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Evaluation of the Biotic Ligand Model for Predicting Metal Bioavailability and Toxicity in SRS Effluents and Surface Waters

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Executive Summary

Researchers have long recognized that the toxicity of metals to aquatic life is a function of water chemistry. Metals can form organic and inorganic complexes and can also sorb onto suspended particles, all of which can reduce the bioavailability and thus the toxicity of the metals. The EPA first addressed metal bioavailability in the 1970's, by issuing hardness-based criteria for selected metals. More recently, the Biotic Ligand Model (BLM) was developed as a computational approach that uses water chemistry to predict metal bioavailability. In 2003, the EPA issued a draft update of ambient water quality criteria for copper, which uses the BLM to calculate safe concentrations of copper for surface waters. However, until the document is finalized (probably in 2006) water quality criteria for copper continue to be hardness-based criteria. Eventually, it is anticipated that water quality criteria for copper, silver, cadmium, and zinc, and possibly nickel, lead, and cobalt will be revised using the BLM.

All of the water quality data that were used to develop the BLM were from waters that have considerably greater hardness than the waters at SRS. Because the cations (primarily calcium and magnesium) that contribute to hardness also influence metal bioavailability, water hardness is an important factor in estimating metal toxicity.

In order to evaluate the performance of the BLM in SRS waters as a means of predicting metal toxicity, water samples were collected from SRS waters that differ with respect to hardness, pH, and concentrations of dissolved organic matter. A number of manipulations were performed on the waters and the BLM was run on each data set. Acute toxicity tests were also performed on each water sample before and after the manipulations and toxicity tests were performed on each sample. By comparing the toxicity predicted by the BLM with the LC50s from the toxicity tests, the accuracy of the BLM in predicting metal toxicity in SRS waters can be determined.

The results indicate that in most instances, the BLM provides an inexpensive and reasonably accurate estimate of copper toxicity in SRS waters. The most important water chemistry factors that influence copper bioavailability in SRS waters are pH and dissolved organic carbon. Copper toxicity is inversely related to pH and to DOC concentrations (as pH increases, copper toxicity decreases; as DOC concentrations increase, copper toxicity decreases). Water hardness is also important, but it appears to be less important in SRS waters than pH and DOC. If the BLM is used to estimate metal toxicity, it is important that the BLM be run on a minimum of 3 to 5 water samples collected from the same location on different days. It may be important to consider seasonal differences in water quality, as well.

1.0 Introduction

Although the EPA water quality criteria for most toxicants are specified concentrations that are identical for all receiving streams, the criteria for seven metals (cadmium, chromium III, copper, lead, nickel, silver, and zinc) are calculated values that are inversely related to water hardness. These metals have hardness-based criteria because it has long been recognized that the toxicity of these metals is inversely related to water hardness (Alabaster and Lloyd, 1980, U.S. EPA, 1986). It is now recognized that hardness was often really a surrogate for other water quality variables (e.g.

alkalinity, pH and specific ions such as calcium, magnesium, chloride, and carbonate), which may have been equally or more important than hardness (Niyogi and Wood, 2004). Other water quality parameters that can also greatly affect metal toxicity include dissolved organic carbon (DOC) and sulfide, which reduce metal toxicity by forming complexes with the metals, thus reducing their bioavailability (Niyogi and Wood, 2004).

Recognizing that hardness-based criteria were often overprotective of receiving streams, the U.S. EPA published interim guidance on the use of Water Effect Ratio (WER) testing in 1994 (U.S. EPA, 1994) and final guidance in 2001 (U.S. EPA, 2001). WER testing involves performing a series of paired metal spiking toxicity tests in site water and standard laboratory water. If a metal is less toxic in site water than in laboratory water, site specific water quality standards can be developed that reflect the reduced toxicity of a metal in site water. Because it is costly and time-consuming to perform WERs, their use has been somewhat limited (Paquin et al., 2002a).

A more recent approach to developing site-specific water quality criteria for metals has been the use of the Biotic Ligand Model (BLM). Initially developed in the late 1990s as a collaborative effort between scientists at the EPA and several research institutes and universities, the BLM is fundamentally a chemical equilibrium-based model that predicts metal bioavailability (and thus toxicity) based on the degree of metal binding at the gill surfaces of fish (Schnoor et al, 1997; U.S. EPA Science Advisory Board, 2000; Di Toro et al, 2001; Santore et al, 2001). The BLM is in essence a computational equivalent of WER testing (Niyogi and Wood, 2004). The U.S. EPA is in the process of revising the hardness-based criteria for several metals using the BLM. A draft of the criteria for copper was released in 2003 (U.S. EPA 2003), and research is currently in progress to develop BLM-based criteria for silver, lead, zinc, nickel, cadmium, and chromium.

The BLM was primarily developed using water quality data from streams in the northeast and north-central regions of the United States. In general, these streams have much higher hardness and much lower DOC than SRS surface waters. This report presents an overview of the mechanisms of metal toxicity and the Biotic Ligand Model and also evaluates the potential use of the BLM at SRS, using data collected from SRS outfalls and receiving streams. In addition, it also evaluates the extent to which several water quality parameters affect metal bioavailability under the low-hardness, high DOC conditions that exist in SRS surface waters and provides recommendations on the use of the BLM at the Savannah River Site.

2.0 Background - Mode of Toxic Action of Heavy Metals to Fish

In order to understand the effects that water chemistry can have on metal toxicity, it is necessary to have some understanding of how metals produce a toxic response in aquatic organisms, such as fish. Gills are the primarily route of uptake for most heavy metals. Metals affect fish gills primarily by disrupting osmoregulation and by reducing oxygen uptake at the gill surface, due to the production of excess mucous by the gills (Mallatt, 1995). These processes are summarized in this section of the report.

The bodies of freshwater organisms have a higher concentration of cations (primarily sodium, potassium, calcium and magnesium) and anions (primarily chloride, sulfate, carbonate and bicarbonate) than the surrounding water. As a result, due to a diffusion gradient, water tends to diffuse inward into the bodies of aquatic organisms, while cations and anions tend to diffuse out of the organisms into the water. To compensate, freshwater organisms produce large volumes of very dilute urine to remove excess water from their bodies and also actively transport cations and chloride across the gill membranes and into the organism. Na^+ uptake is accompanied by NH_4^+ or H^+ efflux and Cl^- uptake by HCO_3^- efflux (Paquin et al. (2003). Calcium is involved in osmoregulation because it stabilizes the epithelial membrane and therefore decreases ionic permeability (Oschman, 1977). When elevated concentrations of metals are present in water, the metals displace calcium from the gill surface, thus increasing gill permeability and resulting in an influx of water into the organism. The displacement of calcium also facilitates movement of the metals across the gill surface and into the organism because cationic metals are transported across the gill surface by the same mechanisms as calcium. Essentially, the cationic transport sites on the surface of the gills cannot distinguish between calcium and many other cationic metals, so the metals are transported across the gills incidentally. In water that has a high hardness, there is sufficient calcium to bind to most of the transport sites, so metal transport across the gill surface is greatly reduced. However, in very soft water there is much less available calcium, and more of the transport sites are available to transport metals across the gill surface and into the bloodstream.

When exposed to water that contains elevated concentrations of metals, a fish's gills will produce excess mucous, as a first-line defense to prevent metal uptake. The mucous binds to the metals and then is sloughed off, thus reducing metal uptake (Playle and Wood, 1989). However, in the presence of prolonged exposure to elevated concentrations of heavy metals, this line of defense breaks down, and toxicity occurs, both directly, by uptake of the metals across the gill surface, and also indirectly, by disruption of osmoregulation and the production of excessive amounts of mucous, which impairs oxygen uptake and ultimately causes the fish to suffocate (Mallatt, 1995).

3.0 Effects of Water Chemistry on Metal Toxicity

Metals in natural waters can exist in many different chemical forms, including the free ion (e.g. Cu^{2+}), dissolved inorganic forms (e.g. hydroxides, carbonates, sulfates), organic complexes (metal bound to dissolved organic matter), and variety of particulate forms (bound to clays or incorporated into the matrix of soil particles; Schnoor et al, 1997). The relative distribution of these forms is a function of environmental conditions, including pH, hardness, and the presence of organic matter and/or inorganic particulates (e.g. clays; Wood et al., 1997). It is the free metal ion that is transported across the gill membrane. Therefore, anything that reduces the concentration of the free metal ion in water or reduces the number of available binding sites for the free metal on the surface of the gill will reduce its toxicity. In general, waterborne metals are most toxic to fish in water that has low hardness, low pH, and low DOC (Playle, Dixon, and Burnison, 1993). Sections 3.1, 3.2 and 3.3 detail the effects of water hardness, dissolved organic carbon, and pH on metal bioavailability.

3.1 Water Hardness

Water hardness is a measure of the combined concentrations of calcium and magnesium salts (primarily carbonates, bicarbonates, and sulfates) that are dissolved in water and is expressed as mg/l as $CaCO_3$. The toxicity of many divalent cationic metals is inversely related to water hardness, primarily because the calcium and magnesium ions that contribute to water hardness compete with divalent cationic metals for binding sites on the surface of gills and other tissues (see Section 2.0 for more details). If the metals cannot bind to these binding sites, uptake and toxicity are reduced. When EPA scientists developed water quality criteria in the 1970's and 1980's, they recognized the inverse relationship between water hardness and metal toxicity and developed hardness-dependent criteria for 7 metals, including cadmium, trivalent chromium, copper, lead, nickel, silver (acute only), and zinc. However, it is well documented in the literature that the toxicity of mercury is also inversely related to water hardness (Boening, 2000), and it is likely that this is true for other cationic heavy metals, as well.

SRS groundwaters are very soft, with hardnesses generally less than 10 mg/l and also have a relatively low pH (generally around 5). SRS surface waters are also very soft, ranging from about 6 mg/l in Upper Three Runs to about 25 mg/l in Lower Three Runs. Due to a dip in a geologic formation underlying SRS, there is a hardness gradient across SRS, with hardnesses increasing in an easterly direction. The hardness of the Savannah River is about 25 mg/l. The ions responsible for water hardness can form scale of the insides of water distribution pipes and other plumbing, which minimizes dissolution of metals from the pipe walls. Very soft waters do not form scale, and as a result tend to dissolve metals present in metal piping. Sources of metal at SRS that have potential to dissolve and be problematic in NPDES discharges include metal piping (including water distribution and cooling systems), galvanized roofing, and galvanized exterior stairs and elevated walkways (catwalks). Copper plumbing leaches not only copper, but lead, since most copper plumbing has lead-sweated joints. Galvanized plumbing leaches zinc, and also small amounts of lead. Most plumbing at SRS is copper or galvanized piping. Cooling towers at SRS also contain copper condenser coils. Any outfall that contains water that has traveled through copper or galvanized piping (rather than PVC or stainless steel) has the potential to contain potentially toxic concentrations of metals.

In summary, the toxicity of copper and many other cationic metals is inversely related to water hardness. In the most recently issued NPDES permit (issued December 2003), SCDHEC calculated metal limits based on a hardness of 25, rather than a hardness of 50 that was used in the pervious permit. Because the water hardness of SRS surface waters range from <10 mg/l to around 25 mg/l, it is reasonable to base water quality criteria for hardness-based metals on hardnesses of <50 mg/l. However, as discussed in Sections 3.2 and 3.3 of this report, there are other water chemistry factors that should be considered, as well.

3.2 Dissolved Organic Carbon

As discussed in Section 2.0, it is the free ion form of a metal that is transported across the gill membrane. Dissolved organic carbon (DOC) includes a complex mixture of naturally occurring organic compounds, many of which are humic and fulvic acids. The primary source of DOC is decomposing plant material (primarily leaves from terrestrial vegetation that fall into water, and degradation products from decaying vegetation in the floodplains of streams that leach into the water). Humic and fulvic acids impart the characteristically dark color to waters that flow through swamps in the southeast and bogs in other locales that are collectively called “blackwater” streams. DOC concentrations in southeastern blackwater streams typically range from about 1 to 8 mg/l. In SRS streams DOC ranges from about 3 to 8 mg/l (Lower, 1987).

In natural waters, fulvic acids generally comprise 20 to 80% of DOC, humic acids roughly 5%, and hydrophilic acids 5 to 20% (Aiken and Cotsaris, 1995). Fulvic acids are comprised of organic molecules with organic weights ranging between 500 and 2000, while humic acids are much larger with molecular weights ranging from >2000 to 100,000 (Thurman, 1985). Hypothetical structures for fulvic and humic acids are shown in Figures 1 and 2. Both fulvic and humic acids contain many negatively charged binding sites that are capable of binding cationic metals. However, other cations, such as Ca^{2+} and Mg^{2+}, can also bind to the negatively charged binding sites of fulvic and humic acids, and reduce their efficacy in binding other metals. Calcium and magnesium can also decrease the solubility of higher molecular weight compounds, causing them to precipitate. Therefore, calcium and magnesium can reduce the ability of DOC to reduce metal toxicity, both by competing for binding sites on the organic molecules, and by reducing the solubility of the DOC. However, calcium and magnesium can also bind clay particles and dissolved organic matter into a relatively stable clay-metal-humus complex (Stevenson, 1985), in which the metals are tightly bound and not bioavailable.

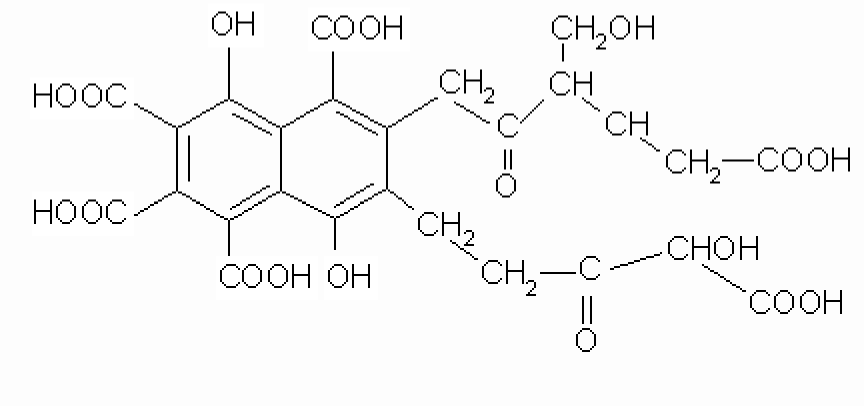


Figure 1. Hypothetical Structure of Fulvic Acids

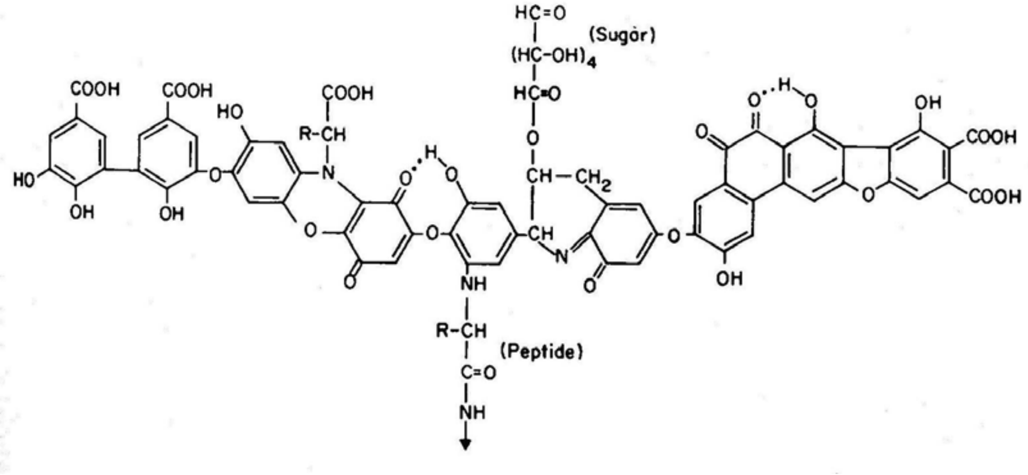


Figure 2. Hypothetical Structure of Humic Acids

Because DOC consists of many different types of organic molecules, DOC can differ widely in its ability to bind heavy metals. Abbt-Braun and Frimmel (1999) evaluated the copper complexation capacity of nine different types of DOC from Norway and reported complexation capacities ranging from 63.6 to 181.8 μg Cu/mg DOC (Table 1), which indicates that the complexation capacities of these nine sources varied by a factor of 3. Klaine et al (personal communication, 2001) performed acute toxicity tests on copper solutions treated with 5 mg/l DOC from the same nine Norwegian sources and reported LC50's ranging from 104 to 216 μg/l total copper (Table 1). Interestingly, there was little relationship between copper complexation capacity and copper toxicity (Figure 3), which suggests that the role of DOC in reducing copper toxicity is not solely related to metal complexation capacity.

Table 1. Copper Complexation Capacity of DOM Isolates and Their Effect on Copper Toxicity

DOM Isolate	Cu Complexation Capacity (μg Cu/mg DOC)	48 hour Copper LC50 (μg/l Cu) for *Daphnia magna*
TRE	99.77	119.4
HEM	73.08	177.8
AUR	81.34	103.9
MAR	73.08	110.0
BIR	63.55	134.4
HUM	77.53	177.8
GJL	68.63	131.5
GJU	181.75	121.8
HEO	83.25	216.2

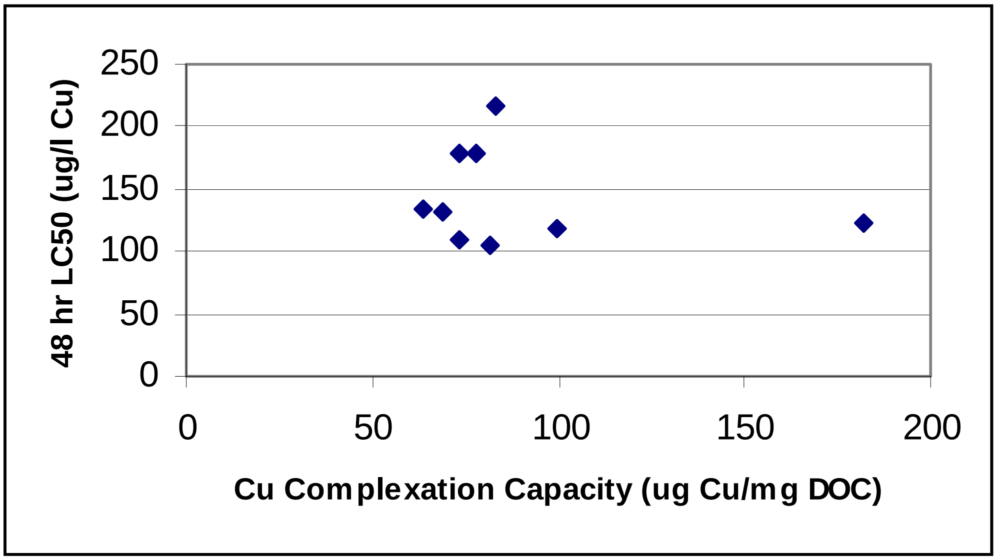
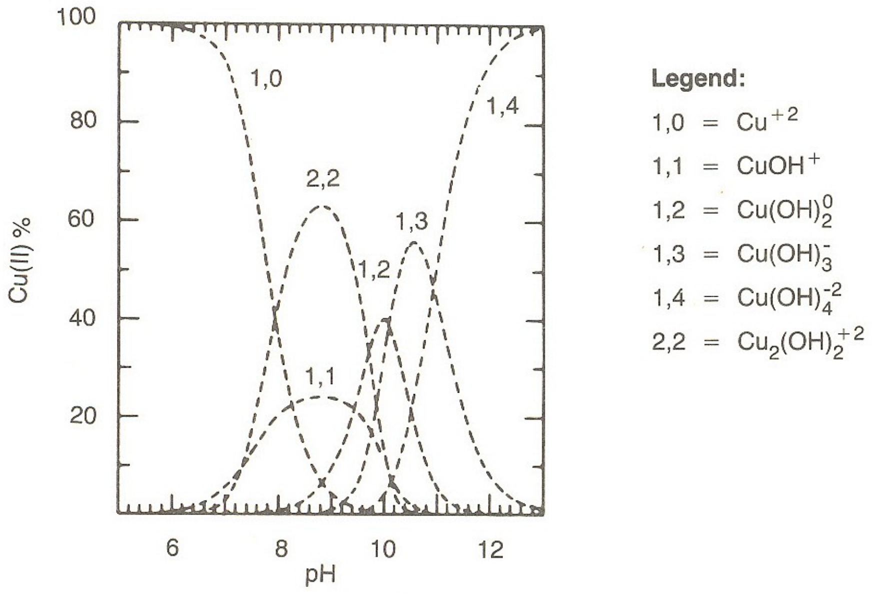


Figure 3. Relationship between Copper Complexation Capacity and Toxicity

There are numerous reports in the literature that indicate that DOC is capable of forming organic complexes with copper and other heavy metals. Since organo-metallic complexes cannot be transported across cell membranes, the toxicity of heavy metals can be greatly reduced by DOC. SRS surface waters are blackwater streams that contain sufficient DOC (3 to 8 mg/l) to complex substantial amounts of copper. Therefore, the EPA hardness-based criteria for metals may be overly protective of SRS surface waters, since they do not address the role of DOC complexation.

3.3 pH

Most cationic metals exist as free ions at low pH. As the pH increases, more of the metal forms complexes with hydroxides and carbonates, and often with chlorides and sulfates (Bodek et al., 1988). For example, at a pH of 6.0, 100% of copper exists as the free ion; at a pH of 8, approximately 40% exists as the free ion, with the remainder existing as $Cu_2(OH)_2^{+2}$ and $CuOH^+$; and at a pH of 9, less than 2% of the copper exists as the free ion (Figure 4).



I = 1 *M*, T = 25°C, 10^{-5} *M* Cu(II)

Dashed curves denote supersaturation with respect to CuO.

Source: Baes and Mesmer [1]. *(Copyright 1976, John Wiley & Sons. Reprinted with permission.)*

Figure 4. Chemical Speciation of Cu (II) in Unpolluted, Organic-free Freshwater Aquatic Systems (from Bodek et al., 1988)

SRS groundwaters and surface waters have relatively low pH, because they are very poorly buffered, due to low hardness, and contain naturally occurring humic and fulvic acids. These naturally occurring organic acids, which are present in blackwater streams, not only lower the pH of the water, but are also the source of DOC in the surface waters.

The pH of most SRS surface waters typically ranges from <5 (in strongly blackwater streams, such as the headwaters of Fourmile Branch) to circumneutral.

3.4 Metal Availability in SRS Surface Waters

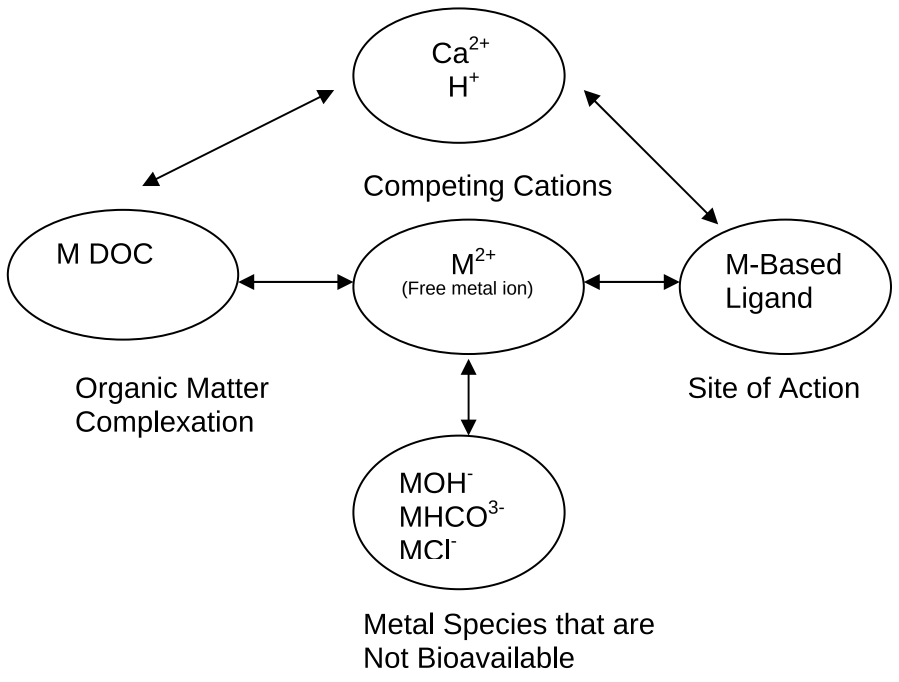
In SRS streams, the low hardness and relatively low pH of the water tends to increase metal bioavailability and toxicity, while the DOC present in the water tends to form organic complexes with metals and thus reduce bioavailability. All three of these water chemistry parameters must be considered when assessing the potential for metal toxicity at NPDES outfalls. However, because many SRS outfalls discharge into very small receiving streams and therefore receive minimal initial dilution, it is unlikely that there will be sufficient DOC present in the headwaters of receiving streams to significantly reduce metal bioavailability.

4.0 Biotic Ligand Model

By the mid-1990's the EPA had acknowledged that water hardness was not the only factor that influenced metal toxicity. A collaborative effort was begun between EPA scientists and scientists at several research institutes and universities to develop an empirical model to predict metal toxicity. The result was the development of the Biotic Ligand Model, which is a mathematical model that describes and quantifies the bioavailability of certain metals to aquatic life (Hydroqual, 2001). The model is based on the theory that toxicity is not only related to total aqueous metal concentration, but that metal complexation and interaction at the site of action (biotic ligand) of toxicity need to be considered (Di Toro, 2001). For fish, the biotic ligand is believed to be the sodium or calcium channel proteins in the gill surface that regulate the ionic composition of the blood. Mortality occurs in aquatic organisms when the concentration of metal bound to the biotic ligand (e.g., fish gill) exceeds a certain threshold concentration (Santore et al., 2001). The amount of metal that binds to the gill surface is determined by a competition for metal ions between the toxic metal ion and other metal cations in solution (i.e. calcium). Figure 5 is a conceptual representation of the biotic ligand model. Parameters needed to run the model include DOC, pH, and measurements of the concentrations all major cations (e.g. Ca, Mg, K, Na) and anions (e.g. Cl, CO_3, HCO_3, SO_4, S^-) in the water of interest . These data are used to calculate binding constants for gill interactions with metals, hydrogen ion, and cations such as calcium, magnesium, and sodium. The model considers both complexation and competition reactions that limit metal binding at the biotic ligands on the gill surface. Critical biotic ligand concentrations are then calculated, based on a dose-response relationship of mortality to accumulation at the gill (MacRae, 1994, as cited by Santore et al., 2001). When suitably calibrated, the BLM can be used to predict the total dissolved metal concentration that is associated with the LA50 (the concentration of the metal-biotic ligand complex that is associated with 50% mortality, based on "lethal accumulation").

The BLM employs two models: The CHESS Model and WHAM, Version 5. The CHESS Model (CHemical Equilibrium in Soils and Solutions), which was developed by Santore and Driscoll (1995), computes metal speciation, but does not take into account complexation by organic matter. WHAM (Windermere Humic Aqueous Model) , which was developed by Tipping (1994) predicts proton binding to carboxyl and phenolic sites on humates. The BLM employs these two models to compute gill copper concentrations (Di Toro et al, 2001). The BLM also incorporates the competitive effects of other

Figure 5. Conceptual Biotic Ligand Model



cations, such as Ca^{2+}, that interact with the biotic ligand to reduce toxicity. For example, at a fixed free metal concentration, as hardness increases, the increased Ca^{2+} competes with the free metal for binding sites at the biotic ligand, thus requiring a higher free metal concentration to achieve the same toxic effect. The BLM uses this competitive mechanism to simulate the change in metal toxicity due to a change in hardness. Thus, the BLM can effectively predict the reduction in metal toxicity due to an increase in the concentrations of hardness cations (Meyer et al., 1999).

Water quality parameters used in the BLM include pH, alkalinity, dissolved organic carbon (DOC), calcium, magnesium, sodium, potassium, chloride, sulfate, sulfide and dissolved concentrations of the metal(s) of interest.

As the BLM was developed, the results of the BLM for various samples of water were compared to WER results for the same waters. Generally, the LC50s predicted by the BLM were within a factor of 2 of the LC50s measured by the WERs (Di Toro et al 2001). Di Toro also used the BLM to estimate the behavior of mixtures of metals that exert toxicity at the same biotic ligand and found good concurrence for a number of metals.

The BLM may assist in developing technically defensible site-specific criteria, waste load allocations, and ecological risk assessments, and may eventually replace the use of Water Effect Ratios. At present, the BLM is under review by regulatory agencies and is being considered for use in refining water quality criteria in the United States and elsewhere. One of the advantages of the BLM over WER testing is that the BLM can be used to estimate the behavior of mixtures of metals much more rapidly and cost-effectively than WER testing. However, as discussed below, BLMs for various metals are in different stages of development, and it may be several years before reliable BLMs are available for routine use, particularly for predicting chronic toxicity.

4.1 Current Status of BLM Development

In 1999, the EPA published a notice of intent to revise the aquatic criteria for certain metals (starting with copper and silver) using the BLM. In 2003, the EPA issued a draft update of ambient quality criteria for copper (U.S. EPA 2003), which uses the BLM to calculate copper criteria. The EPA anticipates that the final copper criteria document will be issued midyear in 2006 (personal communication, Luis Cruz, EPA). In the interim, copper criteria are still calculated using water hardness, rather than the BLM. Revision of the aquatic criteria for silver lags behind that of copper. At present, the criteria for silver have still not been revised, nor has a schedule been released for doing so.

The most recently released version of the BLM (Hydroqual's Version 2.2.1) includes the capability to predict acute toxicity of the following metals to the following organisms:

Species	Copper	Silver	Cadmium	Zinc
Fathead minnow	X	X	X	X
Rainbow trout		X	X	X
Daphnia magna	X	X		X
Daphnia pulex	X			
Ceriodaphnia dubia	X	X	X	

At present, BLM research continues on developing and refining BLMs for copper, silver, zinc, nickel, cadmium, lead, and cobalt. There are currently three versions of the acute Cu-BLM and a single version of a chronic Cu-BLM. The acute Cu-BLMs were developed primarily using toxicity data for the fathead minnow (*Pimephales promelas*). The site of acute copper toxicity for this species has been identified as Na ion uptake channels in the gill membrane. The adsorption of copper on gill surfaces of the fathead minnow has been calibrated to measurements of copper accumulation on the gill in waters that have different chemistry (Playle et al., 1992). The primary difference in the three acute versions of the BLM are: different affinity constants (log K) for some biotic ligand-cation complexes, different assumptions about the forms of copper that may bind to the biotic ligand sites, different constants for inorganic copper complexes, different assumptions with regard to the binding of Cu^{2+} by DOM, and different LA50 values for *Daphnia* sp. Details of these differences can be found in Niyogi and Wood (2004). The newly developed chronic BLM for copper (De Schamphelaere and Janssen, 2004a) includes a factor for simultaneous dietary exposure. The model performed reasonably well in predicting 21-day EC50s and NOECs in natural waters. However, its developers have pointed out that there is still much that is not known about the physiological mechanisms of metal bioavailability.

Silver - Three acute Ag-BLMs have been developed (Schwartz and Playle, 2001; McGeer et al., 2000 and Bury et al., 2002). Like the copper BLM, one of the main differences between the Ag-BLMs are the use of different affinity constants in the models. All three versions show reasonably good success in predicting acute silver toxicity in the laboratory, but none have been validated in natural waters so far (Niyogi and Wood, 2004). Niyogi and Wood (2004) suggest that Ag-BLMs would be much improved by the addition of a stability constant for reduced sulfide, since sulfide plays such an important role in governing silver bioavailability in natural waters. As yet, no chronic BLM has been developed for silver.

Zinc - Although two preliminary versions of an acute ZN-BLM have been developed (Santore et al., 2002; Heijerick et al. 2002), neither are as advanced as the BLMs for copper and silver. Further research is needed to confirm the effects of pH (especially <6.0 and >8.0), DOM, and Na^+, Ca^{2+} and Mg^{2+} on acute toxicity in laboratory water. None of the models have been validated in natural waters so far (Niyogi and Wood, 2004). De Schamphelaere and Janssen (2004c) have developed a chronic Zn BLM, which showed reasonable success in predicting chronic zinc toxicity to juvenile rainbow trout. Their predicted values were within a factor of 2 of the observed values. However, more research is needed to clarify the role of DOM is chronic zinc toxicity and the model has not yet been validated in natural waters.

Nickel - At present, the Water Environment Research Foundation (2002) is in the early stages of developing an acute Ni-BLM using a data set from Meyer et al. (1999). Nickel differs from all of the other metals for which BLMs have been developed, in that it is believed to be a respiratory toxicant and not an ionoregulatory toxicant in fish. However, Pane et al. (2003b) recently demonstrated that the primary mechanism of nickel toxicity is ionoregulatory in *D. magna* (due to inhibition of Mg^{2+} uptake) and not respiratory, as observed in fish. These conflicting findings suggest that considerably more research is needed to understand the physiology involved in nickel toxicity in different species of aquatic life.

Cadmium - To date, there has been no published acute Cd-BLM, although extensive physiological research has documented that cadmium is a potent antagonist of Ca^{2+} uptake in fish gills (Verbost et al, 1987, 1988, 1989). Playle et al (1993a, 1993b) have developed a gill-Cd binding model for the fathead minnow, which may be a starting point for development of an acute Cd-BLM in the future.

Lead - At present, there have been no acute or chronic BLMs published for lead (Niyogi and Wood, 2004). However, McDonald et al. (2002) have proposed a gill-Pb binding model in rainbow trout that provides a basic framework for developing an acute Pb-BLM. The log $K_{gill\text{-}Pb2+}$ estimated by McDonald et al. was intermediate between the Zn and Cd log K values, which concurs with the relative toxicities of the three metals. Recent research on fish suggests that Pb^{2+} is primarily an antagonist of active Ca^{2+} uptake at the gills, like Zn^{2+} and Cd^{2+}. However, lead is also thought to affect Na^+ and Cl^- regulation (Rogers et al., 2003, 2004). The gill-Pb binding model proposed by McDonald et al. considers that Pb^{2+} has about 100 times greater affinity for DOM than does the fish gill, which indicates that the presence of DOM greatly reduces lead toxicity. In order to transform the gill binding model into a BLM, there is a need for more LC50 tests to be conducted on fish so that critical gill-lead burdens can be quantified and also a need to extend these tests to model aquatic invertebrates such as daphnids.

Cobalt - As for lead and cadmium, at present there is no published acute Co-BLM, but there is a gill-Co binding model for the rainbow trout developed by Richards and Playle (1998). They report that Co^{2+} binds to gill sites >1000 times more weakly than Cd^{2+}, 10 times more weakly than Pb^{2+} and about 6 times more weakly than Zn^{2+}. Their research suggests that Co^{2+} is taken up primarily via the active Ca^{2+} transport pathway and that the binding affinities of Co^{2+} are similar for fish gills and DOM. These findings suggest that DOM would have less of an affect in reducing cobalt toxicity than for other metals, such as lead and cadmium, which have much stronger binding affinities for DOM than for fish gills. While this gill-Co binding model provides a basic framework, the same requirements as for the gill-Pb binding model will be needed to transform it into a technically sound BLM that can be used for environmental regulation. The most important area of future focus is to correlate the model-simulated influence of water chemistry on gill-cobalt accumulations with measured acute toxicity (96 h LC50) in fish under well-defined water chemistry so as to quantify the critical gill cobalt burdens. Additional water chemistry variables such as alkalinity and Mg^{2+} should be investigated in this context. The approach should then be extended to model aquatic invertebrates such as daphnids.

Aluminum - Gensemer and Playle (1999) have provided some preliminary modeling information on Al-gill interactions in the fish gill, which could be useful for developing an acute Al-BLM in the future. However, no work is currently in progress on the development of a BLM for aluminum.

4.2 BLM Application in Very Low Hardness Waters

Most BLM development and verification studies have been performed on waters of intermediate hardness (generally 50 to 200 mg/l). With the exception of research published by Long et al. (2004) and Van Genderen et al (2005), there has been very little BLM work done in low hardness waters.

Long et al. (2004) investigated the effects of low hardness and pH on copper toxicity to *Daphnia magna*. They prepared one set of synthetic water that had the same pH and varied the hardness (7.9 to 50 .7 mg/l) and a second set of synthetic waters that either had a hardness of 7.1 or 20.6 mg/l. They adjusted the pH in aliquots of these two waters such that the pH was 5.6, 7.0 or 8.6 (see Table 2). They then performed three replicate copper toxicity tests on each of the 10 waters and reported the mean LC50 for copper in each water. Their results indicated an inverse relationship between hardness and copper toxicity, which is consistent with other reports in the literature. However, most of the toxicity data reported in the literature were for waters with much higher hardness values than those used by Long et al. Long et al.'s data confirm that in general, the inverse relationship between hardness and copper toxicity is maintained in waters that have very low hardness.

Table 2. LC50s for Copper at Various Hardness and pH Values (from Long et al., 2004)

Hardness	pH	48 hr LC50 (total Cu ug/l)
7.9	8.3	2.0 ± 0.6
11.1	8.2	2.0 ± 0.5
22.2	8.3	10.0 ± 1.0
50.7	8.2	11.1 ± 4.6
7.1	5.6	2.0 ± 1.5
7.1	7.0	2.8 ± 1.0
7.1	8.6	4.8 ± 1.1
20.6	5.5	2.0 ± 0.5
20.6	7.0	7.4 ± 1.3
20.6	8.5	6.5 ± 0.7

When pH and hardness covaried, increased copper toxicity was observed under low-pH, low-hardness conditions. Copper speciation was predicted using the MINEQL+ model (Table 3). At a pH of 5.5 to 5.6, most of the copper (71.5 to 81.5%) was present as the Cu^{2+} species, which is widely believed to be the most bioavailable form of copper. At a pH of 7.0 and a hardness of 7.1 mg/l, 17.6% of the copper was present as Cu^{2+}, while at a pH of 7 and a hardness of 20.6, 13.4% of the copper was present as Cu^{2+}. These results suggest that hardness plays a dual role in reducing copper toxicity. Not only does hardness provide a source of calcium and magnesium ions that compete with copper for binding sites on the gill surface, but there is also somewhat of an inverse relationship between hardness and percent Cu^{2+}.

Table 3. Copper Speciation (Percent of Total Copper) Predicted by MINEQL+ for Toxicity Tests Conducted at Varied Hardness and pH Levels (from Long et al., 2004)

Hardness	**pH**	**Cu^{2+}**	**$CuCO_3$**	**$Cu(OH)_2$**	**$Cu(OH)^+$**	**$CuSO_4$**	**$CuHCO_3^+$**
7.1	5.6	71.5	3.5	<1	<1	8.3	16.1
	7.0	17.6	40.9	29.2	1.6	2.1	8.6
	8.6	<1	3.5	96.2	<1	<1	<1
20.6	5.5	81.5	2.0	<1	<1	3.7	12.3
	7.0	13.4	50.2	24.4	1.3	<1	10.0
	8.5	<1	4.3	95.4	<1	<1	<1

Long et al. compared the LC50s from their study to LC50s predicted by the BLM using the water chemistry data from their tests. Only 3 of the 10 LC50 values from their toxicity tests agreed with those predicted by the BLM. Among the values that did not agree, some were lower and some were higher than the values predicted by the BLM. The authors suggest that some of the discrepancy in the results may be due to differences in ionoregulatory demand in very soft waters that are not accounted for by the BLM.

Van Genderen et al. (2005) performed acute copper toxicity tests in low-hardness water (16 ± 8 mg/l) collected from 24 locations in South Carolina using larval fathead minnows. They also performed WERs on the water samples using both standard lab water that had a hardness of 50 mg/l (per EPA protocol) and laboratory water whose hardness matched that of the surface waters. They then compared the LC50s of the toxicity tests to LC50s generated by the BLM using the water chemistry data from the surface waters. They found that approximately 50% WERs performed using laboratory water with a hardness of 50 mg/l produced a WER of <1, which indicates that copper was more toxic in the low-hardness surface water than in the laboratory water (Figure 6). These results indicate that the higher hardness of the laboratory water had more of an effect in reducing copper bioavailability than the naturally occurring DOC (6 ±4 mg/l) in the surface water and suggest that it is inappropriate to use lab water with a hardness of 50 when performing WERs in low-hardness water. The results of the modified WERs indicated that all of the WERs were >1, and the authors suggest that the presence of DOM in the surface water samples reduced copper bioavailability as compared to the laboratory control. The authors made a strong case for performing WERs using laboratory water whose hardness has been adjusted to match that of the surface water

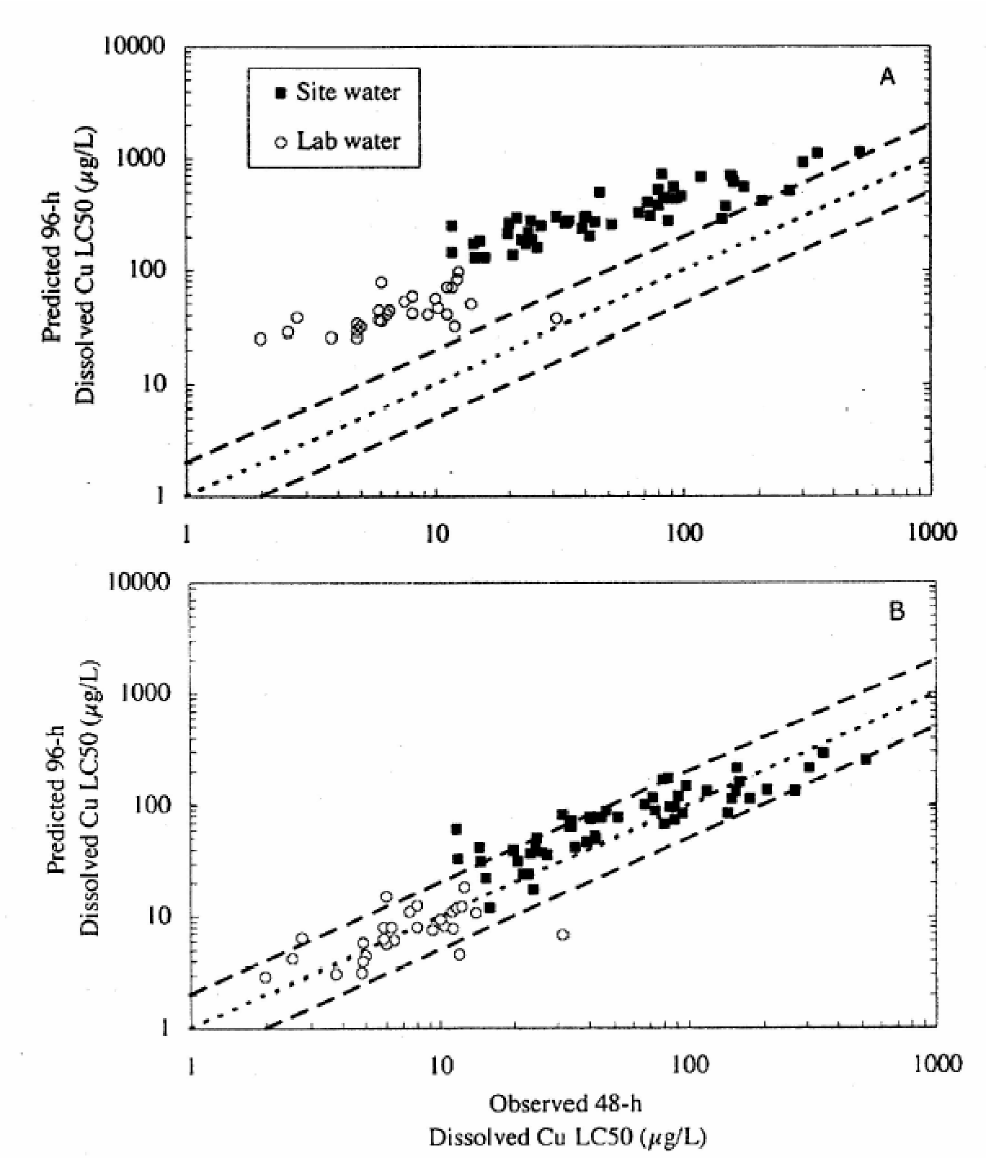


Figure 6. Biotic ligand model results for copper toxicity to larval fathead minnows in low-hardness water.

Dotted line represents the ideal fit (1:1). Dashed lines are plus or minus a factor of two from the ideal fit. Results are shown using the default value for the median lethal gill accumulation (LA50 = 7.32 nmol Cu/g gill wet wt) (**A**) as well as the fitted value (LA50 = 0.2 nmol Cu/g gill wet wt) (**B**). LC50 = median lethal concentration.

that is being tested. When they compared the LC50's of their toxicity tests to the LC50s predicted by the BLM, they found that the LC50s predicted by the BLM were consistently higher than the measured LC50s (BLM was underestimating toxicity; Figure 6). The authors suggest that the fathead minnow larvae may have been somewhat stressed by the very low hardness of the surface waters, which may have increased their sensitivity to another stressor (copper). Their results suggest that LC50s generated by the BLM may be underprotective of low-hardness surface waters. When the BLM was adjusted reducing the critical gill value used in the BLM from 7.32 to 0.2 nmol/g wet tissue, the observed and predicted LC50's were very similar, which suggests that it may be possible to modify the BLM for use in low-hardness water.

5.0 Application of the Biotic Ligand Model at the Savannah River Site

Most of the water quality data used to develop and calibrate the BLM have been in waters that have considerably higher hardness that the surface waters at SRS, where hardness typically averages 6 to 20 µg/l.

Four phases of testing were performed to evaluate the use of the BLM at SRS.

Phase 1 - In 2002, three rounds of water samples were collected from three outfalls and three surface waters and analyzed for the parameters needed to run the BLM. These waters and the rationale for their selection are listed in Table 4.

Table 4. Water Sources Included in Phase 1 Testing and Rationale for Inclusion

Water Source	Rationale
A-01 outfall (downstream from wetland treatment system)	Contains elevated concentrations of DOC that originates from wetland plants and algae (autochthanous DOC); hardness is low for SRS waters; pH is circumneutral; WER results are available
F-05 outfall	Contains elevated concentrations of copper; low DOC; is known to be toxic to *C. dubia*
G-10 outfall	Contains elevated concentrations of DOC from sanitary wastewater treatment plant (allochthanous DOC); contains elevated concentrations of copper and lead; hardness is intermediate for SRS waters; pH is circumneutral; is generally not toxic; WER results are available
H-12 outfall	Contains elevated concentrations of copper; low DOC; at times is marginally toxic to *C. dubia*
Upper Three Runs Rd. C	Contains high DOC; very low hardness
Fourmile Branch	Contains high DOC and relatively low pH; representative of many coastal plain blackwater streams; hardness is intermediate (as compared to other SRS surface waters)
Pen Branch Road B	Contains intermediate DOC, pH, and hardness (as compared to other SRS surface waters)

Phase 2 - In 2005 two rounds of water samples were collected from five of the locations sampled in 2002 and analyzed for the parameters needed to run the BLM. F-05 was not sampled because earlier testing had indicated that it was toxic to *C. dubia*. In Phase 2, in addition to running the BLM, copper spiking toxicity tests were performed in order to determine the LC50 for copper in each water sample. This allows a direct comparison of the copper LC50s that were predicted by the BLM with actual LC50s obtained from the copper spiking tests.

Phase 3 - Following the Phase 2 testing, additional copper spiking studies were performed on effluent collected from the A-01, G-10 and H-12 outfalls. A copper spiking toxicity test was performed on each sample, and then the water chemistry of each sample was modified by adjusting the pH up and down a full pH unit, increasing DOC by

2 and 4 mg/l, and increasing the hardness by 50 and 100 mg/l. pH was adjusted using sulfuric acid or sodium hydroxide; hardness was increased using $NaHCO_3$, $MgSO_4$, $CaSO_4 \cdot 2H_2O$, and KCl in the same proportions that are used to prepare MHSF (moderately hard synthetic formula) lab water (U.S. EPA, 2002), and DOC was increased using Aldrich humic acid (CAS 68131-04-4). A copper spiking test was then performed on each adjusted sample. Each effluent sample was also analyzed for the parameters needed to run the BLM and the BLM was run using the water chemistry data from each treatment. The Phase 3 tests were performed to see how well the BLM performed under various pH, DOC and hardness concentrations.

Phase 4 - H-12 effluent was treated by running a sample of effluent through a peat bed to remove cationic metals. The treated effluent was then mixed with untreated effluent at a ratio of 1:3 to simulate the mixing that would occur if a peat bed were used to treat a portion (25%) of the H-12 effluent stream. Details of the experiment can be found in Nelson and Specht (2005). Peat removes the metals, but also increases DOC and decreases pH. Copper spiking toxicity tests were then run on the blended effluent and MHSF, which is the control water used for laboratory toxicity tests, to compare the toxicity of copper in the two waters.

All toxicity tests and chemical analyses were performed by ETT Environmental, Greenville, SC.

5.1 Results of Phase 1 through 4 Studies

5.1.1 Phase 1 Results

Table 5 contains the water chemistry data for the seven locations that were tested in Phase 1. These data served as input data for the BLM.

Table 6 contains the LC50s predicted by the BLM for copper at the seven locations. Mean predicted LC50's ranged from 11.4 µg/l for H-12 to 119.4 µg/l for G-10. G-10, which is treated effluent from a sanitary treatment plant contains high concentrations of dissolved organic carbon, which reduce the bioavailability of metals. F-05 and H-12 consist primarily of non-contact cooling water, which contain low concentrations of DOC. The BLM predicted very low LC50's in these effluents (means of 13.6 and 11.4 µg/l, respectively). The mean LC50's for the three receiving streams indicated that copper toxicity was quite similar in Upper Three Runs and Fourmile Branch (mean LC50s of 27.5 and 25.1 µg/l, respectively), but that copper was somewhat less toxic in Pen Branch (mean LC50 of 39.0 µg/l).

The results for the individual replicates indicated considerable variability among the three replicates for some locations. For example for the A-01 Wetland, the LC50's ranged from 15.2 to 71.5; at Upper Three Runs from 3.8 to 44.0 and at Fourmile Branch from 14.8 to 43.2. If the BLM results are a good estimate of metal toxicity, these results suggest that metal toxicity varies considerably in some SRS waters, based on changes in water chemistry. The variability in the LC50s for the A-01 wetland are particularly surprising, since this system should have the most consistent water chemistry, due to mixing in the retention basin and the splitter box. The variability appears to be largely due to changes in pH. pH readings in the three A-01 samples ranged from 6.77 to 7.82. pH was highest in July and lowest in October. In the summer, the A-01 wetland has high

Table 5. Water Chemistry Data for Phase 1 Studies

Location	Date	pH	Diss Cu	DOC	Ca	Mg	Na	K	SO4	Cl	Alk
			ug/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
A-01	7/30/2002	7.82	1	3.3	1.10	0.44	1.70	<0.1	0.46	5.36	38.40
	8/27/2002	7.11	3	3	1.20	0.44	1.60	0.1	0.6	5.87	54.20
	10/3/2002	6.77	<1	2.7	1.20	0.42	0.86	<0.1	0.6	2.94	19.70
F-05	7/30/2002	7.87	47	0.5	0.50	0.19	35.00	0.39	1.44	2.97	61.40
	8/27/2002	7.89	2	<0.5	0.55	0.15	37.00	0.49	0.08	2.97	70.80
	10/3/2002	7.92	1	0.6	0.48	0.19	33.00	0.44	1.54	2.91	66.40
G-10	7/30/2002	8.05	16	4	5.00	0.98	120.00	12	11.5	24.6	182.00
	8/27/2002	7.66	13.9	3.4	5.00	1.1	93.00	14	13.28	27.1	99.20
	10/3/2002	8.03	2	3.8	5.20	1	90.00	12	15.64	28.4	107.00
H-12	7/30/2002	7.79	3	0.6	3.80	0.38	7.80	0.67	9.05	4.14	28.50
	8/27/2002	7.31	<1	0.9	1.40	0.19	11.00	0.78	0.03	3.67	82.00
	10/3/2002	7.44	2	0.9	1.80	0.31	14.00	0.78	5.6	2.35	30.60
UTR Rd. C	7/30/2002	7.9	2	1.6	1.30	0.34	1.40	0.26	1.3	1.72	3.67
	8/27/2002	7.84	<1	2.3	2.30	0.37	1.60	0.38	3.05	2.05	8.82
	10/3/2002	6.37	<1	1.7	1.80	0.38	1.60	0.35	1.75	1.83	2.27
FMB Rd. C	7/30/2002	7.78	3	2.4	5.20	0.58	8.50	0.71	4.97	2.7	55.60
	8/27/2002	7.05	1.1	2.4	5.10	0.54	8.30	0.66	3.6	2.81	46.40
	10/3/2002	6.97	<1	2.3	3.90	0.51	7.00	0.66	4.61	2.61	31.80
PB Rd B	7/30/2002	7.41	2	3.5	11.00	0.6	2.00	0.88	2.76	1.85	54.90
	8/27/2002	7.6	<1	3.4	9.70	0.49	2.00	0.84	2.94	2.18	57.70
	10/3/2002	7.19	<1	4.2	12.00	0.57	2.30	0.78	2.39	2.17	54.10

Table 6. Phase 1 Results - *Ceriodaphnia dubia* LC50's Predicted by the BLM

Location	**Rep 1**	**Rep 2**	**Rep 3**	**Mean**
A-01	71.5	27.5	15.2	38.1
F-05	14.4	9.2	17.3	13.6
G-10	150.6	84.1	123.5	119.4
H-12	11.2	10.6	12.4	11.4
UTR Rd C	34.6	44.0	3.8	27.5
FMB Rd. C	43.2	17.3	14.8	25.1
Pen Br. Rd B	37.5	45.7	33.6	39.0

densities of algae, which can increase pH as CO_2 is removed from the water by the algae.

5.1.2 Phase 2 Results

In Phase 2, BLM chemistry (Table 7) and copper-spiking laboratory toxicity tests (Table 8) were performed on water samples collected five of the six locations sampled in Phase 1. F-05 was not included, since a baseline toxicity test indicated that it was toxic. The results indicate reasonably good congruence between the BLM and laboratory results for Fourmile Branch, Pen Branch, and G-10. For A-01, the first test showed good congruence, while the in second test, the BLM underestimated copper toxicity by a factor of 4. Differences in water chemistry at A-01 were largely related to differences in DOC concentrations (1.8 mg/l in the first sample; 7.2 mg/l in the second sample; Table 8). For H-12, the BLM underestimated copper toxicity by a factor of 2 to 4. For Upper Three Runs, the BLM overestimated copper toxicity in the first sample by a factor of 7 and underestimated toxicity in the second sample by a factor of 5. The water chemistry for the Upper Three Runs samples indicates very low DOC in the first sample (1 mg/l) and much higher DOC in the second sample (9.8 mg/l), and also lower pH in the first sample (6.53) than in the second (7.39). There is no obvious explanation for the variations in DOC concentrations, either for A-01 or Upper Three Runs.

Table 7. Water Chemistry Data for Phase 2 Studies

Location	Date	pH	Cu	DOC	Ca	Mg	Na	K	SO4	Chloride	Alkalinity
			ug/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
G-10	7/22/2005	7.72	16.3	3.8	9.2	1.6	124.0	15.0	15.0	39.0	127.0
G-10	8/8/2005	7.81	16	5.8	9.7	1.7	103.0	16.0	16.0	36.0	71.5
A-01	7/22/2005	7.47	3.9	1.8	1.7	0.4	7.2	0.6	3.2	4.0	44.2
A-01	8/8/2005	7.28	2.6	7.2	1.9	0.5	6.9	0.8	2.8	4.6	47.5
H-12	7/22/2005	6.96	3.3	5.6	4.5	0.6	5.2	0.9	6.0	2.2	43.5
H-12	8/8/2005	7.41	3.4	2.3	4.9	0.6	4.0	1.8	8.0	1.7	42.8
UTR Rd. C	7/22/2005	6.53	2.9	1	1.8	0.4	1.5	0.3	3.6	3.4	4.3
UTR Rd. C	8/8/2005	7.39	0.5	9.8	2.1	0.4	1.5	0.2	4.1	4.0	4.9
FMB Rd. C	7/22/2005	7.14	0.5	4.4	4.5	0.5	6.7	0.2	4.4	3.0	47.1
FMB Rd. C	8/8/2005	7.2	0.5	4.9	3.5	0.3	6.6	0.2	6.0	2.2	36.1
PB Rd. B	7/22/2005	7.18	0.5	5.3	6.3	0.5	2.0	0.5	5.0	3.0	42.9
PB Rd. B	8/8/2005	6.96	1.1	5	6.9	0.6	2.3	0.7	4.0	4.0	45.0

Table 8. Phase 2 Results - Laboratory LC50s and BLM LC50s (µg/l) for Copper

Location	Rep 1 LC50	Rep 1 BLM LC50	Rep 2 LC50	Rep 2 BLM LC50
A-01	26.9	24.2	22.2	80.3
G-10	181	104	211	163
H-12	9.15	35.1	14.1	25.4
UTR Rd C	21.8	3.0	26.0	134.6
FMB Rd. C	37.0	36.3	27.9	45.8
Pen Br. Rd B	41.1	42.9	36.3	29.3

5.1.3 Phase 3 Results

In Phase 3, a single sample was collected from each of three outfalls (A-01, G-10 and H-12). Baseline water chemistry (Table 9) and copper spiking tests were run on each outfall. In addition, pH was increased and decreased by one pH unit, hardness was increased by 50 and 100 mg/l, and DOC was increased by 2 and 4 mg/l. Copper spiking toxicity tests were run on each of the altered samples and toxicity was also predicted by the BLM (Table 10).

The results indicate that for A-01 and G-10, the BLM provided a reasonable estimate of copper toxicity. Laboratory and BLM predicted LC50's differed by less than 50%. For the H-12 baseline test, the BLM underestimated toxicity by a factor of 2.7. When pH was increased by one pH unit (which should decrease metal bioavailability, by reducing the amount of free copper), the BLM consistently underestimated copper toxicity. When pH was decreased by one pH unit, the BLM overestimated copper toxicity in the A-01 and G-10 outfalls and underestimated toxicity in H-12 effluent. When hardness was increased in the A-01 and G-10 effluents, the BLM consistently overestimated copper toxicity. In H-12, the BLM provided reasonable predictions of copper toxicity. When DOC was increased in A-01 effluent, the BLM underestimated copper toxicity by a factor of 2. In G-10, which has naturally high DOC, the BLM provided reasonable estimates of toxicity when DOC was artificially increased. In H-12, the BLM greatly underestimated copper toxicity when DOC was increased.

In general, the accuracy of the BLM in predicting copper toxicity was worse in when the effluent samples were chemically altered. These results indicate that not only are pH, DOC and hardness important in determining metal bioavailability, but that there are pronounced differences that occur when these parameters are artificially changed in the laboratory. Apparently, the source of DOC is important in determining metal bioavailability, and probably also the specific ratio of the cations and anions that contributes to water hardness.

Table 9. Water Chemistry for Phase 3 Studies

Location	Date	pH	DOC	Ca	Mg	Na	K	SO4	Cl	Alk.
			mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
A-01 baseline	8/15/05	7.54	3.1	2.1	0.5	8.1	0.8	6	3.0	13.7
A-01 pH up 1	8/15/05	8.54	3.1	2.1	0.5	12.0	0.8	5	3.0	13.7
A-01 pH down 1	8/15/05	6.54	3.1	2.1	0.5	7.5	0.8	18	3.0	13.7
A-01 hardness up 50	8/15/05	7.45	3.1	11.0	7.9	25.0	0.6	7	4.0	58.4
A-01 hardness up 100	8/15/05	7.86	3.1	18.0	15.0	41.0	0.6	16	6.0	109.0
A-01 DOC up 2 mg/l	8/15/05	7.54	5.1	2.1	0.5	9.0	0.8	6	3.0	13.7
A-01 DOC up 4 mg/l	8/15/05	7.54	7.1	2.1	0.5	10.0	0.8	6	3.0	13.7
G-10 baseline	8/23/05	7.7	3.7	7.4	1.3	81.0	11.0	13	26.0	59.4
G-10 pH up 1	8/23/05	8.7	3.7	7.4	1.3	87.0	11.0	15	26.0	59.2
G-10 pH down 1	8/23/05	6.7	3.7	7.4	1.3	84.0	11.0	38	26.0	59.2
G-10 hardness up 50	8/23/05	7.95	3.7	21.0	9.1	98.0	13.0	13	24.0	92.1
G-10 hardness up 100	8/23/05	7.92	3.7	25.0	15.0	130.0	12.0	21	34.0	117.0
G-10 DOC up 2 mg/l	8/23/05	7.7	5.7	7.4	1.3	82.0	11.0	13	26.0	59.2
G-10 DOC up 4 mg/l	8/23/05	7.7	7.7	7.4	1.3	83.0	11.0	13	26.0	59.2
H-12 baseline	8/29/05	7.74	1	1.1	0.2	15.0	0.7	8	9.0	19.5
H-12 pH up 1	8/29/05	8.74	1	1.1	0.2	18.0	0.7	8	9.0	19.5
H-12 pH down 1	8/29/05	6.74	1	1.1	0.2	16.0	0.7	20	9.0	19.5
H-12 hardness up 50	8/29/05	7.92	1	11.0	7.4	32.0	0.7	11	10.0	51.8
H-12 hardness up 100	8/29/05	7.77	1	19.0	14.0	47.0	0.7	11	9.0	85.4
H-12 DOC up 2 mg/l	8/29/05	7.74	3	1.1	0.2	16.0	0.7	8	9.0	19.5
H-12 DOC up 4 mg/l	8/29/05	7.74	5	1.1	0.2	17.0	0.7	8	9.0	19.5

Table 10. Phase 3 Results Laboratory LC50s and BLM LC50s for Copper in Three SRS Waters with pH, Hardness, and DOC Altered

Location	Treatment	Laboratory LC50	BLM Predicted LC50
A-01	None	38	47
	pH up 1 unit	38	119
	pH down 1 unit	26	10
	Hardness up 50 mg/l	97	38
	Hardness up 100 mg/l	169	66
	DOC up 2 mg/l	45	80
	DOC up 4 mg/l	62	115
G-10	None	130	89
	pH up 1 unit	130	197
	pH down 1 unit	119	26
	Hardness up 50 mg/l	176	110
	Hardness up 100 mg/l	230	113
	DOC up 2 mg/l	167	139
	DOC up 4 mg/l	203	190
H-12	None	7.9	21
	pH up 1 unit	9.8	46
	pH down 1 unit	3.1	6
	Hardness up 50 mg/l	19.5	23
	Hardness up 100 mg/l	23.9	21
	DOC up 2 mg/l	17.8	65
	DOC up 4 mg/l	17.4	113

5.1.4 Phase 4 Results

In Phase 4, BLM chemistry and copper spiking toxicity tests were performed on a blended sample of H-12 effluent. The blended sample consisted of 75% unaltered H-12 effluent blended with 25% H-12 effluent that had been treated by running the effluent through a peat bed to remove metals and increase DOC. A copper spiking test was also performed on a sample of synthetic laboratory water (MHSF), which is the water that is routinely used as the control in toxicity tests, and is also the water used as a control when performing Water Effect Ratio studies (WERs). Water chemistry data for the Phase 4 studies is presented in Table 11; toxicity data is presented in Table 12. The laboratory LC50 for the peat bed blend sample was 18.8 µg/l. The BLM overestimated the toxicity by about 50%, predicting an LC50 of 12.6 µg/l. For the MHSF lab water, the laboratory LC50 was considerably lower (6.9 µg/l) than for the peat bed blend sample. The BLM overestimated the LC50 for the MHSF sample by a factor of 3.5, with a predicted LC50 of 2.0 µg/l Cu. This difference may be due, at least in part, to a complete absence of DOC in the MHSF when analyzed, but the addition of some organic matter during the toxicity test, in the form of the algal suspension that is used to feed the test organisms.

Table 11. Water Chemistry for Phase 4 Studies

Location	Date	pH	DOC	Ca	Mg	Na	K	SO4	Cl	Alk.
			mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
Peat bed blend	11/1/05	6.4	4.7	1.5	0.1	5.0	0.5	12	9.0	35.8
MHSF	11/1/05	7.5	0.1	14.0	20.0	26.0	2.1	81	2.0	76.0

Table 12. Phase 4 Results - Copper Toxicity in H-12/Peat bed Effluent and MHSF Lab Water

Water	Laboratory LC50	BLM Predicted LC50
H-12/Peat bed Blend	18.8	12.6
MHSF Lab water	6.9	2.0

5.1.5 Conclusions of SRS-based Studies

In general, the BLM provides a rapid and inexpensive means of estimating copper toxicity in SRS surface waters and effluents. In most instances, the BLM was within a factor of 2 of the laboratory LC50. However, in some instances, the BLM was much less accurate. It was not possible to ascertain whether the differences were due to inaccurate water quality measurements or to a flaw in the model. Certainly the wide range of DOC concentrations measured in A-01 effluent and Upper Three Runs suggest either that the DOC concentrations are extremely variable or that there was an analytical problem associated with measuring DOC. For both A-01 and Upper Three Runs, the laboratory LC50's in both pairs of experiments were very consistent, while the BLM predictions varied. These results suggest an analytical problem in measuring DOC, rather than a problem with the model. However, the variability in results also suggests that the BLM should be run on at least three to five water samples before any conclusions are made regarding metal toxicity at an outfall or stream.

5.1.6 Cost Comparison

In 2005, the cost of performing the toxicity testing and supporting water chemistry for an acute stream-lined WER was approximately $8000. In contrast, the cost of performing three to five rounds of water chemistry for the parameters needed to run the BLM is less than $1000. Obviously, the BLM offers the opportunity to estimate metal bioavailability for a much lower cost. However, at present, SCDHEC will modify metal limits in NPDES permits based on WER results, but has no provisions for modifying limits based on BLM results. It is likely that as the BLM becomes more widely accepted, that SCDHEC may adopt it as an alternative to the WER. Even if SCDHEC will not accept BLM results in lieu of a WER, the BLM still provides an inexpensive and rapid means of assessing whether performing a WER study would be likely to result in higher metal limits.

6.0 Future Directions and Recommendations

This section is largely based on recommendations proposed by Niyogi and Wood (2004), who did a superb job of summarizing the current state of the science for BLMs and made excellent suggestions on areas of future research.

Niyogi and Wood (2004) state that "the scientific underpinnings of the present BLM approach are sound. The mechanistic framework that the BLM models provide for predicting bioavailability and acute toxicity of metals in aquatic environments will lead to significant improvements over current regulatory protocols such as hardness-based adjustments or WERs. The empirical validations performed so far appear very promising, and are important steps toward establishing the BLM concept as a geochemically and biologically robust approach for incorporating bioavailability concepts into Water Quality Criteria. Furthermore, the BLM can serve as a valuable predictive tool in environmental toxicology (i.e., to determine which experiments and which water quality variables are likely to be most important). However, further research or modifications of current approaches are required to enhance the potential of the BLM concept as a practical tool for site-specific ecological risk assessment for metals in the natural waters."

"A fundamental principle of the BLM approach is to relate acute toxicity to the critical metal accumulation at the biotic ligand. For mechanistic understanding, this remains essential, as well as for biological and geochemical correctness because this process defines the receptor site density. Operationally, in geochemical modeling the same endpoint can be achieved by a high log K and a low LA_{50}, or by the opposite combination, but only one is biologically correct. However, in practice, the actual measurement of this critical burden in the organism has proven difficult, and most reported LA_{50} values are probably just surrogate values. It is now clear that a portion of the measured burdens on the apparent biotic ligand may be in nontoxic form and/or at nontoxic site of action. For example, silver complexed with chloride or thiosulfate may accumulate on the gill, yet may not cause acute toxicity in fish (Wood et al, 1999). Similarly, there are Na^+-sensitive (toxic site of action) as well as Na^+-insensitive (nontoxic site of action) components of the high-affinity Cu binding sites at the fish gill (Grosell and Wood, 2002). The true LA_{50} is not necessarily a gill metal burden on a fish or a whole body metal burden in a daphnid but rather the actual amount of metal bound to a "receptor" protein such as an ionoregulatory enzyme or an ion channel. Therefore, incorporation of the true metal binding properties (B_{max}, log $K_{BL\text{-}Me}$, LA_{50}) of the key toxic site (e.g., Na^+-K^+-ATPase for Na^+ antagonists) instead of the gill as a whole, into the present acute BLMs remains an important and as yet unachieved goal. However a significant intermediate step is to quantify the functional inhibition of the protein in question, as demonstrated by McGeer et al. (2000) in version B of the acute Ag-BLM, or its immediate consequence, as in the SBM of Paquin et al. (2002a). This approach could certainly improve current BLMs for other metals (copper, cadmium, zinc, cobalt, and lead) where key toxic sites of interference are now known or are strongly suspected. Future research should also focus on developing the in vitro BLMs as a practical tool for simple and cost-effective way for generating site-specific AWQC. "

"This quest for mechanistic and geochemical accuracy need not and should not impede the regulatory implementation of BLMs, which work well despite an absence of information on critical metal burden. In practice, models calibrated directly to toxicity (which is the end point of regulatory interest) can be as effective as those which employ a measured LA_{50}. The single most important criterion for acceptance of a model should be its validation on natural water data sets different from those which were used to calibrate the model in the first place."

"The process of recalibrating fish based models to more sensitive invertebrates simply by reducing LA_{50} without altering the log *K* values is questionable, irrespective of this issue as to the true LA_{50}. Toxic mechanisms, and therefore the nature of the toxic sites, may be fundamentally different. For example, nickel acts by a respiratory mechanism in fish (Pane et al, 2003a; 2004) but by an ionoregulatory mechanism, inhibition of active Mg^{2+} uptake, in daphnids (Pane et al., 2003b), so log *K* values for protective cations will probably differ. Buffering conditions for pH at the gill surface may also differ substantially between these very different organisms, changing the effective log $K_{BL\text{-}H+}$ or even metal speciation (Playle, 1998). Furthermore, there is accumulating evidence that the protective effects of two major hardness cations differ between fish (where Ca^{2+} is more effective, probably because it regulates the permeability and stability of membrane proteins in the fish gills; Hunn, 1985; Wood, 1992; Perry and Wood, 1985), and daphnids (where Mg^{2+} appears to have equal or even greater protective effects than Ca^{2+}; Heirjerick et al., 2002, Gensemer et al., 2002; Welsh et al, 2000a, 2000b). BLM constants should be worked out directly by experimentation on the organisms of interest.

"In this regard, it is noteworthy that the present set of acute BLMs have been developed based only on studies with two species of fish (rainbow trout, fathead minnow) and assorted daphnids. There is a need for research to extend the BLM approach to a much wider range of freshwater organisms, including other families of fish, other invertebrates both pelagic and benthic, and representative algae. The goal here is 3-fold: (i) to determine the species-sensitivity distributions of BLM predictions for regulatory purposes (analogous to those classically compiled as hardness-adjusted LC_{50} values; U.S. EPA, 1986); (ii) to determine general principles by which BLMs can be recalibrated between related species; and (iii) to detect those classes of organisms where BLM principles and/or constants fundamentally differ. For example, limitations of the present acute BLM approach in freshwater algae have now been reported (Hassler et al., 2003, 2004). These studies suggest that physiology of the biotic ligands in algae is probably somewhat different relative to fish and daphnids, perhaps related to oxygen generation and carbon dioxide consumption in plants versus oxygen consumption and carbon dioxide production in animals. There is also a need to extend the present BLM approach to the marine and estuarine environment where ion transport mechanisms (and therefore biotic ligands) and metal speciation are fundamentally different. "

"A fundamental but usually unstated assumption of the present BLM approach is that the properties of the biotic ligand are unchangeable regardless of water chemistry conditions. However, ion transport and permeability characteristics of the fish gill are known to be sensitive to water chemistry of acclimation, particularly water hardness and pH. There is now ample evidence that these factors as well as chronic sublethal preexposure to the waterborne metal itself, dietary metal levels, dietary ions, and organism age can all lead to large changes in the metal binding properties of the biotic ligand and significantly alter the toxicological sensitivity of the fish. Decreased sensitivity with acclimation to chronic metal exposures has been observed in daphnids as well (Muyssen and Janssen, 2001; Barata et al., 2002). These findings demonstrate that the properties of the biotic ligand are dynamic rather than fixed. If the goal is to make site-specific WQC, these factors should be incorporated-i.e., regulations should be based on the BLM for the most sensitive life stages of organisms that occur in the area, acclimated to typical water chemistry, background metal concentrations, and diet at the site in question."

"DOM character also tends to be site-specific, and one of the greatest areas of present uncertainty is how to deal with DOMs in current acute BLMs, let alone different DOMs. Given that the concentration range of interest for specific metals is limited for predicting acute toxicity, there exists no convincing evidence that multiple site models with multiple log *K* values do any better than single site models (or vice versa) for dealing with DOM in acute BLMs. The more important need at present is to derive adjustment factors for the very different protective abilities of different types of DOM. Measurements of color (specific absorbance and reduced sulfide content hold promise in this regard, though much more research is needed. Furthermore the action of reduced sulfides in toxic waters (either inside or outside DOM) in preventing toxicity has so far only been quantified directly for silver; this may prove to be an important protective factor that should be incorporated into acute BLMs for other metals."

"Present acute BLMs have been developed so far for individual metals, but metals usually exist in mixtures in contaminated aquatic environments and are known to interact with each other. The basic principles of the BLM approach appear to be ideally suited to analyze such phenomena, because once common sites of binding are identified, the biotic ligand constants can be used to predict the outcome of metal interactions. Metals interacting at common sites should follow principles of concentration additivity, and those acting at different sites should exhibit effects additivity (Norwood et al., 2003; Newman and Unger, 2003). Playle (2004) recently simulated the former using two-to-six metal scenarios (silver, copper, cadmium, cobalt, lea*d* and zinc) by combining log *K* values of respective gill binding models into the MINEQL+ V-4.5 framework and using the toxic unit concept (Newman and Unger, 2003). The model simulations yielded greater than strict additivity at low metal concentrations, strict additivity at intermediate metal concentrations, and less than strict additivity at high metal levels, independent of metal combinations. Interestingly, Newman and McCloskey (1996) reached similar conclusions based on their review of previously published literature dealing with acute toxic effects of metal mixtures on aquatic animals. Moreover, multiple metal-gill models behaved predictably against alterations of water pH and Ca^{2+}, although not against alteration of DOM. However, it should be noted that Playle (2004) adopted a simplified assumption for simulation purposes-that both Ca^{2+} antagonists (Cd^{2+}, Zn^{2+}, Pb^{2+}, and Co^{2+}) and Na^{+} antagonists (Cu^{+} and Ag^{+}) were competing for the same binding sites (concentration additivity only). "

"Finally, in many jurisdictions, AWQC are designed to protect against chronic toxicity, not just acute toxicity, so as to provide lifetime protection to the resident fauna. There is an urgent need for more research to derive BLMs that predict chronic toxicity in site-specific waters. It would be unfortunate if regulatory authorities apply the "acute-to-chronic ratio" (ACR), as done traditionally in hardness-based criteria to acute BLM predictions to generate chronic AWQC. The strength of the BLM is its mechanistic foundation, and only in cases where the mechanism of chronic toxicity is the same as that of acute toxicity will the approach be valid. Already, a chronic Cu-BLM for daphnia has been developed very recently and is showing reasonable success in predicting chronic reproductive effects. Interestingly, the protective actions of Na^{+} seemed to be similar in the chronic model and the acute model, suggesting that the toxic mechanisms of acute and chronic copper toxicity are similar in nature. Moreover, the predictions of the chronic model did not seem to be altered by simultaneous dietary copper exposure (De Schamphelaere and Janssen, 2004d). Furthermore, a chronic Zn-BLM has also been developed for fish (De Schamphelaere and Janssen, 2004c), and its similarity to the acute Zn-BLM in the dominant protective role of Ca^{2+} in predicting mortality (the most sensitive endpoint in the chronic history) again suggest that the acute and chronic toxic mechanisms of zinc in fish are similar (i.e., disruption of Ca^{2+} homeostasis). In case of silver also, the mechanism does appear similar (Na^{+} balance pathology). However, the protective actions of some factors (Ca^{2+}, Mg^{2+}, DOM, pH, and Cl^{-}), which are effective against acute toxicity, are either lessened or non-existent during chronic exposure for copper and silver. This may well be because the equilibrium assumptions of the present BLM are violated during prolonged exposures, pointing to a need to incorporate kinetic adjustments and to avoid ACR approach. Regardless, chronic BLMs should be based on chronic tests that, with skill and care, will be as useful as the present acute BLMs based on acute toxicity tests."

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