



2003 DRAFT UPDATE OF AMBIENT WATER QUALITY CRITERIA FOR COPPER

**2003 UPDATE OF AMBIENT WATER QUALITY CRITERIA FOR
COPPER**

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NOTICES

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CONTENTS

| | |
|--|-----------|
| Notices | ii |
| Acknowledgments | iii |
| Acronyms | vi |
| 1.0 INTRODUCTION | 1 |
| 1.1 Background | 1 |
| 1.2 Copper in the Environment | 1 |
| 1.3 Update of Copper Criteria with the Biotic Ligand Model | 1 |
| 1.4 Copper Criteria Document Information | 2 |
| 2.0 THE CONCEPT OF BIOAVAILABILITY AND REGULATORY APPROACHES FOR COPPER | 2 |
| 2.1 Empirical Models Relating Water Chemistry to Toxicity | 4 |
| 2.2 Mechanistic Models—Relating Water Chemistry to Toxicity | 5 |
| 3.0 INCORPORATION OF BLM INTO CRITERIA DEVELOPMENT PROCEDURES | 7 |
| 3.1 Implications for Criteria—Criteria Calculations | 7 |
| 3.2 BLM Input Parameters | 7 |
| 3.3 Model Prediction Modes | 8 |
| 3.4 Data Acceptability and Screening Procedures | 8 |
| 3.5 Estimation of Test Water Chemistry | 9 |
| 3.6 Water Chemistry Data Acquisition | 9 |
| 3.7 Ranking of Quality of Test Chemistry Characterization | 9 |
| 3.8 Criteria Computations | 10 |
| 4.0 CONVERSION FACTORS | 11 |
| 5.0 DATA SUMMARY AND CRITERIA CALCULATION | 11 |
| 5.1 Summary of Acute Toxicity to Freshwater Animals and Criteria Calculation | 11 |
| 5.1.1 Comparison with Hardness-Adjusted Values | 15 |
| 5.2 Summary of Acute Toxicity to Saltwater Animals and Criteria Calculation | 16 |
| 5.3 Formulation of the CCC | 17 |
| 5.3.1 Statistical Evaluation of Chronic Toxicity Data | 17 |
| 5.3.2 Calculation of Freshwater CCC | 19 |
| 5.3.3 Evaluation of the Chronic Data Available for Saltwater Species | 21 |
| 6.0 PLANT DATA | 21 |
| 7.0 BIOACCUMULATION OF COPPER | 23 |
| 8.0 OTHER DATA | 23 |
| 9.0 NATIONAL CRITERIA STATEMENT | 24 |
| 10.0 IMPLEMENTATION | 24 |
| 11.0 REFERENCES | 58 |

FIGURES

| | |
|--|----|
| Figure 1. Conceptual Diagram of Copper Speciation and Copper-Gill Model | 5 |
| Figure 2. Comparison of Predicted and Measured Acute Copper Toxicity to <i>P. promelas</i> | 6 |
| Figure 3. Quality Scale for <i>D. magna</i> BLM Input Data | 12 |
| Figure 4. Ranges and Distribution of Normalized LC50 Values for Species Listed in Table 1 | 13 |
| Figure 5. Ranked Freshwater Genus Mean Acute Values (GMAVs) | 14 |
| Figure 6. Comparison of Existing Hardness Based WQC and BLM Based WQC in Synthetic Laboratory Water and EPA Standard Recipe Water for DOC = 2.3 mg/L | 15 |
| Figure 7. Ranked Saltwater Genus Mean Acute Values (GMAVs) | 18 |
| Figure 8. Relationship Between Freshwater Acute Copper Sensitivity (LC50 or EC50) and Acute-Chronic Ratios | 20 |

TABLES

| | |
|--|----|
| Table 1a. Acute Toxicity of Copper to Freshwater Animals | 26 |
| Table 1b. Acute Toxicity of Copper to Saltwater Animals | 36 |
| Table 2a. Chronic Toxicity of Copper to Freshwater Animals | 42 |
| Table 2b. Chronic Toxicity of Copper to Saltwater Animals | 44 |
| Table 2c. Acute-Chronic Ratios | 45 |
| Table 3a. Ranked Freshwater Genus Mean Acute Values with Species Mean Acute-Chronic Ratios | 46 |
| Table 3b. Ranked Saltwater Genus Mean Acute Values with Species Mean Acute-Chronic Ratios | 47 |
| Table 3c. Freshwater and Saltwater Final Acute Value (FAV) and Criteria Calculations | 49 |
| Table 4a. Toxicity of Copper to Freshwater Plants | 50 |
| Table 4b. Toxicity of Copper to Saltwater Plants | 53 |
| Table 5a. Bioaccumulation of Copper by Freshwater Organisms | 55 |
| Table 5b. Bioaccumulation of Copper by Saltwater Organisms | 56 |
| Table 6. Species Numbers Used in Figure 4 | 57 |

APPENDICES

| | |
|--|-----|
| Appendix A. Ranges in Calibration and Application Data Sets | A-1 |
| Appendix B. Biotic Ligand Model (BLM) User's Guide | B-1 |
| Appendix C. Other Data on Effects of Copper on Freshwater and Saltwater Organisms | C-1 |
| Appendix D. Estimation of Water Chemistry Parameters for Acute Copper Toxicity Tests | D-1 |
| Appendix E. Saltwater Conversion Factors for Dissolved Values | E-1 |
| Appendix F. BLM Input Data and Notes | F-1 |
| Appendix G. Hardness Slopes | G-1 |
| Appendix H. Regression Plots | H-1 |
| Appendix I. Unused Data | I-1 |

ACRONYMS

| | |
|--------|---|
| ACR | Acute-Chronic Ratio |
| BL | Biotic Ligand |
| BLM | Biotic Ligand Model |
| CCC | Criterion Continuous Concentration |
| CF | Conversion Factors |
| CHESS | Chemical Equilibria in Soils and Solutions |
| CMC | Criterion Maximum Concentration |
| CWA | Clean Water Act |
| DIC | Dissolved Inorganic Carbon |
| DOC | Dissolved Organic Carbon |
| DOM | Dissolved Organic Matter |
| ELS | Early Life Stage |
| EPA | Environmental Protection Agency |
| FACR | Final Acute-Chronic Ratio |
| FAV | Final Acute Value OR Final Accumulation Value |
| FCV | Final Chronic Value |
| FIAM | Free Ion Activity Model |
| GMAV | Genus Mean Acute Value |
| GSIM | Gill Surface Interaction Model |
| HA | Humic Acid |
| LA50 | Lethal Level of Accumulation at 50 Percent Effect Level |
| LOAEC | Lowest Observed Adverse Effect Concentration |
| Me:BL | Metal-Biotic Ligand Complex |
| MSE | Mean Square Error |
| NASQAN | National Stream Quality Accounting Network |
| NOAEC | No Observed Adverse Effect Concentration |
| NOM | Natural Organic Matter |
| PLC | Partial Life-Cycle |
| SMAV | Species Mean Acute Values |
| TSS | Total Suspended Solids |
| WER | Water-Effect Ratio |
| WET | Whole Effluent Toxicity |
| WHAM | Windermere Humic Aqueous Model |
| WQC | Water Quality Criteria |

1.0 INTRODUCTION

1.1 Background

Over the past 20 years the U.S. Environmental Protection Agency (EPA) has published a number of guidance documents containing aquatic life criteria recommendations for copper (e.g., U.S. EPA 1980, 1985, 1986, 1996). The present document contains EPA's latest criteria recommendations for protection of aquatic life in ambient water from acute and chronic toxic effects from copper. These criteria are based on the latest available scientific information and supersede EPA's previously published recommendations for copper.

This document provides updated guidance to States and authorized Tribes to establish water quality standards under the Clean Water Act (CWA) to protect aquatic life from copper. Under the CWA, States and authorized Tribes are to establish water quality criteria to protect designated uses. Although this document constitutes EPA's scientific recommendations regarding ambient concentrations of copper, it does not substitute for the CWA or EPA's regulations, nor is it a regulation itself. Thus, it cannot impose legally binding requirements on EPA, States, Tribes, or the regulated community, and might not apply to a particular situation based on the circumstances. State and Tribal decisionmakers retain the discretion in adopting approaches, on a case-by-case basis, that differ from this guidance when appropriate. EPA may change this guidance in the future.

1.2 Copper in the Environment

Copper is an abundant trace element found in the earth's crust and is also a naturally occurring element that is generally present in surface waters (Nriagu 1979). Copper is a micronutrient for both plants and animals at low concentrations; however, it may become toxic to some forms of aquatic life at elevated concentrations. Thus, copper concentrations in natural environments, and its biological availability, are important. Naturally occurring concentrations of copper have been reported from 0.03 to 0.23 µg/L in surface seawaters and from 0.2 to 30 µg/L in freshwater systems (Bowen 1985). Copper concentrations in locations receiving anthropogenic inputs such as mine tailing discharges can vary anywhere from natural background to 100 µg/L (Hem 1989; Lopez and Lee 1977) and have in some cases been reported in the 200,000 µg/L range in mining areas (Davis and Ashenberg 1989; Robins et al. 1997). Mining, leather and leather products, fabricated metal products, and electric equipment are a few of the industries with copper-bearing discharges that contribute to anthropogenic inputs of copper to surface waters (Patterson et al. 1998).

1.3 Update of Copper Criteria with the Biotic Ligand Model

The freshwater criteria in this document differ from EPA's previous metals criteria primarily with regard to how metal availability to organisms is addressed. Previous criteria were based on empirical relationships of toxicity to water hardness. These criteria combine the effects of various water quality variables correlated with hardness. Such criteria are most applicable to waters where these correlations were similar to the data set used to derive the relationships. The criteria presented here instead use the biotic ligand model (BLM) (Di Toro et al. 2001). The BLM is based on the premise that toxicity is related to metal bound to a biochemical site (the biotic ligand) and that binding is related to total dissolved metal concentrations and complexing ligands in the water. The complexing ligands compete with the biotic ligand for metals and other cations in the water. Unlike the empirical hardness relationships, the BLM explicitly accounts for individual water quality variables, is not linked to a particular correlation among these variables, and can address variables that were not a factor in the hardness relationship.

1.4 Copper Criteria Document Information

Although the new BLM model has now been adopted for use in place of the formerly applied hardness-based approach the updated freshwater criteria derivations in this document are still based on the principles set forth in the 1985 Guidelines (or Guidelines, Stephan et al. 1985). Therefore, it is useful to have some understanding of how the Guidelines are ordinarily applied: (1) Acute toxicity test data must be available for species from a minimum of eight genera with a minimum required taxonomic diversity. The diversity of tested species is intended to ensure protection of various components of an aquatic ecosystem. (2) The final acute value (FAV) is an estimate of the fifth percentile of a sensitivity distribution represented by the average LC50s and EC50s, the Genus Mean Acute Values (GMAVs), of the tested genera. The criterion maximum concentration (CMC) is set to one-half of the FAV to correspond to a lower level of effect than the LC50s/EC50s used to derive the FAV. (3) Chronic toxicity test data (longer term survival, growth, or reproduction) must be available for at least three taxa to derive a final chronic value (FCV). A criterion continuous concentration (CCC) can be established from an FCV calculated similarly to an FAV, if chronic toxicity data are available for eight genera with a minimum required taxonomic diversity; or most often the chronic criterion is set by determining an appropriate acute-chronic ratio (ACR) (the ratio of acutely toxic concentrations to the chronically toxic concentrations) and applying that ratio to the FAV. (4) When necessary, the acute and/or chronic criterion may be lowered to protect recreationally or commercially important species.

The body of this document contains information on acute and chronic toxicity of copper relevant to the derivation of the freshwater and saltwater acute and chronic criteria. It also includes information on the effects of water quality parameters on bioavailability and toxicity of copper as well as some BLM development information. Additional information on the generalized BLM framework, theoretical background, model calibration, and application can be found in the Technical Support Document for the BLM or in the published literature. The data that were reviewed and not used to derive the criteria and other supporting information are also provided in tables and appendices.

2.0 THE CONCEPT OF BIOAVAILABILITY AND REGULATORY APPROACHES FOR COPPER

Copper occurs in natural waters primarily as Cu (II) predominately in complexed form. Free Cu may be present, but is generally a minor species (Stumm and Morgan 1981). Copper reacts with both inorganic and organic chemicals in solution and in suspension, resulting in a multitude of chemical forms. Because the cupric ion is highly reactive, it forms moderate to strongly complexed solutes and precipitates with many inorganic and organic constituents of natural waters (e.g., carbonate, phosphate, and organic materials) and is readily sorbed onto surfaces of suspended solids. Even though it is present in water in many forms, the toxicity of copper to aquatic life has been shown to be related primarily to activity of the cupric ion, and possibly to some of the hydroxy complexes (Allen and Hansen 1996; Andrew 1976; Andrew et al. 1977; Borgmann and Ralph 1983; Chakoumakos et al. 1979; Chapman and McCrady 1977; Dodge and Theis 1979; Howarth and Sprague 1978; Pagenkopf 1983; Petersen 1982; Rueter 1983). Many examples of this classic response of organisms to cupric ion activity, as well as some limited exceptions, are reviewed by Campbell (1995). A formal description of these metal-organism interactions, now commonly referred to as the Free Ion Activity Model (FIAM), was first provided by Morel (1983). Pagenkopf (1983) using a similar approach applied the Gill Surface Interaction Model (GSIM) to predict metal effect levels over a range of water quality characteristics.

Based on the mechanistic principles underlying the BLM, the following general trends of copper toxicity are expected because individual water quality parameters and their combinations are varied among exposure waters. Any changes in water quality that would be expected to decrease the activity of

the free copper ion would be expected to decrease the bioavailability of copper. For example, increases in pH, increases in alkalinity, and increases in natural organic matter would all tend to decrease copper bioavailability and would therefore tend to be associated with increased copper LC50 values. Metal bioavailability may also be modified by competitive interactions at the biotic ligand. Increased concentrations of sodium and calcium, for example, can result in reduced binding of copper to physiologically active gill binding sites and can thereby reduce copper bioavailability. Competition with protons is included in the copper model and could result in lower bioavailability at low pH. But these effects occur at relatively lower pH values than are typically used in toxicity tests and, as a result, the primary effect of changing pH is to decrease bioavailability at high pH. Cation competition also has an effect on complexation of Cu by natural organic matter (NOM), and this interaction will to some degree offset competitive interactions that occur at the gill or other sites of action of toxicity.

Historically, aqueous discharges of metals have been regulated based on concentrations of total metal—usually measured as the concentration of total recoverable metal (i.e., the sum of the dissolved metal and the metal that can be liberated from solids during extraction in hot, dilute mineral acid). This regulatory approach was the basis for previous EPA water quality criteria for copper. In 1993, EPA altered the traditional regulatory approach for protection of aquatic life to account for the influence of suspended solids on metal toxicity. EPA authorized States to regulate discharges based on dissolved metal concentration instead of total recoverable metal concentration (Prothro 1993). This change was an attempt to incorporate into the regulatory process the notion that the concentration of dissolved metal better approximates the toxic fraction than does the concentration of total metal (i.e., the presence of suspended solids tends to decrease metal toxicity; see review by Meyer et al. 2002). Nevertheless, a regulatory approach based solely on the concentration of dissolved metal did not address concerns that other water quality parameters besides total suspended solids (TSS) concentration alter metal toxicity.

EPA has already incorporated linear regression equations into criteria calculation procedures to account for decreases of acute and chronic toxicity of copper to freshwater organisms as water hardness increases. However, these regression equations account for other parameters that vary in addition to hardness (at least among some of the data) but do not explicitly account for effects of these other water quality parameters on toxicity.

In response to concerns that the metal criteria did not provide a mechanism to account for the modifying effects of water quality parameters other than hardness on metal toxicity, EPA issued guidance in the early 1980s on the use of a water-effect ratio (WER) method (Carlson et al. 1984; U.S. EPA 1983, 1992, 1994). The WER is “a biological method to compare bioavailability and toxicity in receiving waters versus laboratory test waters” (U.S. EPA 1992). Extensive guidance has been developed on how to evaluate a WER (U.S. EPA 1994). The essence of the approach is as follows. The WER is calculated by dividing the acute LC50 of the metal, determined in water collected from the receiving water of interest, by the LC50 of the metal determined in a standard laboratory water, after adjusting both test waters to the same hardness. The national hardness-based acute criterion concentration is then multiplied by this ratio (i.e., the WER) to establish a site-specific criterion that reflects the effect of site water characteristics on toxicity.

However, a WER accounts only for interactions of water quality parameters and their effects on metal toxicity to the species tested, in the water sample collected at a specific location and at a specific time. Although the WER approach remains an important component in establishing site-specific variations to ambient water quality criteria for metals, a complementary approach is needed that (1) explicitly accounts for water quality parameters that modify metal toxicity and (2) can be applied more frequently across spatial and temporal scales.

Because of the influence of water quality parameters such as pH, alkalinity, and organic matter on the formation of compounds that affect the amount of cupric ion present, not all of the copper in the water column contributes directly to toxicity. In other words, not all of the copper appears to be bioavailable. Although the term “bioavailability” eludes a consensus definition (Dickson et al. 1994), in the context of this document it is used to convey the general concept that total Cu (or, more generally, the total concentration of any metal in an exposure water) is not a good predictor of toxicity (Campbell 1995; Meyer 2002; Morel 1983). This concept has led to research and regulatory activity to develop better ways to predict metal toxicity and regulate aqueous discharges (Bergman and Dorward-King 1997; Di Toro et al. 2001; Hamelink et al. 1994; Morel 1983).

2.1 Empirical Models Relating Water Chemistry to Toxicity

Early copper criteria documents (U.S. EPA 1980, 1985, 1996) incorporated linear regression equations into the criterion-calculation procedure to account for attenuation of acute and chronic toxicity of copper to freshwater biota as water hardness increases. Previously though, the only parameter with enough useful data to provide an acceptable predictive capability of copper toxicity was hardness. Temperature ranges were not sufficiently wide with most species, pH values were often not reported or were highly variable, and alkalinity and dissolved organic carbon (DOC) were rarely reported. As a result, criteria for copper, and those for several other metals, were established as functions of water hardness. These equations were determined from meta-analyses in which variables other than hardness varied among at least some of the data sets that were used. Therefore, the regression coefficients for hardness did not only reflect how hardness affected copper toxicity; additionally, hardness was a surrogate for other co-varying water quality parameters not explicitly included in the regression analyses. Moreover, these criteria did not include methods to explicitly account for modifying effects of other water quality parameters when those parameters varied and hardness did not.

An alternate approach that has been proposed to predict metal toxicity is to (1) identify the bioavailable fraction of the metal; (2) analyze or calculate the concentration(s) of the bioavailable form(s) in the exposure water; and (3) predict the toxicity based on an empirical relationship between the biological response and the concentration(s) of the bioavailable form(s). According to this approach, only direct measurement of the concentration of the free metal ion or calculation of its concentration (using a geochemical-speciation model) is needed. Supporting this bioavailable-fraction approach, the concentration of cupric ion is a constant predictor of acute toxicity even in the presence of varying levels of inorganic or organic ligands, which complex copper and alter the cupric ion concentration (i.e., the cupric ion LC50 remains constant even though the concentrations of the ligands differ considerably in different exposure waters) (e.g., Borgmann 1983; Santore et al. 2001). However, this approach is not correct when other cations in the water can interact with the biota. For example, the LC50 of Cu^{2+} increases significantly as the concentration of Ca^{2+} (a major component of water hardness) is increased (Meyer et al. 1999). Thus, the concentration of cupric ion alone is not always sufficient to predict toxicity.

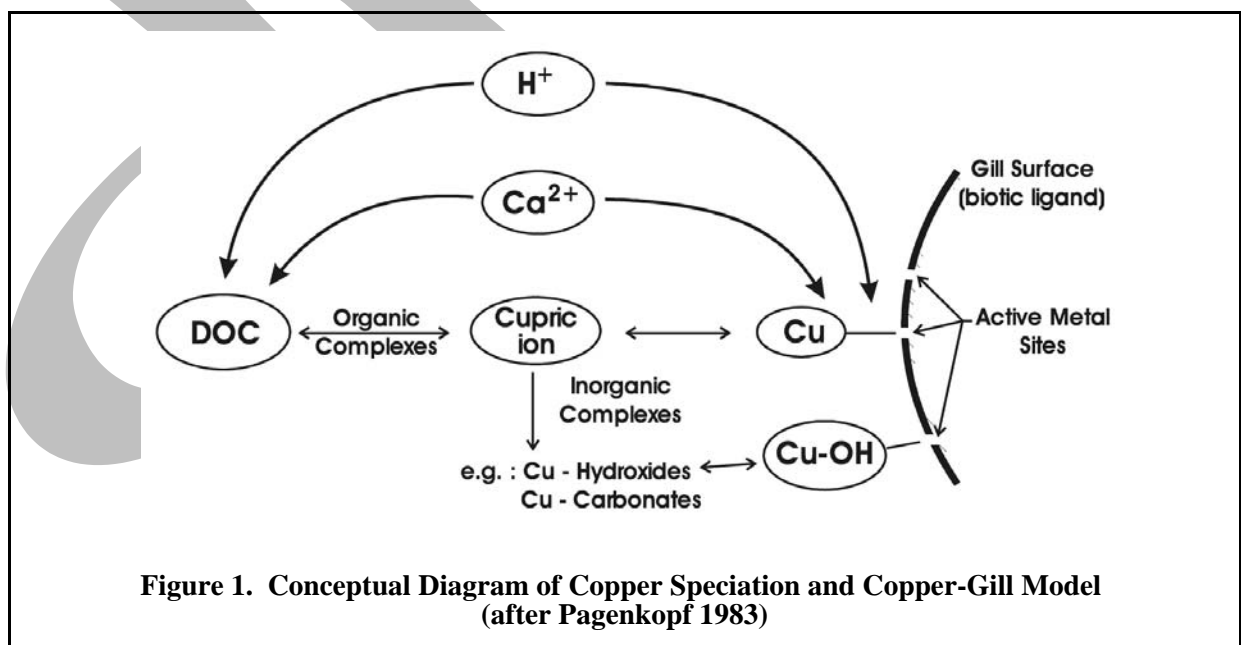
More generally, there is no universally constant bioavailable fraction of a metal that can be identified by chemical analyses (Meyer et al. 2002). The interactions among the abiotic components in the exposure water are important to consider, as well as the interactions of those components with the biota. Hence, although the simple concept of predicting metal toxicity based on the chemical analysis of a bioavailable fraction is qualitatively appealing, in practice, it is quantitatively elusive (Meyer 2002). Instead, the complex interactions of Cu^{2+} with dissolved components, suspended particles, and the biota must be simultaneously considered in order to accurately predict copper toxicity (see Mechanistic Models section).

2.2 Mechanistic Models—Relating Water Chemistry to Toxicity

Although the current water quality criteria for several metals, including copper, are hardness-dependent, it has long been recognized that many other factors affect copper toxicity. The chemical speciation of copper in natural waters and the explanatory power of the free copper ion in determining copper toxicity were first recognized more than 30 years ago (Anderson and Morel 1978; Sunda and Gillespie 1979; Sunda and Guillard 1976; Sunda and Lewis 1978; Zitko et al. 1973). These concepts were eventually formalized in models that linked metal chemistry and biological effects including the gill surface interaction model (GSIM) (Pagenkopf 1983) and the free ion activity model (FIAM) (Morel 1983). Playle and others demonstrated that copper binding to fish gills can be modeled using a chemical speciation approach (Playle et al. 1993a, b). Recently, MacRae and others demonstrated that copper accumulation at the gill shows a dose-response relationship with mortality (MacRae et al. 1999). A more comprehensive review of these historical developments is presented in Paquin et al. (2002).

Although early models showed remarkable utility, several critical issues remained. A considerable amount of information about speciation of metals in the environment has become available and computing techniques have been developed to simulate metal speciation (Nordstrom et al. 1979). Still, the interactions of metals with natural organic matter remained a topic of intense research and debate for the next few decades. Until recently, few available models could predict metal chemistry in the presence of natural organic matter over a range of environmental conditions.

The biotic ligand model is a recent attempt to develop a metal bioavailability model based on the latest chemical and physiological effects information of metals in aquatic environments (Di Toro et al. 2001; Paquin et al. 1999; Santore et al. 2001). The approach was presented to EPA's Science Advisory Board during 1999 and it received a generally favorable response (U.S. EPA 1999, 2000). Like the FIAM and GSIM, the BLM is based on a description of the chemical speciation of metals in aqueous systems (Figure 1). Chemical speciation is simulated as an equilibrium system that includes complexation of inorganic ions and NOM. The chemical system is simulated by the chemical equilibria in soils and solutions (CHESS) model (Santore and Driscoll 1995), including a description of metal interactions with NOM based on the Windermere humic aqueous model (WHAM) (Tipping 1994). A significant advantage



of the NOM chemistry developed for WHAM is that reactions and parameter values were developed by simultaneously considering numerous NOM samples and numerous metals.

The BLM also includes reactions that describe the chemical interactions of copper and other cations to physiologically active sites (or “biotic ligands”) that correspond to the proximate site of action of toxicity. The model parameters define the degree of interaction based on binding affinity characteristics measured in gill-loading experiments (Playle et al. 1993a, b). That is, the biotic ligand (BL) is represented by a characteristic binding site density and conditional stability constant for each of the dissolved chemical species with which it reacts. Predictions of metal toxicity are made by assuming that the dissolved metal LC50, which varies with water chemistry, is always associated with a fixed critical level of metal accumulation at the biotic ligand. This fixed level of accumulation at 50 percent mortality, referred to as the LA50, is the concentration of the metal-biotic ligand complex (Me:BL) that is associated with 50 percent mortality for a fixed exposure. It is assumed to be constant, regardless of the chemical characteristics of the water (Meyer et al. 1999, 2002). This combination of reactions that describe aqueous metal speciation and organism interactions allows the BLM to predict copper toxicity to a variety of organisms over a variety of water quality conditions (Santore et al. 2001). Appendix A describes the range of water quality values and species to which the model has been applied.

A significant advantage of the BLM is that most of the parameters are invariant for different organisms, despite the complexity of the modeling framework. All of the thermodynamic constants used to simulate inorganic and organic chemical equilibrium reactions are determined by characteristics of the metal and the available ligands. As such, the constants do not change for simulations involving different organisms. Binding constants for copper and other cations to the biotic ligand were developed from data reported by Playle and others using fathead minnow (Playle et al. 1993a, b). Similar measurements would be difficult or impossible to obtain for many organisms, especially invertebrates, because of the difficulty associated with isolating and excising gill tissue, or an appropriate analog. Nevertheless, the parameter values developed from fathead minnow measurements appear to work adequately for other organisms (Santore et al. 2001). Figure 2 shows the predictive capabilities of the model with fathead minnows.

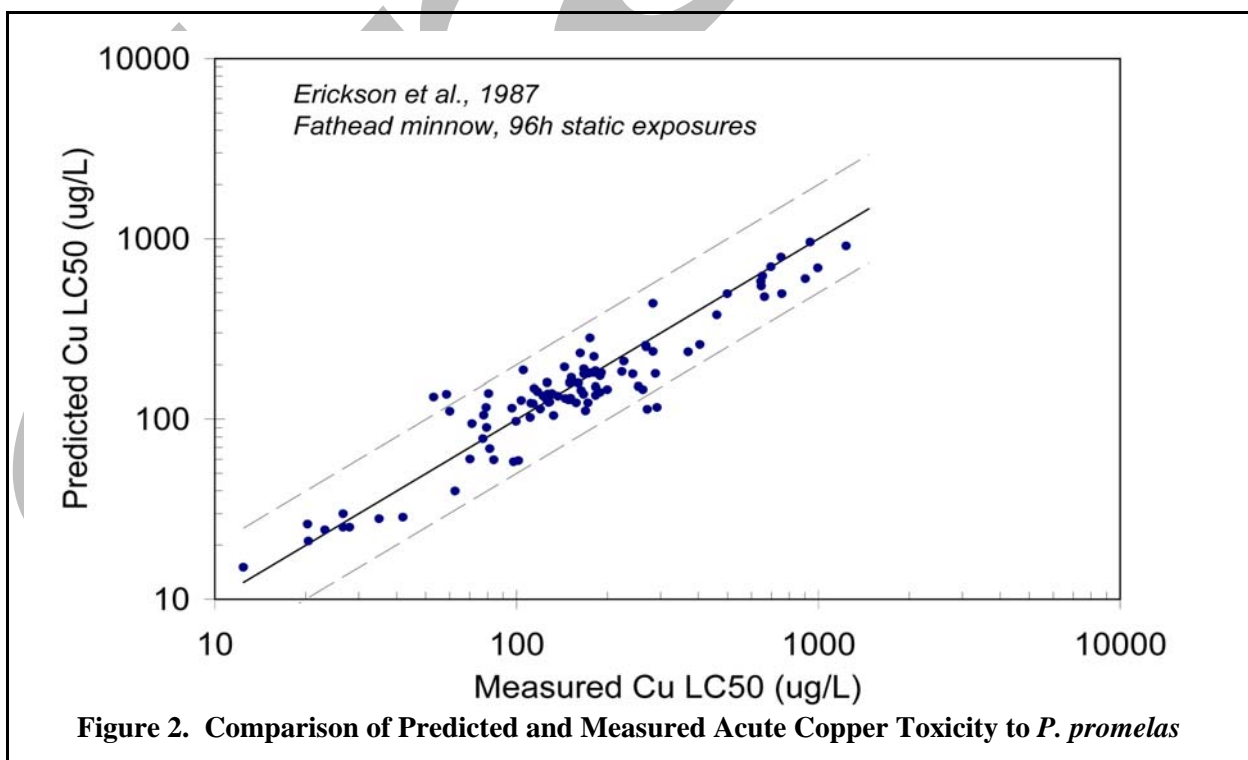


Figure 2. Comparison of Predicted and Measured Acute Copper Toxicity to *P. promelas*

3.0 INCORPORATION OF BLM INTO CRITERIA DEVELOPMENT PROCEDURES

3.1 Implications for Criteria—Criteria Calculations

The use of the BLM to predict the bioavailability and toxicity of copper to aquatic organisms under site-specific conditions is a significant change from the previous CMC derivation methodology. Previous aquatic life criteria documents for copper (e.g., U.S. EPA 1980, 1985, 1996) expressed the CMC as a function of water hardness. Now, EPA chooses to utilize the BLM to update its freshwater acute criterion because the BLM accounts for all important inorganic and organic ligand interactions of copper while also considering competitive interactions that influence binding of copper at the site of toxicity, or the “biotic ligand.” The BLM’s ability to incorporate metal speciation reactions and organism interactions allows prediction of metal effect levels to a variety of organisms over a wide range of water quality conditions. Accordingly, the BLM is an attractive tool for deriving water quality criteria. Application of the BLM may reduce, if not eliminate, the need for site-specific modifications, such as Water Effect Ratios, to account for site-specific chemistry influences on metal toxicity.

While the BLM is currently considered appropriate for use to derive an updated freshwater CMC, further development is required before it will be suitable for use to evaluate a saltwater CMC or a CCC or chronic value.

3.2 BLM Input Parameters

For copper simulations, the necessary water quality input parameters are: pH; dissolved organic carbon (DOC) (in mg/L); percent humic acid; temperature; major cations (Ca^+ , Mg^+ , Na^+ , and K^+); major anions (SO_4^- , Cl^-); dissolved inorganic carbon (DIC); and sulfide.

Dissolved cations compete with Cu^{2+} for dissolved organic matter (DOM) binding sites. For example, pH is important in determining the metal complexation capacity of dissolved organic matter (DOM). It also is important in determining speciation of inorganic carbon, which relates to formation of metal carbonate complexes. DOM can likewise play a critical role in determining metal speciation and bioavailability. Its concentration is entered into the BLM in terms of the concentration of DOC. Because the representation of metal-NOM complexes in the BLM adopted from WHAM, characterizes metal complexation with both humic and fulvic organic matter, it is necessary to specify the distribution of these two humic acid forms of natural organic matter. Ca and Na can directly compete with copper at DOM and biotic ligand binding sites, and these cations will therefore have a direct effect on model predictions. Magnesium may have a critical role as well for some organisms. In that SO_4 may be the dominant anion in freshwater, it is important for determining the charge balance and ionic strength in BLM calculations. Chloride can also contribute to ionic strength computations for copper. The sum of three inorganic species in the BLM—carbonate (CO_3), bicarbonate (HCO_3), and carbonic acid (H_2CO_3)—is considered inorganic carbon. Inorganic carbon is a critical input to the BLM because many metals including copper form carbonate complexes. DIC measurements are typically not made in the environment, so even though it is the preferred measurement, DIC can be estimated from alkalinity and pH when a DIC measurement is not available. Sulfide has a strong affinity for many metals, and although the sulfide concentration is traditionally assumed to be negligible in aerated waters; its concentration may be impacted by wastewater treatment plant effluents.

A number of fixed parameters or constants are also used in the BLM along with the input parameters specified above for speciation or toxicity mode computations. Some of the key fixed constants are the binding constants for the interactions between copper and protons and the “biotic ligand.” The

values contained in the model were derived by Playle and coworkers by conducting gill-loading experiments (Janes and Playle 1995; Playle et al. 1992, 1993a, b). Playle et al. (1993a, b) also developed the gill site density parameter of 30 nmol/g wet weight used in the model from measured copper gill concentrations.

3.3 Model Prediction Modes

The graphical user interface that has been developed for the BLM allows the user to run the model in either the “Metal Toxicity Mode” or in the “Metal Speciation Mode.” Run in the toxicity mode, the BLM predicts the dissolved concentration of copper required to cause acute mortality for water characteristics specified by the user. Run in the speciation mode, the BLM calculates the chemical speciation of a dissolved metal, including complexation with inorganic and organic ligands, and the biotic ligand. Each computational mode requires the user to specify the chemical parameters discussed above and either a dissolved copper concentration or a copper accumulation associated with the biotic ligand.

The biotic ligand represents a discrete receptor or the site of action of toxicity to an organism, where accumulation of metal at or above a critical threshold concentration leads to acute toxicity. The lethal accumulation level on the BL that results in an effect on 50 percent of the individuals is termed the “LA50” for that species. The LA50 concentration of copper on the BL is expected to result in 50 percent mortality in a toxicological exposure for a fixed exposure duration. The LA50 is expressed in units of nmol Cu/g wet weight of the BL. Since the BLM includes inorganic and organic speciation and competitive complexation of copper with the BL, the amount of dissolved copper required to reach this threshold will vary, depending on the water chemistry. Therefore, in addition to calculating chemical speciation, use of the BLM to evaluate the dissolved Cu concentration that is associated with the LA50 provides a prediction of the concentration of copper that would result in acute toxicity (e.g., LC50) for a given set of water quality characteristics.

When run in the metal toxicity mode, the BLM will predict the LC50 of copper using an LA50 value from a parameter file specific to a particular species for all of the observations with a complete set of BLM input parameters. However, the BLM can also be run with “User Defined” LA50s. That is, the BLM will predict LC50s based on the LA50 values specified by the user rather than the default LA50 value specified in the parameter files for particular organisms. Instructions for constructing BLM input files and running the model can be found in the Biotic Ligand Model User’s Guide (Appendix B).

3.4 Data Acceptability and Screening Procedures

Data screening procedures for this effort differed from data screening procedures for previous copper criteria documents, in that studies previously considered unacceptable for deriving criteria are acceptable when utilizing the BLM. For example, studies with DOC content exceeding 5 mg/L or studies that were fed were not always acceptable in the past, but are now acceptable for use with the BLM, because the BLM is designed to account for these differences. Conversely, some previously acceptable freshwater acute toxicity tests were relegated to Appendix C (other data) because of poor chemical characterization, together with several other freshwater tests in which copper concentrations in the test chambers were not measured. Detailed chemical analyses of the dilution water, test water, and measured copper concentrations are critical parameters for the BLM (see Mechanistic Models section). The lack of any or all of these major ion concentrations, including measurements of total or dissolved copper, without reliable estimates of surrogate values, precludes the use of a particular study’s results (see next section, Estimation of Test Water Chemistry).

3.5 Estimation of Test Water Chemistry

To incorporate the BLM into the copper aquatic life criteria document, a data table was generated summarizing the acute toxicity of copper to freshwater organisms that included the necessary BLM water chemistry parameters. Studies lacking measured copper concentrations were not considered for further evaluation. A literature review was conducted, searching AQUIRE, BIOSYS, and CAS. The literature was reviewed, and the appropriate measurements were tabulated.

As the understanding by the scientific community of the important influence of water chemistry on metals toxicity has increased, measurements (and reporting) of relevant water quality parameters has also increased. Still, much of the currently available aquatic toxicity literature for metals does not include measurements for all of the key BLM inputs. Many of these key BLM inputs were not measured or reported in the published material reviewed for this update of the WQC. Consequently, additional data were obtained from the authors; additional measurements were made in relevant water sources; or, finally, input parameters were estimated. A detailed description of the methods used to obtain or estimate these input parameters is included in *Estimation of Water Chemistry Parameters for Acute Copper Toxicity Tests* (Appendix D). Below is a summary of the effort undertaken to estimate the various test water chemistry conditions.

3.6 Water Chemistry Data Acquisition

Studies included in Table 1a of the ambient water quality criteria document for copper were reviewed to record all reported information on dilution and test water chemistry. Any additional references to which the authors referred while describing their test waters were retrieved. When critical water chemistry parameters were not available, authors were asked to measure missing water chemistry parameters in the toxicity test source waters. If primary or corresponding authors could not be contacted, an attempt was made to contact secondary authors or personnel from the laboratories where the studies had been conducted. Failing this, the U.S. Geological Survey National Stream Quality Accounting Network (NASQAN) and the EPA STORage and RETrieval (STORET) data were used to obtain data for tests conducted in ambient surface water. Where actual water chemistry data were unavailable, data from other studies with the same water were used as surrogate values if appropriate. In some instances, other available sources were contacted to obtain water chemistry data (e.g., city drinking water treatment officials). The acquired data were scrutinized for representativeness and usefulness for estimating surrogate values to complete the water quality information for the dilution and/or test water that was used in the original studies. When the above sources could not be used geochemical ion input parameters were based on the reported hardness measurement and regression relationships constructed for various input parameters from NASQAN data.

As with any modeling effort, the reliability of model output depends on the reliability of model input. Although the input data have been carefully scrutinized and filtered, the reliability of the BLM-derived accumulation and toxicity values for this project are subject to the limitations of the input measurements and estimation procedures described above.

3.7 Ranking of Quality of Test Chemistry Characterization

A ranking system was devised to evaluate only the quality of the chemical characterization of the test water, not the overall quality of the study itself. Studies with a rank of 1 contain all of the necessary parameters for BLM input based on measurements from either the test chambers or the water source. In general, studies in which the BLM input parameters were reported for test chamber samples take precedence over studies in which the parameters were reported only for the source water. A

characterization ranking of 2 denotes those studies where not all parameters were measured, but reliable estimates of the requisite concentrations could be made. Similarly, a rank of 3 denotes studies in which all parameters except DOC were measured, but reliable estimates of DOC could be made. For the majority of the tests, a chemical characterization of 4+ was assigned because hardness, alkalinity, and pH were measured, and the ionic composition could be reliably estimated or calculated. A 4- was assigned to those studies conducted using standard reconstituted water in which hardness, alkalinity, or pH was either measured or referenced, and the recipe for the water is known (ASTM 2000; U.S. EPA 1993). The chemical characterization rank of 5 was ascribed to studies in which one of the key parameters (DOC, Ca, pH, alkalinity) was not measured, and when it could not be reliably estimated. If two or more key parameters (DOC, Ca, pH, alkalinity) were not measured and could not be reliably estimated, a study was given a chemical characterization rank of 6. Studies receiving a quality rating of greater than 4 were not used in the criteria development procedures because the estimates for some of the key input parameters were not thought to be reliable.

3.8 Criteria Computations

To calculate the acute criterion or CMC, reported acute toxicity values (e.g., LC50s) (Table 1a) and individual test water chemistry parameters were used to calculate LA50 values by running the model in the speciation mode. These LA50 values were then normalized to a standard water condition (Table 1a, footnote d) by running the model in the toxicity mode and specifying user-defined LA50s. As used here, “normalization” refers to the procedure whereby all of the measured effect levels were adjusted, via use of the BLM, to the predicted LC50 that would have been expected in a standard test water. These normalized LC50s were used to calculate Species Mean Acute Values (SMAVs), Genus Mean Acute Values (GMAVs), and a Final Acute Value (FAV) pursuant to the 1985 Guidelines procedure. The FAV represents a hypothetical genus more sensitive than 95 percent of the tested genera. The FAV was derived from the four GMAVs that have cumulative probabilities closest to the 5th percentile toxicity value for all the tested genera (Table 3a). Inputting this FAV as an LC50 concentration and running the model in speciation mode determines the lethal accumulation associated with the FAV in the standard test water. Since it is assumed that the LA50 does not vary with changes in water chemistry, this LA50 is programmed into the model as a constant. To derive a criterion for a specific site, the site water chemistry data are input to the model. The model then uses an iterative approach to determine the dissolved copper concentration needed to achieve a Cu-biotic ligand concentration equal to the criterion LA50. This dissolved Cu concentration is in effect the FAV based on site water chemistry. The site-specific CMC is this predicted dissolved metal concentration divided by two. The site-specific CCC is the CMC divided by the final acute-chronic ratio (FACR).

The LA50s used in criteria computations were calculated for each test in which water quality characteristics could be reasonably well characterized. Because an underlying premise of the BLM is that the LA50 is invariant for a given organism, for any test condition, the fact that some residual variability in LA50s exists may reflect model uncertainty, including: (1) among-strain variability; (2) among-life-stage variability; and (3) potential physiological effects of the site water on the test organism that alter organism sensitivity rather than metal bioavailability.

Ultimately, the final freshwater criteria depend on a number of varying water quality parameters (e.g., Ca⁺, Mg⁺, and DOC), and any number of test water chemistries could be used to normalize the Table 1a data. Table 1a data (LC50s and EC50s) are standardized to the water chemistry condition specified in footnote f, for illustrative purposes only as is typical in hardness-dependent metals criteria documents. Be that as it may, the normalization chemistry selected may influence the species sensitivity distribution, particularly when two or more species have similar sensitivities to copper toxicity. Example criteria for several water chemistry conditions are provided in Figure 6.

4.0 CONVERSION FACTORS

Although past water quality criteria for copper (and other metals) had been established upon total metals' concentrations, EPA made the decision to allow the expression of metals criteria on the basis of dissolved metal (operationally defined as metal that passes through a 0.45-micron filter, [U.S. EPA 1993]) because it was thought to better represent the bioavailable fraction of the metal. At that time, most data in existing databases were from tests that were either conducted using nominal concentrations, or provided only total copper measurements, such that some procedure was required to estimate their dissolved equivalents. Now, dissolved metals toxicity values are required as BLM input in order to obtain lethal accumulation values. EPA used conversion factors (CF) that when multiplied by the total metal concentrations result in a dissolved metal concentration. CF corresponds to the percentage of the total recoverable metal that is dissolved.

CFs for the conversion of total copper concentrations in water from freshwater toxicity tests to dissolved copper concentrations were developed by conducting a number of laboratory toxicity tests (Stephan 1995; University of Wisconsin-Superior 1995). Simulation tests were conducted to determine the influence of copper concentrations, presence or absence of food, duration of the test, hardness, and species of test organism on the concentration of dissolved copper in the test water. The simulation tests were designed to mimic conditions that existed during the toxicity tests used to derive the earlier metals criteria, such as sorption of metal onto test chambers, uptake of metal by test organisms, and precipitation. The recommended conversion factors from the Stephan (1995) report (0.96 for both the CMC and CCC) were utilized to convert total recoverable measurements to dissolved values, when necessary.

In the case of saltwater, several studies are available that report nominal, total, and dissolved concentrations of copper in laboratory water (Table 1b) from site-specific WER studies (refer to Appendix E for further details). These studies show relatively consistent ratios for the nominal-to-dissolved concentrations and for total-to-dissolved concentrations. The dissolved-to-nominal conversion requires a larger correction factor than does the dissolved-to-total correction. The data provided in Appendix E bear this out in all but one case (SAIC 1993 data for the blue mussel). Nominal copper concentrations for this series of tests may have been overstated or the measured total copper concentrations may have been proportionally lower than for the other studies. The overall ratio for correcting saltwater total copper concentrations to dissolved copper concentrations is 0.909, based on the results of six studies (Appendix E). This is comparable to its equivalent conversion factor in freshwater, which is 0.960 (Stephan 1995). When it is necessary to convert nominal saltwater copper concentrations to dissolved copper concentrations, the conversion factor is 0.838 based on the same six studies.

5.0 DATA SUMMARY AND CRITERIA CALCULATION

5.1 Summary of Acute Toxicity to Freshwater Animals and Criteria Calculation

This effort identified approximately 600 acute freshwater toxicity tests with aquatic organisms and copper considered acceptable for deriving criteria. Of these acceptable studies, approximately 100 were eliminated from the criteria derivation process because they did not report measured copper concentrations. Nearly 150 additional studies were eliminated from the calculation of the FAV because they received a quality rating of greater than 4 in the quality rating scheme described above.

The BLM version AP08-Build 2002-05-07 was used to calculate lethal accumulation values for each individual test result included in Table 1a by running the model in the metal speciation mode (see Appendix B, BLM User's Guide). Reported effect levels (i.e., LC50s or EC50s) and the chemistry characterization for each test were input parameters for the model (Appendix F). LC50s or EC50s

reported in terms of total recoverable metal were converted to dissolved concentrations as discussed above in the Conversion Factors section. Lethal accumulation values were then converted to toxicity values (e.g., LC50s) at standard water condition by running the model in the metal toxicity mode.

Data from approximately 350 test were used to derive normalized LC50 values, including 15 species of invertebrates, 22 species of fish, and 1 amphibian species (Table 1a). Large variations in toxicity values were observed for some species. Examination of the nature of these individual values showed that a majority of them corresponded to observations where key BLM parameters were missing and thus estimated (i.e., a quality ranking of 3 or 4 range is typical for these values), and for many species the variation in LC50 was seen to increase in observations with more missing BLM parameters (e.g., *D. magna*, Figure 3). The large variability in LC50 for some species, therefore, seems to be related to the use of estimated BLM parameters for some of the data. For other organisms (such as rainbow trout), significant variations in LC50s were likely due to the mixture of life-stages represented in the acute toxicity datasets. In general, an objective approach that could be used to automatically screen anomalous LC50 values was needed. For a given species with more than five test results, relatively extreme values

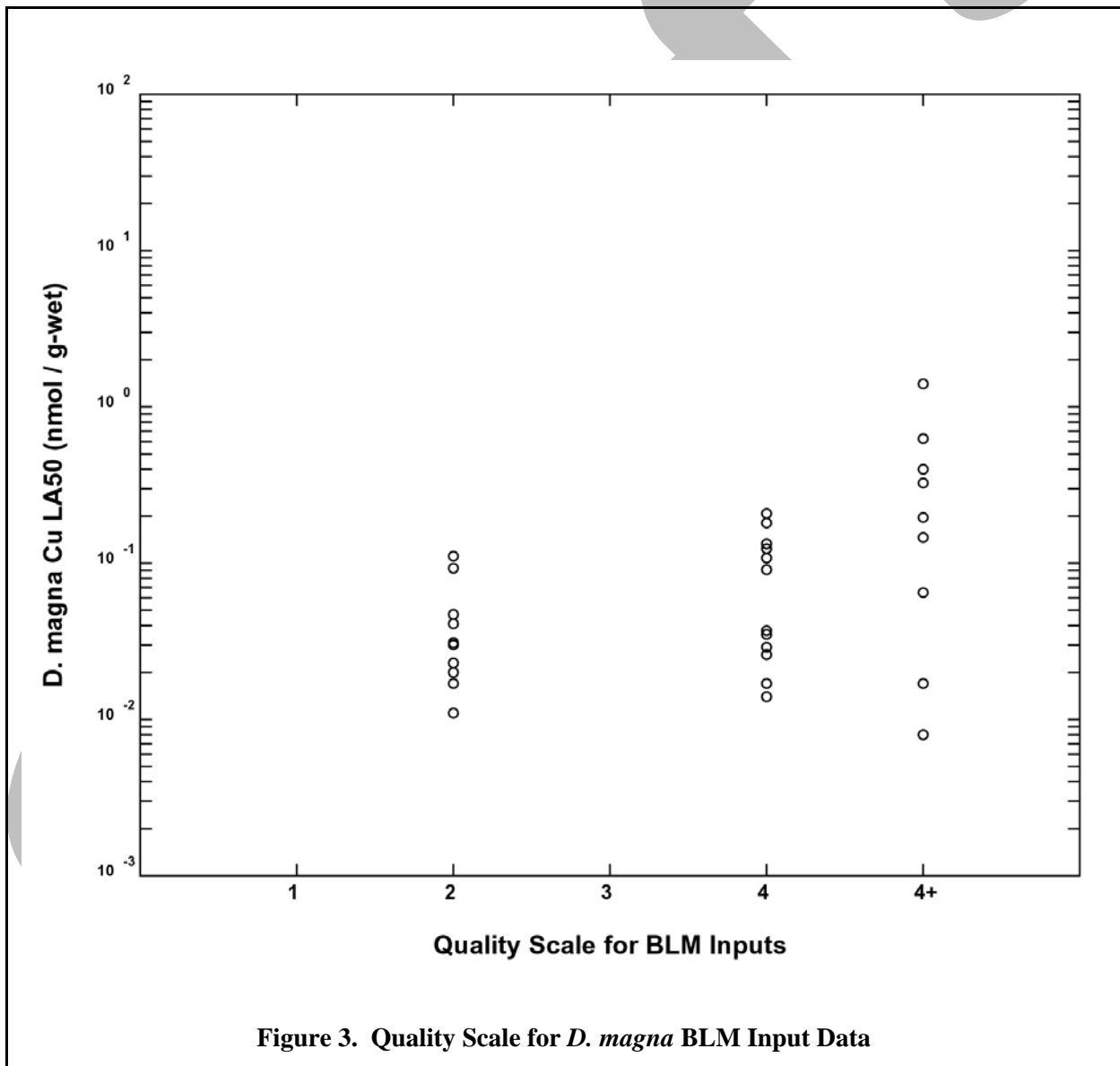


Figure 3. Quality Scale for *D. magna* BLM Input Data

were defined within the distribution of LC50 values using a simple statistical method that identifies those individual values that are far from most of the rest of the population of values (Chambers et al. 1983). To characterize these extreme values, a range was established by first calculating the difference between the 1st and 3rd quartiles for the entire dataset. This difference was then multiplied by 1.5 and either added to the 3rd quartile, or subtracted from the 1st quartile to establish the “inside range.” Any points falling outside this range were identified as extreme values. While data limitations preclude the application of a more formal evaluation of “statistical outliers,” this simplified procedure was considered to be a reasonable way to account for what appeared to be anomalous results.

As an example of this method applied to the LC50 data, box plots are shown of the range of LC50 values for each of the species in Table 1a. Species are identified with numbers, as shown in Table 6. For each species, the geometric mean is shown as the center symbol, the first set of ranges represent the 1st and 3rd quartile. The second set of ranges represent the minimum and maximum values excluding extreme values. Data corresponding to extreme values are individually plotted as separate plotting symbols (Figure 4). For the extreme values, the number of vertices in the plotting symbol represents the

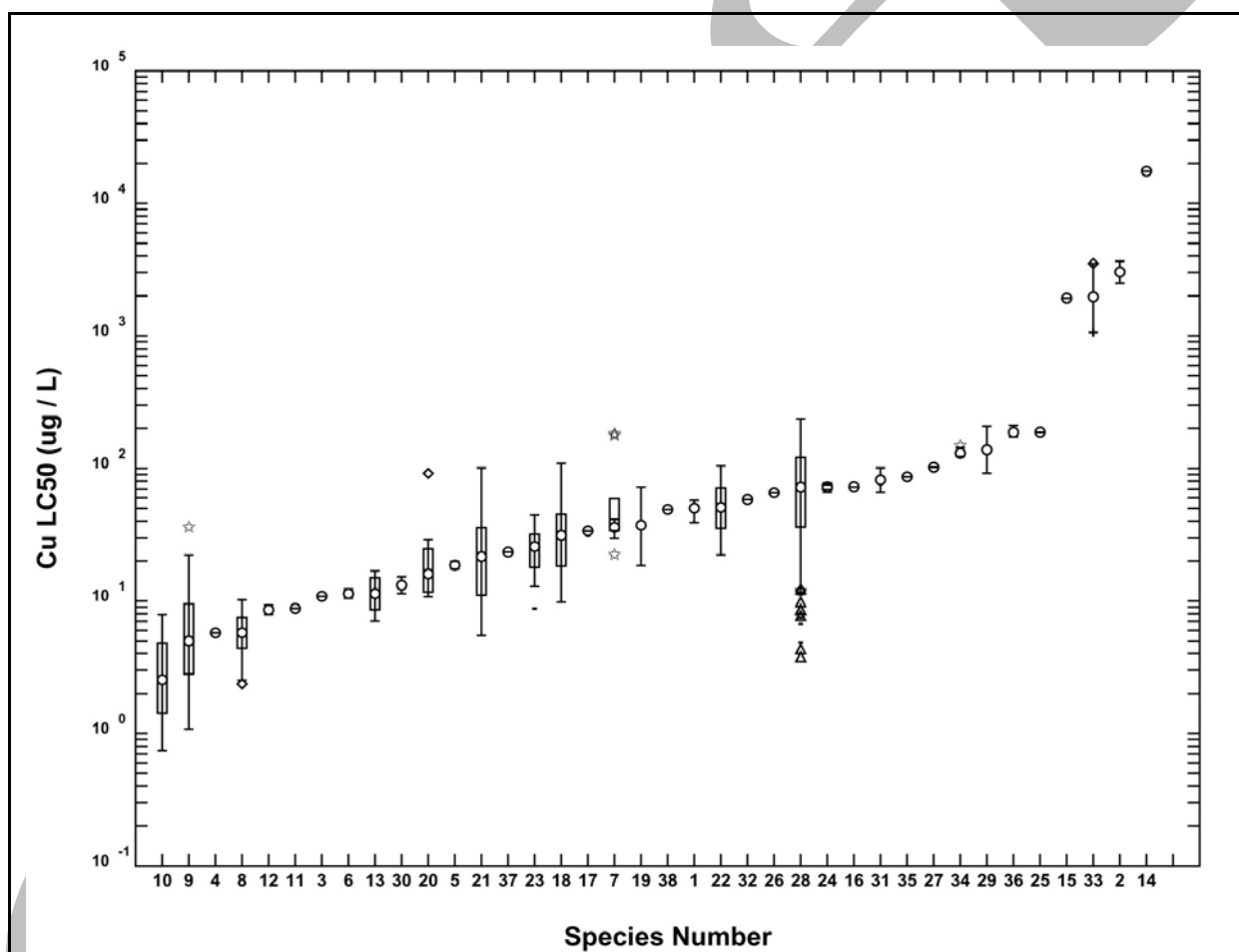


Figure 4. Ranges and Distribution of Normalized LC50 Values for Species Listed in Table 1

Species are identified by unique species number listed in Table 6. For each species the range between the 1st and 3rd quartile of all available normalized LC50 values is represented by the box. Extreme values are plotted as individual symbols, with the number of vertices indicating the quality scale (extreme values and quality scales are discussed in Section 5.1). Statistics shown for normalized LC50 values after excluding extreme values include the geometric mean shown as a circle, and minimum and maximum values shown as whisker bars around the mean.

quality ranking (e.g., a triangle represents an observation with a quality ranking of three, a diamond represents an observation with a quality ranking of 4+, a star represents a quality ranking of 4 or 4-). The LC50 values that corresponded to “extreme values” were therefore not considered in subsequent calculation of the 5th-percentile LC50 value.

SMAVs ranged from 2.54 µg/L for the most sensitive species, *Daphnia pulicaria*, to 101,999 µg/L for the least sensitive species, *Notemigonus crysoleucas*. Cladocerans were among the most sensitive species, with *D. pulicaria*, *D. magna*, *Ceriodaphnia dubia*, and *Scapholeberis sp.* being four out of the six most sensitive species. Invertebrates in general were more sensitive than fish, representing the 10 lowest SMAVs.

The 27 GMAVs calculated from the above-mentioned SMAVs ranged from 3.56 µg/L for *Daphnids* to 101,999 µg/L for the *Notemigonus* genus. Nine of the 10 most sensitive genera were invertebrates. The salmonid genus *Oncorhynchus* was the most sensitive fish genus, with a GMAV of 29.11 µg/L and an overall GMAV ranking of 10.

Toxicity values are available for more than one species in eight different taxonomic families. The ranked GMAVs are presented in Figure 5. Pursuant to procedures used to calculate a FAV, a FAV of 4.2 : g/L was derived from the four GMAVs with cumulative probabilities closest to the 5th percentile toxicity value for all the tested genera (Table 3c). The presumption is that this acute toxicity value represents the LC50 for an organism that is sensitive at the 5 percentile level of the GMAV distribution. The four lowest GMAVs vary by less than a factor of three from the highest to the lowest value. The CMC is the FAV divided by two, and rounded to two significant figures. Therefore, the freshwater dissolved copper CMC for the normalization chemistry presented is 2.1 µg/L.

Site-water chemistry parameters are needed to evaluate a criterion. This is analogous to the situation that previously existed for the hardness-based WQC, where a hardness concentration was necessary in order to derive a criterion. Examples of CMC calculations at various water chemistry conditions are presented in Figure 6.

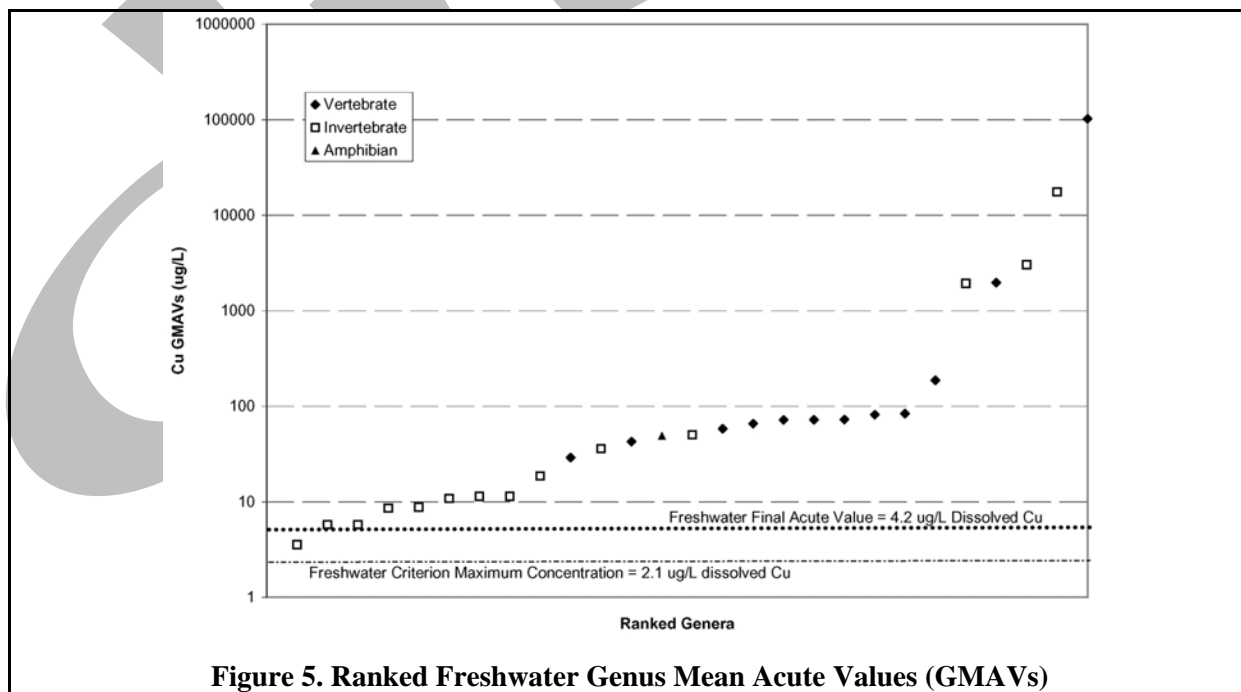
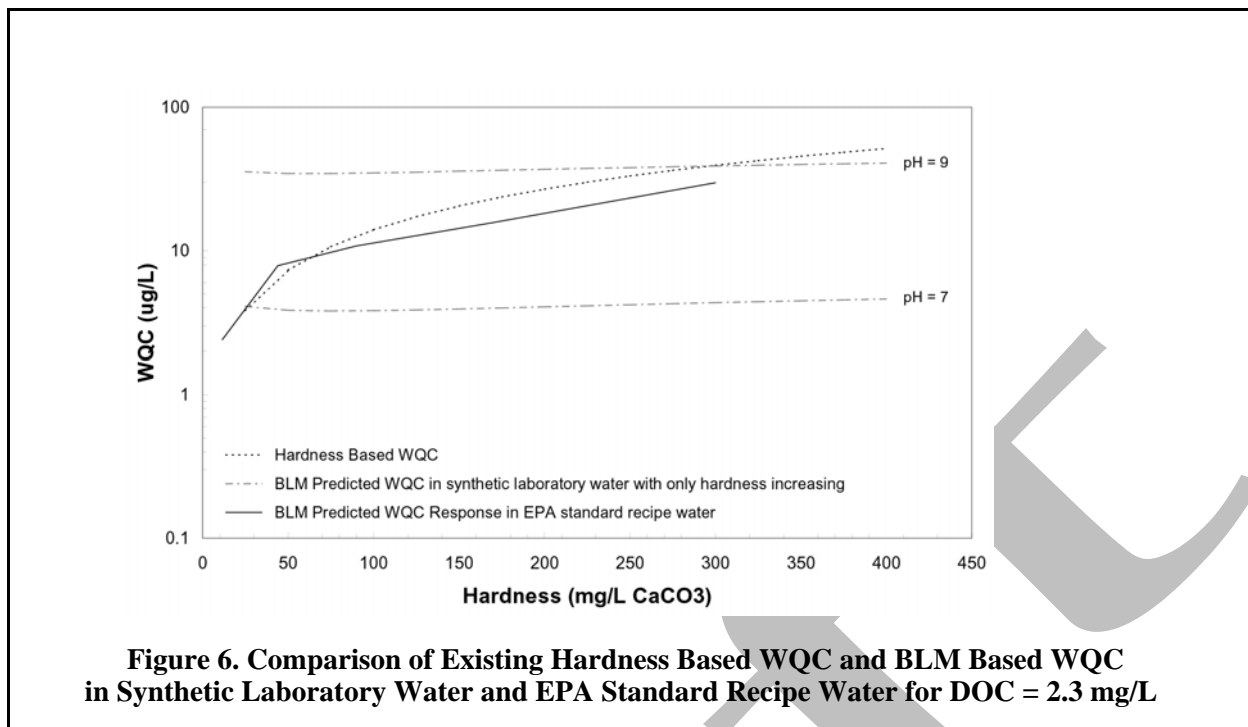


Figure 5. Ranked Freshwater Genus Mean Acute Values (GMAVs)



5.1.1 Comparison With Hardness-Adjusted Values

As discussed previously, EPA's earlier freshwater copper criteria recommendations were hardness-dependent values. One would expect a BLM-based criterion calculation procedure to yield the more appropriate criterion—appropriate in the sense that it accounts for the important water chemistry factors that affect toxicity, including DOC complexation, where the hardness correction does not. While in principle the BLM is expected to improve the criteria calculation method, the BLM's ability to accurately predict LC50s or metal speciation is limited by the quality of the input data. For this effort, many input parameters were estimated. To ascertain if the BLM-based criterion is an improvement over a hardness-dependent criterion in light of the necessity of estimating several of the required input parameters, the variations between measured versus predicted values for each of these approaches were compared.

For the first comparison, lethal accumulation values were calculated for each study result (uncensored data) utilizing the measured or estimated chemistry input parameters. Average accumulation values for each species were calculated and used to run the BLM with "User Defined" LA50s, specifying the species average accumulation value for all study results for that species and the original input chemistry parameters. The predicted LC50 values at each chemistry condition were compared with the originally measured values by regressing the natural logarithm of the predicted toxicity value versus the natural logarithm of the measured toxicity value.

A similar procedure was performed for the hardness adjustment. A pooled hardness slope was calculated using all appropriate Table 1a data (considering all quality ratings) based on the 1985 Guideline procedure (Appendix G). This pooled slope was used to normalize all Table 1a data used for the BLM analysis to a standard hardness of 50 mg/L (measured as CaCO_3). Species mean acute values were calculated and used to predict LC50s for each test result, for that same species, at the test hardness. Again, the natural logarithms of the measured versus hardness predicted values were regressed.

The mean square of error (MSE) from these two least squares regression procedures were compared. The MSE from the BLM measured versus predicted analysis (0.403) was only slightly lower than the MSE from the comparable hardness analysis (0.420). The small reduction in the MSE for the BLM analysis is interpreted to mean that the BLM, in this case, was a slightly better predictor of LC50 values and somewhat better at reducing variability among species mean values compared with the hardness adjustment for these laboratory water studies. Application of the BLM in field situations where DOC is expected to be present at higher concentrations than those observed in laboratory studies would likely improve the performance of the BLM compared with the hardness adjustment. The reason is that the BLM would reasonably account for the typically observed increase in effect levels under such conditions, while the hardness-based approach would not.

As a comparison between the hardness typical of the previous copper criterion and this revised criterion using the BLM, both procedures were used to calculate criterion values for waters with a range in hardness as specified by the standard EPA recipes (U.S. EPA 1993). The EPA recipes specify the concentration of various salts and reagents to be used in the synthesis of laboratory test waters with specific hardness values (e.g., very soft, soft, moderately hard, hard, or very hard). As the water hardness increases in these recipes, pH and alkalinity also increase. This has implications for the BLM because the bioavailability of copper would be expected to decrease with increasing pH and alkalinity due to the increasing degree of complexation of copper with hydroxides and carbonates and decreasing proton competition with the metal at both DOM and biotic ligand binding sites. The BLM was used to predict the WQC with a DOC concentration of 2.3 mg/L (the average value in the data used in Table 1) for the five standard hardness waters. The BLM criterion for these waters agrees very well with that calculated by the hardness equation used in previous copper criterion documents (Figure 6). However, alkalinity and pH change as hardness changes in the EPA recipes. The BLM prediction is taking all of these changes in water quality into account. It is possible to use the BLM to look only at the change in predicted WQC with changes in hardness (e.g., alkalinity and pH remaining constant). Also shown in Figure 6 are BLM predictions with only hardness varying. As can be seen, these predictions show a much flatter response with increasing hardness, and do not match the response seen in the hardness equation at all. The hardness equation, therefore, is based on waters where changes in hardness are accompanied by changes in pH and alkalinity. However, there are many possible natural waters where changes in hardness are not accompanied by changes in pH and alkalinity (such as water draining a region rich in gypsum). In these cases, the hardness equation based criterion will still assume a response that is characteristic of waters where hardness, alkalinity, and pH co-vary, and will likely be underprotective relative to the level of protection intended by the Guidelines, in high hardness waters. Conversely, in waters where the covariation between hardness, pH, and alkalinity is greater than is typical for data in Table 1, the hardness equation based criteria may be overprotective.

5.2 Summary of Acute Toxicity to Saltwater Animals and Criteria Calculation

Tests of the acute toxicity of copper to saltwater organisms (acceptable for deriving criteria) have been conducted with 34 species of invertebrates and 18 species of fish (Table 1b). In general, where relationships were apparent between life stage and sensitivity, values only for the most sensitive life stage were considered in deriving SMAVs. The censoring procedure used for the freshwater toxicity values was also considered for use in censoring saltwater acute toxicity values. However, it was not applied. The freshwater censoring procedure was not used because, in one case, it resulted in eliminating only data for the most sensitive life-stage, rather than the insensitive life-stage. In situations where data indicate that a particular life-stage for the species is at least a factor or two more resistant than another, the Guidelines recommend that the data for the more resistant life-stage not be used in the calculation of the SMAV.

Embryo-larval life-stages of bivalve mollusc genera represent the first two of the four most sensitive genera, including, by sensitivity rank, the genera *Mytilus*-11.5 µg/L and *Crassostrea*-12.6 µg/L. Toxicity data for *Mytilus edulis* were distinguished from data for *Mytilus spp.* based on the molecular

genetics work presented by Gaffney (1997) and information about the collection locations of the test organisms for the *Mytilus* studies. The fourth most sensitive genera (the sea urchin genus *Strongylocentrotus*) is also represented by the embryo-larval life-stage (Table 1b). Comparing the data for older mussels (Nelson et al. 1988) and oysters (Okazaki 1976) with data for embryo-larval forms indicates that these early life stages (ELs) are appreciably more sensitive than the older forms. This is probably true for marine invertebrates in general, although data for the red abalone (Martin et al. 1977) indicate that 48-hour larvae are perhaps slightly more resistant than larger forms. The mysid, *Holmesimysis costata*, and the copepods, *Eurytemora affinis* and *Acartia tonsa*, are among the most sensitive crustacean species tested.

Except for the summer flounder and the cabezon, with GMAVs of 12.7 and 86.4 µg/L, respectively, no other saltwater fish had a GMAV below 100 µg/L. Fourteen other genera of marine fish had GMAVs from 117 to 4,743 µg/L dissolved copper. Two of the lowest fish GMAVs were based on tests with early life stages, and the higher fish GMAVs did not include tests with early life stages. These results suggest that acute tests with early (post-hatch) life stages can generally be protective of acute toxicity to older life stages, but not necessarily the reverse.

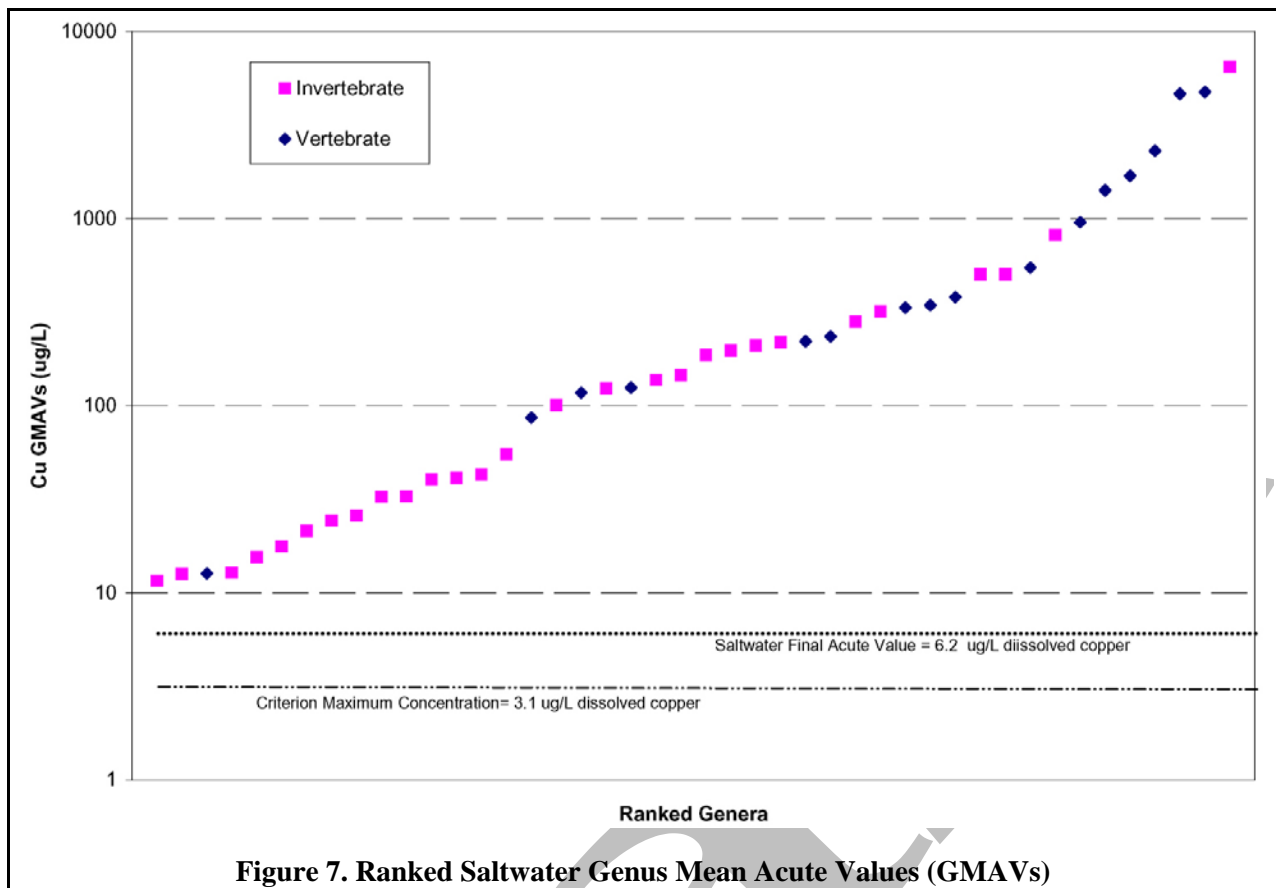
In sum, several studies indicate that salinity affects copper toxicity and those effects are species-dependent. The brackish water clam, *Rangia cuneata*, was very sensitive to copper in freshwater (LC50 210 µg/L at <1 g/kg salinity), but 35 to 38 times more resistant at salinities of 5.5 and 22 g/kg (Olson and Harrel 1973). Similarly, young striped bass were about three times more sensitive to copper at a salinity of 5 g/kg than at 10 or 15 g/kg (Reardon and Harrel 1990). An influence of salinity was observed by Ozoh (1992a) in the previously cited study of the influence of temperature and salinity on copper toxicity to the polychaete worm, *Hediste diversicolor*. Effects of salinity were more consistent than those for temperature. A regression of log LC50 versus log salinity indicated a slope of 0.245 for young worms, and a slope of 0.596 for mature worms. Increasing salinity over the range tested (7–30 g/kg) increased LC50s by factors of approximately 1.4 and 2.4 for young worms and mature worms, respectively. Establishing salinity-dependent criteria on the basis of these limited data is not possible. Furthermore, salinity-based criteria should be based only on tests with organisms and life stages that would be present at lower salinities.

Acute values are available for more than one species in the eight different taxonomic families recommended in the Guidelines. The 44 available saltwater GMAVs ranged from 11.5 µg/L dissolved copper for *Mytilus* to 6,448 µg/L for *Rangia*, a factor of over 500 difference (Table 3b, Figure 7). In each of six genera with a range of SMAVs, all SMAVs within the genus are within a factor of 3.5. A saltwater FAV of 12.3 µg/L dissolved copper was obtained using the four lowest GMAVs in Table 3b and the calculation procedure described in the Guidelines. This FAV was lowered to 6.19 µg/L to protect commercially and recreationally important mussel species. The CMC is the FAV divided by two, and rounded to two significant figures. Therefore, the new saltwater dissolved copper CMC is 3.1 µg/L.

5.3 Formulation of the CCC

5.3.1 Statistical Evaluation of Chronic Toxicity Data

In aquatic toxicity tests, chronic values are usually defined as the geometric mean of the highest concentration of a toxic substance at which no adverse effect is observed (highest no observed adverse effect concentration, or NOAEC) and the lowest concentration of the toxic substance that causes an adverse effect (lowest observed adverse effect concentration, or LOAEC). The significance of the observed effects is determined by statistical tests comparing responses of organisms exposed to low-level (control) concentrations of the toxic substance against responses of organisms exposed to elevated concentrations. Analysis of variance is the most common test employed for such comparisons. This



approach, however, has limitations; it has the disadvantage of resulting in marked differences between the magnitudes of the effects corresponding to the individual chronic values, because of variation in the power of the statistical tests used, the concentrations tested, and the size and variability of the samples used (Stephan and Rogers 1985).

An alternative approach to calculate chronic values focuses on the use of point estimates such as regression analysis to define the dose-response relationship. With a regression equation or probit analysis, which defines the level of adverse effects as a function of increasing concentrations of the toxic substance, it is possible to determine the concentration that causes a relatively small effect, for example a 5 to 30 percent reduction in response. To make chronic values reflect a uniform level of effect, regression and probit analyses were used, where possible, both to demonstrate that a significant concentration-effect relationship was present and to estimate chronic values with a consistent level of effect. The most precise estimates of effect concentrations can generally be made for 50 percent reduction (EC50); however, such a major reduction is not necessarily consistent with criteria providing adequate protection. In contrast, a concentration that causes a low level of reduction, such as an EC5 or EC10, is rarely statistically significantly different from the control treatment. As a compromise, the EC20 is used here to represent a low level of effect that is generally significantly different from the control treatment across the useful chronic datasets that are available for copper.

Regression or probit analysis was utilized to evaluate a chronic dataset only in cases where the necessary data were available and the dataset met the following conditions: (1) it contained a control treatment (or low exposure data point) to anchor the curve at the low end, (2) it contained at least three concentrations, and (3) two of the data points had effect variable values below the control and above zero (i.e., "partial effects"). Control concentrations of copper were estimated in cases where no measurements were reported. These analyses were performed using the Toxicity Relationship Analysis Program software

(version 1.0; U.S. EPA). Additional detail regarding the aforementioned statistical procedures is available in the cited program.

When the data from an acceptable chronic test met the conditions for the logistic regression or probit analysis, the EC20 was the preferred chronic value. When data did not meet the conditions, was not available, or did not lend itself to regression analysis, best scientific judgment was used to determine the chronic value. In this case, the chronic value is usually the geometric mean of the NOAEC and the LOAEC. But when no treatment concentration was an NOAEC, the chronic value was less than the lowest tested concentration.

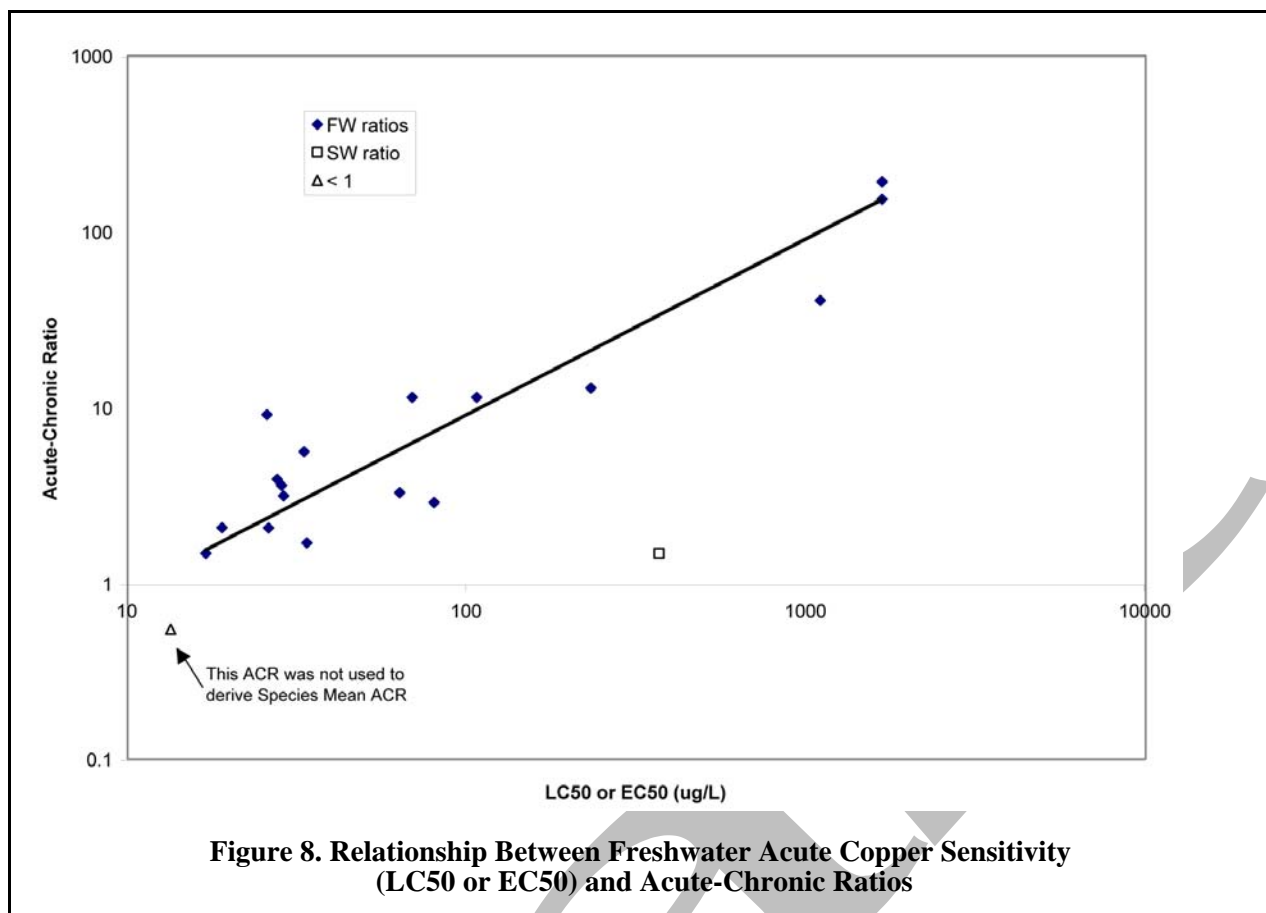
For life-cycle, partial life-cycle, and early life stage tests, the toxicological variable used in chronic value analyses was survival, reproduction, growth, emergence, or intrinsic growth rate. If copper apparently reduced both survival and growth (weight or length), the product of variables (biomass) was analyzed, rather than analyzing the variables separately. The most sensitive of the toxicological variables was selected, for the most part, as the chronic value for the particular study.

A species-by-species discussion of each acceptable chronic test on copper evaluated for this document is presented in Appendix H. Figures that presents the data and regression/probability distribution line for each of the acceptable chronic test which contained sufficient acceptable data are also provided in Appendix H.

5.3.2 Calculation of Freshwater CCC

Acceptable freshwater chronic toxicity data from early life stage tests, partial life-cycle tests, and full life-cycle tests are currently available for 29 tests including data for 6 invertebrate species and 10 fish species (Table 2a). The 17 chronic values for invertebrate species range from 2.83 (*D. pulex*) to 34.6 µg/L (*C. dubia*); and the 12 chronic values for the fish species range from <5 (brook trout) to 60.4 µg/L (northern pike). Of the 29 chronic tests, comparable acute values are available for 17 of the tests (Table 2c). The relationship between acute toxicity values and ACRs is presented in Figure 8. The supporting acute and chronic test values for the ACRs and the species mean ACRs are presented in Table 3c.

The general effect of hardness on chronic toxicity is not evident upon inspection of the limited hardness-chronic toxicity data for the species for which such evaluations are marginally possible. Five tests over a range of hardness values were conducted with *D. magna* (Blaylock et al. 1985; Chapman et al. unpublished manuscript; van Leeuwen et al. 1988). Five tests over a range of hardness values were also conducted with *C. dubia* (Belanger et al. 1989; Carlson et al. 1986; Oris et al. 1991). Winner (1985) conducted eight tests with *D. pulex* over a range of hardness values, but humic acid was also varied in these tests. In the *D. magna* tests, chronic values increased when hardness increased from about 50 to about 100 mg/L; however, in one of the tests, the chronic value decreased when hardness was further raised to about 200 mg/L. In a second test conducted at a hardness of 225 mg/L, the chronic value was not much higher than those in the 100 mg/L hardness tests. The resulting overall slope for *D. magna* based on these data is negative. The *C. dubia* test exhibited no discernible trends between hardness and toxicity. One possibility is that daphnids may be ingesting precipitated copper that might form at high hardness and high pH. Alternatively, Winner et al. (1985) suggest that Ca^{2+} and Mg^{2+} ions in hard water may be displacing Cu^{2+} from binding sites on humic acid, making more copper bioavailable. Because the hardness relationship with chronic toxicity is equivocal, no overall chronic slope was derived.



Because the minimum eight family data requirements for chronic toxicity data were not met in order to use the FAV approach and because the relationship between hardness and chronic toxicity is equivocal, EPA elected to derive the CCC utilizing the ACR approach from the Guidelines. Moreover, this was a means of incorporating the improvements of the acute BLM calculations into the chronic criterion derivation procedures even though, as previously mentioned, additional development is required before the BLM will be suitable for use in evaluating chronic toxicity data directly. To calculate the FCV, the FAV is divided by the FACR; thus, no chronic hardness slope is necessary to derive a CCC.

The freshwater FCV is derived using acute chronic ratios in conjunction with the FAV. However, the FAV is site-water specific. To derive a FCV, the BLM is run in the toxicity mode, which utilizes the accumulation value constant incorporated in the model to calculate an LC50 based on the site water chemistry composition. This LC50 is then divided by the freshwater FACR to generate an FCV, which is the basis for the CCC.

Overall, individual ACRs varied from <1 (0.55) for *C. dubia* (Oris et al. 1991) to 191.6 for the snail, *Campeloma decisum* (Arthur and Leonard 1970). Species mean acute-chronic ratios ranged from 1.48 in saltwater for the sheepshead minnow (Hughes et al. 1989) to 171.2 in freshwater for the snail, *C. decisum*. The FACR of 3.23 was calculated as the geometric mean of the ACRs for sensitive freshwater species, *C. dubia*, *D. magna*, *D. pulex*, *O. tshawytscha*, and *O. mykiss* along with the one saltwater ACR for *C. variegatus*. Pursuant to the Guidelines, consideration was given to calculating the FACR based on all ACRs within a factor of 10, but because there appeared to be a relationship between acutely sensitive species and increases in ACRs as sensitivity decreased, the FACR was derived from data for species whose SMAVs were close to the FAV. Based on the normalization water chemistry conditions used for

illustrative purposes in the document, the freshwater CMC value is 4.2, which divided by the FACR of 3.23 results in a freshwater CCC of 1.3 µg/L dissolved Cu.

5.3.3 Evaluation of the Chronic Data Available for Saltwater Species

Only one acceptable saltwater chronic copper value is available for the sheepshead minnow (Table 2b). This chronic toxicity value was obtained from a flow-through early life stage test in which the concentrations of copper in the test chamber were measured.

The ELS test with sheepshead minnow was one of the tests for which the chronic value and most sensitive effect are reported without providing concentration-response data. Thus, regression analysis was not an option for statistical evaluation of the data in this case. In the 28-day ELS test, growth was reported to be a more sensitive endpoint than mortality, and the chronic value for growth was 249 µg/L. The 96-hour LC50 reported for copper in this study was 368 µg/L, and the two values provide an acute-chronic ratio of 1.48.

A life-cycle test was conducted with the mysid, *Americamysis bahia* (formerly *Mysidopsis bahia*). Survival of mysids was reduced at 140 µg/L, and production of young virtually ceased at 77 µg/L (significant at $P < 0.05$), but reproduction at 24 and 38 µg/L was not different from that of controls. Based on reproductive data, unacceptable effects were observed at 77 µg/L, but not at 38 µg/L, resulting in a chronic value of 54.09 µg/L. Using the acute value of 181 µg/L, an ACR for this mysid would be 3.346. Control survival in this test however, was considered inadequate; thus, the chronic value was not used to derive the final chronic criterion.

The ACR value for saltwater is for a relatively acutely insensitive saltwater species, with a GMAV falling in the upper half of all tested saltwater genera. The lowest saltwater acute values are from tests with embryos and larvae of molluscs and embryos of summer flounder, which are possibly the most sensitive life stages of these species. Although saltwater ACRs for acutely sensitive saltwater species are not available, ACRs for acutely sensitive freshwater species are available. Some of the most acutely sensitive freshwater species for which ACRs are available are cladocerans *C. dubia*, *D. magna*, and *D. pulex*. (Data for *D. pulex* are not listed in Table 1a because of the ranking based on the chemical characterization of the test water for the BLM. *D. pulex* would be among the most acutely sensitive species if a hardness adjustment were utilized instead of the BLM.) On the basis of data for the five sensitive freshwater species along with the one available saltwater ACR for the sheepshead minnow, the saltwater FACR is the same as the freshwater ACR of 3.23. Thus, for saltwater, the final chronic value for copper is equal to the FAV of 6.188 µg/L divided by the ACR of 3.23, or 1.9 µg/L (Table 3c).

6.0 PLANT DATA

Copper has been widely used as an algicide and herbicide for nuisance aquatic plants (McKnight et al. 1983). Although copper is known as an inhibitor of photosynthesis and plant growth, toxicity data on individual species suitable for deriving aquatic life criteria (Table 4a, b) are not numerous.

The relationship of copper toxicity to the complexing capacity of the water or the culture medium is now widely recognized (Gächter et al. 1973; Petersen 1982), and several studies have used algae to “assay” the copper complexing capacity of both fresh and salt waters (Allen et al. 1983; Lumsden and Florence 1983; Rueter 1983). It has also been shown that algae are capable of excreting complexing substances in response to copper stress (McKnight and Morel 1979; Swallow et al. 1978; van den Berg et al. 1979). Foster (1982) and Stokes and Hutchinson (1976) have identified resistant strains and/or species of algae from copper (or other metal) impacted environments. A portion of this resistance probably results from induction of the chelate-excretion mechanism. Chelate excretion by algae may also serve as a

protective mechanism for other aquatic organisms in eutrophic waters; that is, where algae are capable of maintaining free copper activities below harmful concentrations.

Copper concentrations from 1 to 8,000 µg/L have been shown to inhibit growth of various freshwater plant species. Very few of these tests, though, were accompanied by analysis of actual copper exposure concentrations. Notable exceptions are freshwater tests with green alga, including *Chlamydomonas reinhardtii* (Schafer et al. 1993; Winner and Owen 1991b), which is the only flow-through, measured test with an aquatic plant, *Chlorella vulgaris* and *Selenastrum capricornutum* (Blaylock et al. 1985). There is also a measured test with duckweed (Taraldsen and Norberg-King 1990).

A direct comparison between the freshwater plant data and the BLM derived criteria is difficult to make without a better understanding of the composition of the algal media used for different studies (e.g., DOC, hardness, and pH) because these factors influence the applicable criteria comparison. BLM derived criteria for certain water conditions, such as low to mid-range pH, hardness up to 100 mg/L as CaCO₃, and low DOC are in the range of, if not lower than, the lowest reported toxic endpoints for freshwater algal species and would therefore appear protective of plant species. In other water quality conditions BLM-derived criteria may be significantly higher (see Figure 6).

Data are available on the toxicity of copper in saltwater to several species of macroalgae and microalgae (Table 4b). A comparison of effect levels seen in tests with saltwater plants and the CMC and CCC established to protect saltwater animals indicates that only one test result falls slightly below the CCC. One static unmeasured test, with the microalgae *Scrippsiella faeroense*, provides an 8-day growth EC50 of <1 µg/L (Saifullah 1978). However, this result failed to include a reported background copper concentration of 1.86-4.18 µg/L, placing this response in the range of <2.86-5.18. In addition, the study included a second experiment with the same species and an 8-day growth EC50 of 5 µg/L; adding in the reported background range brings this EC50 to 6.86-9.18 µg/L. Thus, the animal CCC appears adequate for protecting against chronic seawater plant effects observed in tests included in Table 4b.

Two publications provide data for the red algae *Champia parvula* that indicate that reproduction of this species is especially sensitive to copper. The methods manual (U.S. EPA 1988) for whole effluent toxicity (WET) testing contains the results of six experiments showing nominal reproduction LOECs from 48-hr exposures to 1.0 to 2.5 µg/L copper (mean 2.0 µg/L); these tests used a mixture of 50 percent sterile seawater and 50 percent GP2 medium copper. The second study by Morrison et al. (1989) evaluated interlaboratory variation of the 48-hr WET test procedure; this six-test study gave growth EC50 values from 0.8 to 1.9 µg/L (mean 1.0 µg/L). Thus, there are actually 12 tests that provide evidence of significant reproductive impairment in *C. parvula* at nominal copper concentrations between 0.8 and 2.5 µg/L, which is in the range of the saltwater CCC. For these studies though, the dilution water source was not identified.

One difficulty in assessing these data is the uncertainty of the copper concentration in the test solutions, primarily with respect to any background copper that might be found in the dilution water, especially with solutions compounded from sea salts or reagents. Thus, with a CCC of 1.9 µg/L dissolved copper, the significance of a 1 or 2 µg/L background copper level to a 1 to 3 µg/L nominal effect level can be considerable.

The reproduction of other macroalgae appears to be generally sensitive to copper, but not to the extent of *Champia*. Many of these other macroalgae appear to have greater ecological significance than *Champia*, several forming significant intertidal and subtidal habitats for other saltwater organisms, as well as being a major food source for grazers. Reproductive and growth effects on the other species of macroalgae sometimes appear to occur at copper concentrations between 5 and 10 µg/L (Appendix C, Other Data). Thus, most major macrophyte groups seem to be adequately protected by the CMC and CCC, but appear similar in sensitivity to some of the more sensitive groups of saltwater animals.

7.0 BIOACCUMULATION OF COPPER

Because no regulatory action levels for copper and human health are applicable to aquatic organisms, and no consumption limits are established for wildlife, there is no basis for developing a residue-based criterion (or final residue value) for copper based on EPA's current Guidelines.

As more information is acquired about food consumption as a route of copper exposure to fish and macroinvertebrates, bioaccumulation potential—and the link to environmental source concentrations—may become a considerably more important factor in establishing criteria. Currently, the database available for calculating potential bioconcentration (from the water) or bioaccumulation (from all sources) is limited. This is especially true given the current Guidelines requirement for deriving BCFs that all water concentrations be adequately quantitated, and that tissue levels be approaching steady state or else that tests be at least 28 days in duration. Additionally, bioconcentration factors for copper usually are not constant; instead, they generally decrease as aqueous copper concentrations increase (McGeer et al. 2003).

After culling the data according to the Guidelines, the only acceptable bioaccumulation factors for copper (Table 5a, b) were juvenile fathead minnows (464), Asiatic clams (45,300), polychaete worms (1,006–2,950), mussels (2,491–7,730), and Pacific oysters (33,400–57,000).

8.0 OTHER DATA

Many of the data identified for this effort are listed in Appendix C, Other Data, for various reasons, including exposure durations other than 96 hours with the same species reported in Tables 1a and 1b, with some exposures lasting up to 30 days. Acute values for test durations less than 96 hours are available for several species not shown in Tables 1a and 1b. Still, these species have approximately the same sensitivities to copper as species in the same families listed in Tables 1a and 1b. Reported LC50s at 200 hours for chinook salmon and rainbow trout (Chapman 1978) differ only slightly from 96-hour LC50s reported for these same species in the same water.

A number of other acute tests in Appendix C were conducted in dilution waters that were not considered appropriate for criteria development. Brungs et al. (1976) and Geckler et al. (1976) conducted tests with many species in stream water that contained a large amount of effluent from a sewage treatment plant. Wallen et al. (1957) tested mosquitofish in a turbid pond water. Until chemical measurements that correlate well with the toxicity of copper in a wide variety of waters are identified and widely used, results of tests in unusual dilution waters, such as those in Appendix C, will not be very useful for deriving water quality criteria.

Appendix C also includes tests based on physiological effects, such as changes in growth, appetite, blood parameters, stamina, etc. These were included in Appendix C because they could not be directly interpreted for derivation of criteria.

A direct comparison of a particular test result to a BLM-derived criterion is not always straightforward, particularly if complete chemical characterization of the test water is not available. Such is the case for a number of studies included in Appendix C. While there are some test results with effect concentrations below the example criteria concentrations presented in this document, these same effect concentrations could be above criteria derived for other normalization chemistries, raising the question as to what is the appropriate comparison to make. For example, Appendix C includes an EC50 for *D. Pulex* of 3.6 µg/L (Koivisto et al. 1992) at an approximate hardness of 25 mg/L (33 mg/L as CaCO₃). Yet, example criteria at a hardness of 25 mg/L (as CaCO₃) (including those in Figure 6) range from 0.23 µg/L (DOC = 0.1 mg/L) to 4.09 µg/L (DOC = 2.3 mg/L) based on the DOC concentration selected for the

synthetic water recipe. The chemical composition for the Koivisto et al. (1992) study would dictate what the appropriate BLM criteria comparison should be.

Based on the expectation that many of the test results presented in Appendix C were conducted in laboratory dilution water with low levels of DOC, the appropriate comparison would be to the criteria derived from low DOC waters. Comparing many of the values in Appendix C to the example criteria presented in this document, it appears that a large proportion of Appendix C values are above these concentration levels. This is a broad generalization though and as stated previously, all important water chemistry variables that affect toxicity of copper to aquatic organisms should be considered before making these types of comparisons.

Studies not considered suitable for criteria development were placed in Appendix I, Unused Data.

9.0 NATIONAL CRITERIA STATEMENT

The procedures described in the “Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses” indicate that, except where a locally important species is very sensitive, freshwater aquatic organisms and their uses should not be affected unacceptably if the 4-day average concentration of dissolved copper does not exceed the BLM-derived site-water LC50 (i.e., FAV) divided by the FACR more than once every 3 years on the average (i.e., the CCC) and if the 24-hour average dissolved copper concentration does not exceed the BLM-derived site-LC50 (or FAV) divided by two, more than once every 3 years on the average (i.e., the CMC).

The procedures described in the “Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses” indicate that, except where a locally important species is very sensitive, saltwater aquatic organisms and their uses should not be affected unacceptably if the 4-day average concentration of dissolved copper does not exceed 1.9 µg/L more than once every 3 years on the average and if the 24-hour average concentration does not exceed 3.1 µg/L more than once every 3 years on the average.

A return interval of 3 years continues to be EPA’s general recommendation. However, the resilience of ecosystems and their ability to recover differ greatly. Therefore, a site-specific return interval for the criteria may be established if adequate justification is provided.

10.0 IMPLEMENTATION

The use of criteria in designing waste treatment facilities requires selection of an appropriate wasteload allocation model. Dynamic models are preferred for application of these criteria. Limited data or other factors may make their use impractical, in which case one should rely on a steady-state model. EPA recommends the interim use of 1Q5 or 1Q10 for criterion maximum concentration design flow and 7Q5 or 7Q10 for the criterion continuous concentration design flow in steady-state models for unstressed and stressed systems, respectively. These matters are discussed in more detail in the Technical Support Document for Water Quality-Based Toxics Control (U.S. EPA 1991).

With regard to BLM-derived freshwater criteria, to develop a site-specific criterion for a stream reach, one is faced with determining what single criterion is appropriate even though a BLM-calculated “instantaneous criterion” (i.e., a criterion value appropriate for specific water chemistry conditions at a particular instant) will be time-variable. This is not a new problem unique to the BLM—hardness-dependent metals criteria are also time-variable values. Although the variability of hardness over time can be characterized, EPA has not provided guidance on how to calculate site-specific criteria considering this variability. Multiple input parameters for the BLM complicate the calculation of site-specific criteria because of their combined effects on variability. EPA is currently in the process of developing guidance

on how to address these factors. Presently, EPA expects that few sites have sufficient data for all the input parameters to enable adequate characterization of the inherent variation at a site. Therefore, EPA is currently evaluating probabilistic techniques (Monte Carlo techniques) and statistical analyses to address this issue and anticipates publishing separate BLM implementation guidance.

DRAFT

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Appendices

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Appendix A. Ranges in Calibration and Application Data Sets

Appendix B. Biotic Ligand Model (BLM) User's Guide

**Appendix C. Other Data on Effects of Copper on
Freshwater and Saltwater Organisms**

**Appendix D. Estimation of Water Chemistry Parameters for
Acute Copper Toxicity Tests**

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Appendix E. Saltwater Conversion Factors for Dissolved Values

Appendix F. BLM Input Data and Notes

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Appendix G. Hardness Slopes

Appendix H. Regression Plots

Appendix I. Unused Data