM and the probabilistic dilution model.

Note that the probabilistic analysis presented in Figure 13 need not represent only various times in one receiving water. Regional risk assessments, which attempt to evaluate the risk of a particular chemical at many places, can also be thought of in terms of a probabilistic analysis. The frequency distribution can represent various exposure conditions at many locations at one time or for one season, or they can represent the entire ensemble of data from all the locations. The only requirement is that the assumed form of the probability distribution (e.g., lognormal) be representative. The necessary statistical parameters could be established using regional monitoring data, for example. Once they are established, the analysis proceeds as discussed previously. The violation frequency would represent the extent to which a particular metal represents a potential hazard in the region.

**SUMMARY AND CONCLUSIONS**

We have presented the BLM and demonstrated its utility using portions of two available data sets for copper. The model considers both the aqueous speciation of the metal and cation–metal competition at the biotic ligand. It is constructed from previously available formulations: the gill site interaction model of Pagenkopf [9] and, more recently, of Playle and coworkers [10–17]. The organic carbon complexation model of Tipping and coworkers is used for the aqueous speciation computation [31–33]. The data analyses presented in this paper and its companions [6,7] demonstrate that the BLM can predict the copper and silver LC50s for both fish and Daphnia to within a factor of two for observed LC50s that range over two orders of magnitude because of water chemistry variations.

The application of the BLM to analyzing the additivity of metals is examined. Although the copper and silver fathead minnow data suggest that the toxicity of these metals is interchangeable, the remaining data, nickel in particular, do not. The condition for aqueous concentration toxic unit additivity is presented. Finally, the use of the BLM for risk assessment evaluations is presented. An analysis framework is suggested in which lognormal probability distributions are used. This approach requires only modest data sets of metal concentrations, pH, alkalinity, hardness, and DOC concentrations to establish the statistical parameters.

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