

NEXUS BETWEEN GEOCHEMISTRY AND AQUATIC TOXICOLOGY IN DEVELOPMENT OF SITE-SPECIFIC COPPER CRITERIA ACROSS MULTIPLE DRAINAGES IN AN ARID LANDSCAPE

Barry A. Fulton, Joseph S. Meyer¹

¹ARCADIS U.S., Inc., 1687 Cole Blvd., Suite 200, Lakewood, Colorado 80401, USA
(Joseph.Meyer@arcadis-us.com)

Introduction

The default aquatic life criteria for Cu in all 50 states of the USA are based on site-specific water hardness. However, other water quality parameters [e.g., pH, alkalinity, dissolved organic carbon (DOC) concentration] can have equal or greater effects on Cu toxicity than hardness alone, as a result of two principal types of geochemical/biogeochemical interactions: 1) complexation of Cu by ligands in the water (e.g., HCO_3^- , CO_3^{2-} , DOC) and 2) competition between Cu and other cations (e.g., H^+ , Na^+) for binding to DOC and to sites of toxic action on organisms (Meyer et al. 2007). These water chemistry parameters should be incorporated into the development of site-specific criteria for Cu, thereby reflecting mechanisms by which Cu toxicity is modified and providing a more appropriate level of protectiveness than the default hardness-based criteria. In this presentation, we demonstrate how non-hardness water chemistry parameters can be incorporated into derivation of site-specific criteria at a site in the southwestern USA, where several non-traditional challenges related to an arid environment, nonpoint sources of Cu, multiple watersheds across a large geographic scale, and a remote and rugged landscape had to be overcome.

In recognition of the limitations of hardness-based criteria for Cu and other metals, the U.S. Environmental Protection Agency (USEPA) and all 50 states allow for an empirical procedure to implicitly incorporate water chemistry parameters into site-specific criteria for Cu. That process is called the water effect ratio (WER) procedure, whereby the toxicity of Cu in receiving water collected from a field site is compared to the toxicity of Cu in a hardness-matched laboratory water (USEPA 1994, 2001). The WER represents the extent to which the site water decreases (or increases) the toxicity of Cu, and this is the extent to which the site-specific criteria can exceed (or should be less than) the default hardness-based aquatic life criteria while still maintaining the intended level of protection.

Despite its simplicity, the WER procedure has several drawbacks. First, WER studies generally are time-consuming and relatively expensive. Second, they historically have been applied to point-source discharges into a single receiving water, instead of dealing with nonpoint sources of Cu flowing into multiple

watersheds. And third, guidance for conducting WER studies was developed in mesic (i.e., moist) climates without specific consideration of challenges posed in arid, monsoon-dominated climates. As a result, challenges for applying the WER in the present study include non-perennial surface waters characterized by seasonally-limited flow (i.e., intermittent or ephemeral) influenced by nonpoint sources of Cu.

To provide a less time-consuming and less-expensive process than the WER procedure for taking water chemistry into account, in 2007 the USEPA adopted the biotic ligand model (BLM) for derivation of national freshwater Cu criteria (USEPA 2007). The BLM is a computerized calculation procedure that predicts the toxicity of cationic metals (e.g., Ag, Cd, Cu, Ni, Pb, Zn) to aquatic organisms, by explicitly taking into account a variety of water chemistry parameters that can modify the toxicity of metals to aquatic organisms. Those important water chemistry parameters include pH, alkalinity, hardness, major ions, and DOC concentration. However, the BLM relies on generic parameterization of the affinity and capacity of DOC for binding metals, an important process by which the metals can become less bioavailable. That parameterization relies on geochemical-speciation data generated mostly in mesic climates, and it is not known whether the BLM's parameterization of metal-DOC interactions is applicable to DOC in surface waters in arid regions like the southwestern USA. Moreover, none of the 50 states has yet replaced the hardness-based aquatic life criteria with BLM-based criteria for Cu, although several states in the southwestern USA allow for potential use of the BLM (and the WER procedure) to derive alternate site-specific criteria for Cu.

Herein, we present a case study that illustrates challenges associated with applying the WER procedure to a complex network of ephemeral and intermittent drainages influenced by diffuse Cu contamination in an arid landscape. To expand the application of the WER procedure to these non-perennial systems that lack point sources and cover a relatively large landscape, we developed a WER model that is based on multiple-regression analyses between results of empirical Cu toxicity tests and receiving-water chemistries and used that model to derive site-specific Cu criteria. As a key component of the derivation of and justification for the criteria, we used fundamentals of geochemical interactions (as explicitly incorporated in the BLM) to provide a mechanistic underpinning to the empirical WER model.

Methodology

The study site contained approximately 60-km² of arid, mostly mountainous terrain, ranging from approximately 1500m asl (in the lower-elevation desert grasslands and shrub lands) to 2300m asl (in the higher elevations). Diffuse sources of Cu in some of the soils result from historical industrial emissions and natural mineralization.

We categorized the study site into a total of 9 sub-watersheds that encompass 12 drainages. Most of the surface waters are ephemeral, flowing only in

direct response to intense, short-duration monsoonal precipitation events; however, surface water can remain in isolated pools for up to several weeks. Water chemistry varied considerably across the study site in response to differences in geology, geomorphology, hydrology, and surrounding upland landscapes.

Eighteen surface-water grab samples were collected from pools during summer 2011 (12 locations in August and 6 repeat samples in September). Collection and processing followed USEPA (1994) guidelines. Samples were shipped on ice to the toxicity-testing laboratory, where they were maintained in the dark at <4 °C until test initiation. Additionally, water samples were shipped to an analytical laboratory for chemical characterization.

Standard WER toxicity tests (USEPA 1994, 2001) were conducted with *Daphnia magna* (an aquatic invertebrate routinely used in toxicity tests) exposed to Cu for 48 h in the site waters and hardness-matched laboratory water. The laboratory-water toxicity tests were performed concurrent with the site-water toxicity tests under identical exposure conditions, except the tested Cu concentrations differed as appropriate for the toxicity-modifying factors in the site waters. Exposure waters were mixed thoroughly and allowed to stand for a minimum of 20 h at 4 °C before test initiation to allow the metal chemistry to equilibrate, as recommended by USEPA (2001) and Ma et al. (1999). The observation endpoint was immobilization; thus, toxicity results are reported herein as median effect concentrations (EC50 values) instead of median lethal concentrations (LC50 values). Water chemistry parameters measured in all tests included dissolved oxygen, pH, conductivity, temperature, hardness, alkalinity, organic carbon, Cu, and major inorganic ions (Ca^{2+} , Mg^{2+} , Na^+ , K^+ , Cl^- , SO_4^{2-}); and concentrations of Al, Cd, Cu, Fe, Mn, Pb, and Zn were measured in the site waters.

The 48-h Cu EC50 values for immobilization/death and their 95% confidence limits were computed by maximum likelihood probit analysis. To evaluate the influence of individual water chemistry parameters on Cu toxicity, univariate linear-regression analyses were performed using measured dissolved Cu EC50 values and measured water chemistry parameters. Then, step-wise multiple linear regression analyses were performed to determine the best combination of water chemistry parameters for predicting measured Cu toxicity. Water effect ratios normally are calculated as the ratio $\text{EC50}_{\text{site water}} / \text{EC50}_{\text{lab water}}$, to determine the toxicity-modifying effect of the non-hardness water chemistry constituents in the site water. However, at the request of the regulatory agency involved in this site-specific criteria derivation, the $\text{EC50}_{\text{lab water}}$ in the denominator of that ratio was replaced by the USEPA-recommended Species Mean Acute Value (SMAV, which is the geometric mean of all EC50 values in the USEPA toxicity database for *D. magna* exposed to Cu) for dissolved Cu at a hardness of 100 mg/L as CaCO_3 [i.e., 19.31 $\mu\text{g Cu/L}$; Appendix B in USEPA (2001)].

The toxicity-modifying effects of water chemistry parameters on acute Cu toxicity were also evaluated using the HydroQual Cu BLM (Ver 2.2.3; http://hydroqual.com/wr_blm.html). Concentrations of pH, alkalinity, DOC, Ca²⁺, Mg²⁺, Na⁺, K⁺, Cl⁻, and SO₄²⁻ measured in toxicity-test exposure waters were used as the BLM input parameters. We ran the BLM in toxicity mode (1) to produce BLM-predicted EC50 values, for comparison with measured and regression model-predicted EC50 values; and (2) to calculate BLM-based site-specific aquatic life criteria for Cu, for comparison to the WER-based site-specific criteria.

Details of all the methods are presented in Fulton and Meyer (2014).

Results and Discussion

Water chemistry varied considerably among the site waters. Concentrations of DOC ranged from 1.2 to 15.7 mg C/L and were greater than or equal to 10 mg C/L in 9 of the 17 samples. Water hardness ranged from soft (42 mg/L as CaCO₃) to hard (262 mg/L as CaCO₃), and alkalinity ranged from 27 to 250 mg/L as CaCO₃. Although hardness and alkalinity concentrations in site samples were moderately correlated ($r=0.82$), the hardness-to-alkalinity ratio ranged from 0.71 to 2.8. The pH values ranged from 7.14 to 9.35.

All but one of the 17 calculated WER values were greater than 1.0 (range = 0.99–14.4), indicating that non-hardness water chemistry constituents in most of the site waters considerably decreased Cu toxicity relative to standard laboratory water that is used in toxicity tests from which the default hardness-based Cu criteria are derived. The wide range of WER values can be explained by the wide range of site water chemistries tested and by the known influence of DOC and inorganic parameters (major cations, alkalinity) on Cu bioavailability to aquatic organisms (Meyer et al. 2007, USEPA 2007). However, the variability in WER values presents a challenge to implement site-specific criteria, especially in arid landscapes that contain ephemeral drainages not influenced by point-source discharges of Cu. A one-number approach might not be protective in all of the waters if a geometric mean WER were selected, or it might be overly protective if the lowest calculated WER were selected. Therefore, we developed a regression equation to predict a site-specific WER value in the water chemistry of each drainage, to provide the intended level of regulatory protection against adverse effects of Cu without being overly conservative.

The best-fit WER predictor for these waters is:

$$\text{WER} = \frac{10^{[0.588 + (0.703 * \log \text{DOC}) + (0.395 * \log \text{Alkalinity})]} \cdot \left(\frac{100}{\text{Hardness}} \right)^{0.9422}}{19.31}$$

where DOC concentration is in mg C/L and alkalinity and hardness are in mg/L as CaCO₃. This equation accounts for 85% of the variance in the *D. magna* Cu EC50

values in these waters, and both predictor variables are statistically significant ($p < 0.001$ for DOC, and $p = 0.007$ for alkalinity). Note that although the default aquatic life criteria for Cu are based only on water hardness, hardness alone was a poor predictor of the toxicity ($R^2 = 0.102$, $p = 0.211$) and did not load significantly into the multiple-regression equation. Additionally, pH was an equally poor predictor of the toxicity and also did not load significantly into the multiple-regression equation.

The WER-based site-specific Cu criterion for a given receiving water is then calculated as:

$$\text{S-S Cu criterion } (\mu\text{g Cu/L}) = \text{WER} \cdot \text{default hardness-based Cu criterion } (\mu\text{g Cu/L}) .$$

This allows each water body within the landscape to be assigned a unique site-specific Cu criterion based on its own water chemistry, wherein that water chemistry (and its associated geochemical-speciation interactions with Cu) determines the WER value and the default hardness-based Cu criterion. This avoids the problem of choosing a “one-size-fits-all” Cu criterion for all these water that inherently would be either too over-conservative or too under-conservative (depending on how it would be chosen), thereby providing a cost-effective approach that still maintains the intended level of regulatory protection.

These Cu toxicity results are consistent with the published literature, wherein DOC and alkalinity are usually identified as strong predictors of Cu toxicity; however, hardness usually is not identified as a strong predictor of Cu toxicity, and pH is not always identified as a strong predictor (De Schamphelaere et al. 2004, Sciera et al. 2004, Van Genderen et al. 2005). These results are also consistent with a basic understanding of geochemical speciation of cationic metals like Cu, wherein Cu is complexed by alkalinity ions (HCO_3^- and CO_3^{2-}) and DOC. Because the “free” cupric ion (Cu^{2+}) is considered a major cause of Cu toxicity, complexation of Cu with anionic ligands decreases the bioavailability and toxicity of Cu to aquatic organisms. It is interesting that competition of Cu^{2+} with the hardness cations (Ca^{2+} and Mg^{2+}) for binding to biotic ligands (i.e., sites of toxic action) on the *D. magna* did not appear to play an important role in modifying the toxicity of Cu in these site waters.

The “out-of-the-box” BLM-predicted 48-h dissolved Cu EC50 values in site waters always exceeded the corresponding measured EC50 values and in most cases were more than 2-fold greater than observed values, implying that the “out-of-the-box” BLM under-predicted Cu toxicity in these site waters. This systematic bias in BLM predictions might suggest (1) a sensitivity difference between tested organisms and those used to develop the BLM, and/or (2) a difference in the quality of DOC in site waters compared with those used to develop the BLM (i.e., different Cu-binding affinity or different binding-site density). We were able to remove that systematic bias by adjusting for organism sensitivity (using the toxicity results in the laboratory waters) and by assuming only 43% of the DOC in the site waters interacted with Cu. These adjustments are consistent with results from previous

studies (De Schamphelaere et al 2002, 2003, Meyer and Adams 2010, Constantino et al. 2011).

Although the “out-of-the-box” BLM-predicted EC50 values under-predicted Cu toxicity to *D. magna* in these waters, the “out-of-the-box” BLM-based site-specific Cu criteria did not differ considerably from the empirical WER-based site-specific Cu criteria. This difference in predictability can be attributed to separate components of the BLM being used to predict Cu EC50s to *D. magna* and to calculate site-specific criteria, and to the fact that several other species in addition to *D. magna* are used to calculate the BLM-based Cu criteria.

Conclusions

Water hardness alone is not usually a good predictor of Cu toxicity to aquatic organisms. In the site waters tested in this study, hardness appeared to play only a minor role in determining Cu toxicity to *D. magna*, whereas a combination of other water chemistry constituents (DOC and alkalinity) was a much stronger predictor of toxicity. Because the DOC-and-alkalinity combination accounted for 85% of the variance in *D. magna* EC50 values in the site waters, the multiple-regression equation provides a strong regulatory tool for calculating WER-based site-specific Cu criteria in a variety of water chemistries across this arid southwestern landscape. However, we caution that this WER-based regression equation might not necessarily be applicable to waters at other arid southwestern sites, and additional testing would be needed to determine the accuracy of the model's toxicity predictions. Although the WER-based Cu criteria are more justifiable for this site because they are based on toxicity tests conducted with site waters and were found to be highly predictive of toxicity, the site-specific criteria based on the BLM (and thus based on fundamental geochemical principles) did not differ considerably from the empirical WER-based site-specific criteria (i.e., the BLM-based predictions were highly correlated with the WER-based predictions). This finding further validates the use of the WER model in deriving site-specific copper criteria. The take-home message is: Water chemistry matters in modifying the toxicity of Cu to aquatic organisms, and geochemical interactions of Cu with chemical constituents provide the mechanistic underpinning for differences in toxicity among water bodies whose chemistries differ.

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