Validation and Update of a Model Used to Predict Copper Toxicity to the Marine Bivalve *Mytilus* sp.

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ABSTRACT: A model was recently published that relates the toxicity of copper to the most sensitive taxa in the U.S. Environmental Protection Agency's criteria database (*Mytilus* sp.) with dissolved organic carbon concentrations in saltwater. This model was developed for potential use in risk assessment and in the development of site-specific criteria (SSC) for copper in saltwater environments where *Mytilus* sp. is considered an appropriate indicator species. This manuscript presents the results of a field validation study of that model. Effective concentration 50% (EC_{50}) values ($n = 21$) for seven sites were all predicted by the model within the previously established range of acceptability. Slopes and intercepts of the two data sets were not significantly different. Consequently, the data were pooled, and new equations were developed. Dissolved copper EC_{50} values were highly correlated ($r^2 = 0.76$, $n = 75$, $P < 0.0001$) across a wide range of sample dissolved organic carbon (DOC) concentrations (0.3–12 mg C/L) and were explained by the equation $EC_{50} = 11.22\ DOC^{0.60}$. Two updated equations are proposed for consideration as a means of estimating site-specific final chronic criteria (FCC) and final acute criteria (FAC) for copper in marine and estuarine environments (copper $FCC_{DOC} = 3.59\ DOC^{0.60}$; copper $FAC_{DOC} = 5.61\ DOC^{0.60}$). © 2006 Wiley Periodicals, Inc. Environ Toxicol 21: 65–70, 2006.

Keywords: copper; toxicity; model; saltwater; *Mytilus*

INTRODUCTION

The concentration of copper in U.S. estuaries is a widespread concern. The U.S. Environmental Protection Agency (U.S. EPA) maintains a database of information (http://www.epa.gov/owow/tmdl/) on water bodies that do not attain beneficial uses because of chemical concentrations exceeding water quality criteria limits. A review of that database indicates that copper concentrations above water quality criteria limits have been measured in segments of numerous estuaries around the United States. Exceeding the water quality criteria limits implies that there is an unacceptable risk of negative impact on taxa in those waters. Consequently, these waters are deemed impaired and have been placed on the U.S. Clean Water Act (CWA) 303(d) list. Presently, 41 estuaries are listed explicitly because of copper. An additional 15 estuaries are listed because of high concentrations of "metals," and thus additional bodies of water also may have high levels of copper. Moreover, the database is updated and adds new sites every 2 years, when states are required to file new listings.

Regulatory authorities are required to take the steps necessary to correct the problem and to attain the designated water quality standards set for waters placed on the 303(d) list. Once listed, a variety of studies are required to facilitate the attainment of acceptable concentrations of copper and to assure that taxa inhabiting those waters are protected. One of the first studies is to determine the scope of

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the impairment. In some cases, this analysis may conclude that in fact standards are being attained and that water quality is not impaired. This may result from finding that the assumptions underlying the listing were incorrect or that the impairment has been corrected. If the studies using promulgated water quality criteria suggest that the waters remain impaired, then a site-specific study may be conducted to determine if the criteria are appropriate. If the criteria are not appropriate, then appropriate and protective SSC are determined and implemented. Since 1996, only 23 estuaries (an approximate number because of the vagueness of the database) have undergone the necessary studies and have been removed from the 303(d) list. Thus, much work remains.

The U.S. EPA recognizes the limitations of metal criteria that have been developed on the basis of laboratory tests and that there is a need to account for the effects of natural water chemistry on metal bioavailability and thus toxicity (U.S. EPA, 1984, 2003). It also realizes that the process of site-specific criteria (SSC) development can be resource intensive and expensive and may delay or prohibit their widespread determination (Renner, 1997). Nonetheless, the U.S. EPA provides guidance for several methods that can be used to determine SSC.

One commonly used tool for the development of SSC is the water-effects ratio (WER) test method (U.S. EPA, 1994). WER methods are used to test both water samples collected from the site of interest and laboratory water similar to that which was used to develop the U.S. EPA water quality criteria. WER toxicity tests are performed by spiking copper in both laboratory and site waters and determining the respective 50% lethal or effective concentrations (LC_{50}, EC_{50}). The site water LC_{50} or EC_{50} is divided by the laboratory water LC_{50} or EC_{50} in order to derive the WER. This is repeated for several periods or seasons to account for water quality variability and its effect on copper bioavailability. The SSC is derived by multiplying the existing water quality criteria by the WER. Consequently, if the WER is <1, the SSC decreases; if the WER is >1, the SSC increases. However, because of the high cost of WER tests, the number of sites and samples tested are often limited.

There is a need for techniques that reduce costs, increase certainty, and decrease time frames in order to address the numerous saltwater sites currently on the CWA 303(d) list. In recognition of the need for increased efficiency, the U.S. EPA has published streamlined procedures for determining copper WERs in freshwater (U.S. EPA, 2001). However, because of the ongoing process of assessing water bodies for placement on the 303(d) list and of the number of ongoing SSC development efforts in estuaries around the United States, there is an additional need for a simple method to aid in assessing risk as well as for use in modifying existing copper criteria in saltwater systems. Arnold (2005) has suggested a model and a relatively simple approach to aid in addressing this need. This regression-based model, in principle, is similar to that promulgated by the U.S. EPA (1984) and is widely used to adjust freshwater copper criteria to account for the effects of water hardness. For saltwater, the role of dissolved organic carbon (DOC) in mediating the bioavailability (and thus toxicity) of copper is used to predicted copper toxicity levels in a cost-effective and technically sound fashion. This method potentially represents a practical alternative for developing SSC using WER methods. It was shown to be strongly correlated with results of WER studies conducted in the San Francisco Bay, where U.S. EPA WER procedures (U.S. EPA, 1994) were used (Arnold, 2005).

In this study, the DOC-based model for estimating copper SSC in saltwater was applied to water samples collected from a location that was not used in the development of the original model, that was listed on the EPA 303(d) list as impaired due to copper, and for which copper SSC were being developed using U.S. EPA WER procedures. The purpose of the study was to field-validate the model and to determine if it predicts toxicity within a predetermined acceptability range of a factor of ± 2. Last, if the model calibration and field validation data proved to be statistically similar, then the data sets would be pooled and a recalibrated model developed.

MATERIALS AND METHODS

Toxicity Measurement

Data were collected as part of a cooperative effort with regulatory authorities, dischargers, and nongovernmental organizations performing WER tests to establish copper SSC for Mugu Lagoon and lower sections of Callegaus Creek in Ventura County, California, USA. Samples were collected three times (August 2003, January 2004, and March 2004) at five sites and twice at one additional site (Fig. 1). Reference site water was collected from the Pacific Ocean at the Granite Canyon Marine Laboratory, Carmel, California, USA, and tested concurrently with the site water, resulting in data for a total of seven sites. Granite Canyon water has been used by the California Marine Bioassay Project since 1984 to develop methods for toxicity testing of marine waters (U.S. EPA, 1995a). In August 2003, Mugu Lagoon samples were tested at a different time than the samples from Callegaus Creek. Consequently, a sample of reference water from Granite Canyon was tested concurrently with each, yielding two reference-site copper EC_{50} values for August 2003. In January and March 2004, site samples were not split, and a single reference site sample was concurrently tested. Thus, a total of 21 site- and date-specific copper EC_{50} values were developed.

Toxicity tests for all samples were conducted by the same laboratory (Pacific EcoRisk, Martinez, CA, USA) using embryos of the estuarine bivalve *Mytilus galloprovincialis* (not genetically verified: referred to hereafter as *Mytilus* sp.) obtained from Carlsbad Aquafarms (Carlsbad, CA, USA).

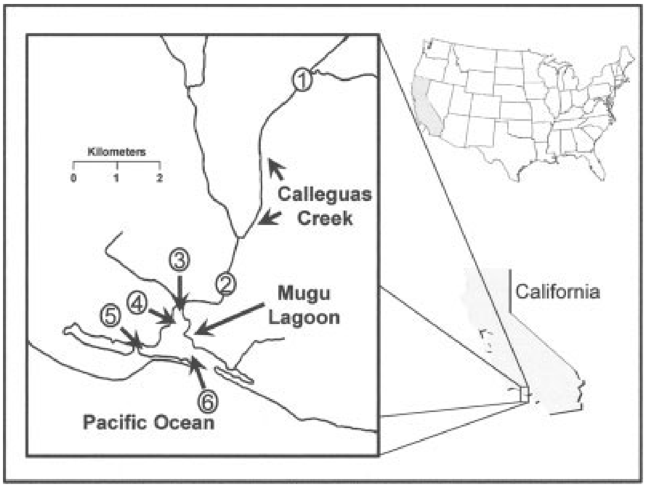

Fig. 1. Map of study sites in Calleguas Creek and Mugu Lagoon, Ventura County, California.

The salinity of all samples was adjusted upward to a constant 30% ± 2% as per U.S. EPA guidelines (1995a) using reagent-grade GP2 artificial sea salts, or the salinity was adjusted downward using reverse-osmosis deionized water. Samples from Mugu Lagoon and Calleguas Creek and the Granite Canyon reference water were split into aliquots and spiked with copper to concentrations encompassing the EC_{50} of *Mytilus* sp. Toxicity tests were conducted using guidelines by the U.S. EPA (1995a) for the *Mytilus* 48-h static embryo-larval development test. The end points for this test include normal shell development and mortality as measures of adverse effects. Positive, negative, and sea salt control tests were performed, according to U.S. EPA guidelines (1995a).

Water Chemistry

Chemical analyses for all parameters used in this study were made after the site water samples had been salinity adjusted. Sampling post–salinity adjustment is important in order to account for modification of copper bioavailability from changes in the water chemistry of the site water test solution caused by this procedure. Dissolved copper (i.e., copper that passes through a 0.45 μm filter) determinations were made using ICP-MS according to U.S. EPA method 1640 (1995c). Dissolved organic carbon determinations were made using an O.I. Analytical Corporation Model 1020A organic carbon analyzer according to U.S. EPA method 415.1 (1979).

RESULTS AND DISCUSSION

The DOC concentrations ranged between 1.0 and 12.0 mg C/L and varied considerably both among sites and sampling periods (Table I). All positive, negative, and sea salt control as well as ambient water toxicity tests performed showed acceptable results according to U.S. EPA guidelines (1995a).

The toxicity of copper to *Mytilus* sp. in the Mugu Lagoon and Calleguas Creek site waters (Table I) varied with DOC concentration in a manner similar to that reported by Arnold (2005; Fig. 2). Regression analysis of log-transformed data for Mugu Lagoon and Calleguas Creek indicated a highly statistically significant relationship ($r^2 = 0.76$, $n = 21$, $F = 61.3$, $P < 0.0001$) between DOC concentrations and dissolved copper EC_{50} values in salinity-adjusted samples (Fig. 3). Moreover, the 21 observations fell within the bounds of acceptability of the original DOC-based toxicity model proposed by Arnold (2005; Fig. 3).

Had the previously reported DOC-based model and recommended procedures been used to estimate the copper SSC for Mugu Lagoon and Calleguas Creek, it would have provided statistically similar results when compared to the WER approach (Fig. 4). Estimates of site- and date-specific WERs were made by dividing the dissolved copper EC_{50}

TABLE I. Dissolved organic carbon and *Mytilus* sp. copper EC_{50} values measured in salinity adjusted samples

	August 2003		January 2004		March 2004	
Site	DOC mg C/L	EC_{50} μg Cu/L	DOC mg C/L	EC_{50} μg Cu/L	DOC mg C/L	EC_{50} μg Cu/L
Calleguas Creek 1	5.1	52.9	8.6	59.4	9.7	47.9
Calleguas Creek 2	4.8	48.2	6.6	54.0	10.0	47.8
Mugu Lagoon 3	1.5	19.6	7.3	34.4	11.0	56.8
Mugu Lagoon 4	1.4	15.9	6.2	33.8	ND	ND
Mugu Lagoon 5	2.0	20.0	4.7	22.5	12.0	54.4
Mugu Lagoon 6	1.7	14.7	3.4	16.1	9.9	41.6
Granite Canyon	1.0[1], 1.2[2]	11.8[1], 11.7[2]	2.9	12.3	3.2	14.1

ND - not determined.
[1] Tested with Calleguas Creek 1 and 2.
[2] Tested with Mugu Lagoon 3–6.

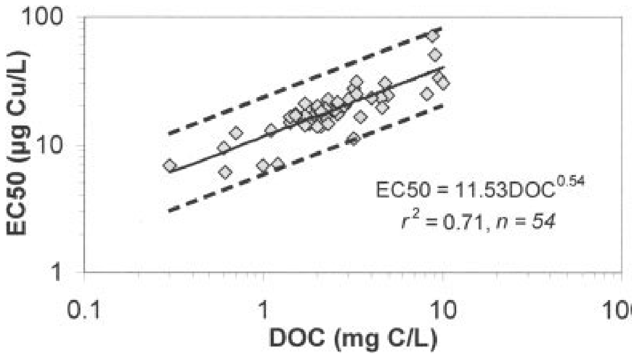

Fig. 2. Plot from Arnold (2005) of dissolved copper EC_{50} values for embryos of *Mytilus* sp. as a function of dissolved organic carbon (DOC) in salinity-adjusted water samples (r^2 = 0.71, n = 54, $P < 0.0001$). The solid line is the line of best fit; dotted lines represent a predetermined level of acceptable predictability (i.e., line of best fit ± a factor of 2) based on the approximate range of *Mytilus* sp. copper EC_{50} values measured in reference toxicity tests (Arnold, 2005).

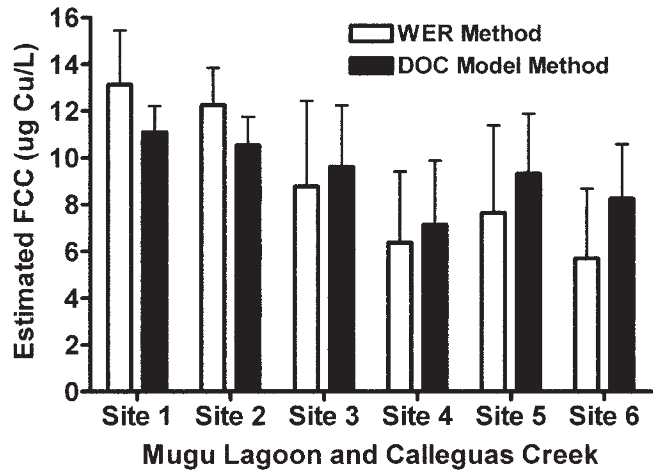

Fig. 4. Geometric means and geometric standard deviations of estimates of site-specific saltwater copper chronic criteria for Mugu Lagoon and Calleguas Creek derived using the water-effects ratio and dissolved organic carbon model methods.

for each site and collection date by the corresponding Granite Canyon reference site water EC_{50}. The chronic saltwater copper criterion of 3.1 μg Cu/L was then multiplied by each WER to estimate the site- and date-specific WER-based chronic saltwater copper criterion. For comparison, the original DOC model recommended by Arnold (2005) was used to estimate corresponding site- and date-specific criteria using the equation $FCC_{DOC} = 3.71\ DOC^{0.54}$. A two-way analysis of variance performed on log-transformed data indicated no overall significant difference ($\alpha = 0.05$) in criteria determined by either method ($P = 0.65$), a not quite significant difference in criteria between sites ($P = 0.06$), and no significant interaction in criteria according to model used and site analyzed ($P = 0.85$).

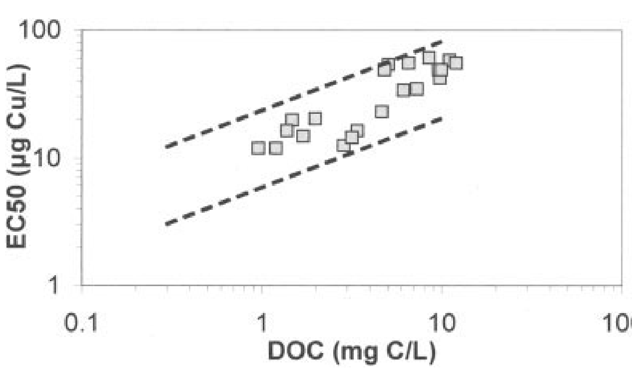

Fig. 3. Plot of dissolved copper EC_{50} values for embryos of *Mytilus* sp. as a function of dissolved organic carbon (DOC) in salinity-adjusted water samples from Mugu Lagoon, Calleguas Creek and Granite Canyon, California (r^2 = 0.76, n = 21, $P < 0.0001$). Dotted lines are lines of acceptable predictability (i.e., line of best fit ± a factor of 2) of the original DOC-based model (Arnold, 2005) being validated.

The original and the Mugu Lagoon and Calleguas Creek DOC models were statistically similar enough to pool the two data sets and recalibrate the model (Fig. 5). Regression analysis of the combined EC_{50} and DOC data from Mugu Lagoon and Calleguas Creek yielded the model $EC_{50} = 10.91\ DOC^{0.66}$ (equation with 95% confidence intervals: $EC_{50} = 10^{1.038\pm0.059}\ DOC^{0.658\pm0.084}$). The model reported by Arnold (2005) was $EC_{50} = 11.53\ DOC^{0.54}$ (the equation with 95% confidence intervals is $EC_{50} = 10^{1.062\pm0.046}\ DOC^{0.542\pm0.097}$). Tests for differences of slopes and y intercepts between the two models indicated no significant

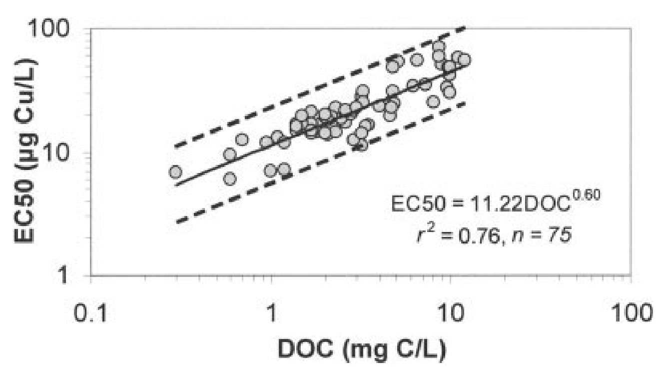

Fig. 5. Plot of pooled results from this study and that of Arnold (2005). The circles represent EC_{50} values of dissolved copper for *Mytilus* sp. embryos as a function of dissolved organic carbon (DOC) in salinity-adjusted water samples (r^2 = 0.76, n = 75, $P < 0.0001$). The solid line is the line of best fit; dotted lines represent a predetermined level of acceptable predictability (i.e., line of best fit ± a factor of 2) based on the approximate range of *Mytilus* sp. copper EC_{50} values measured in reference toxicity tests (Arnold, 2005).

differences (slopes: $F = 1.72$, $P = 0.19$; y intercepts: $F = 1.52$, $P = 0.22$). Thus, the data were pooled and an update of the model calculated. Regression analysis of the pooled data set yielded highly statistically significant results ($r = 0.76$, $n = 75$, $F = 225$, $P < 0.0001$) and yielded the model $EC_{50} = 11.22\ DOC^{0.60}$ (the equation with 95% confidence intervals is $EC_{50} = 10^{1.050 \pm 0.022}\ DOC^{0.600 \pm 0.040}$).

The recalibrated DOC-based toxicity model is easily modified to estimate acute and chronic saltwater copper criteria. Because the final acute value (FAV) for copper is equivalent to the species mean acute value for *Mytilus* (U.S. EPA, 1995b, 2003), the DOC-based model essentially predicts the copper saltwater FAV. The final acute criterion (FAC) is calculated by dividing the FAV by 2 and the final chronic criterion is calculated by dividing the FAV by the copper acute-to-chronic ratio (ACR). The current ACR used by the U.S. EPA in the current copper saltwater criteria document (U.S. EPA, 1995b) is 3.127. However, a new ACR of 3.232 was recently proposed (U.S. EPA, 2003). Thus, the DOC-based toxicity model divided by 2, 3.127, or 3.232 yields the following criteria estimation models: $FAC_{DOC} = 5.61\ DOC^{0.60}$, $FCC_{DOC-3.127} = 3.59\ DOC^{0.60}$, or $FCC_{DOC-3.232} = 3.47\ DOC^{0.60}$. Estimations of criteria based on these DOC-based models are illustrated in Figure 6.

Field validation and recalibration of the DOC-based model serve several purposes. First, they increase the confidence that the toxicity of copper to *Mytilus* sp. can be effectively predicted at a variety of locations using DOC concentrations. The recalibrated model includes data for six locations around the United States (San Francisco Bay, CA; Granite Canyon, CA; Mugu Lagoon and Calleguas Creek, CA; Puget Sound, WA; Galveston Bay, CA; and Narragansett Bay, RI). Second, they extend the utility of the model beyond that of the initial model. The range of DOCs that were used to derive the original model was 0.3–10 mg C/L. The upper limit of this range has been increased to 12 mg C/L now that the data have been pooled.

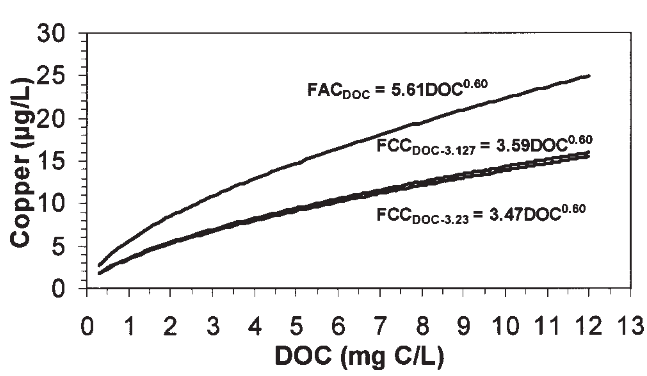

Fig. 6. Saltwater chronic (FCC) and acute (FAC) copper criteria estimated as a function of dissolved organic carbon (DOC) concentrations. Lines labeled $FCC_{DOC-3.23}$ (bottom line) and $FCC_{DOC-3.127}$ (middle line) were calculated using acute-to-chronic ratios of 3.23 and 3.127, respectively.

Although there is a movement toward use of mechanistic-based models, such as the biotic ligand model (BLM; Di Toro et al., 2001; Santore et al., 2001; Paquin et al., 2002) for predicting metal toxicity in freshwater and work is ongoing to develop a BLM for saltwater, there remains utility in the regression-based DOC model in SSC development and risk assessment. First, it has been developed, validated, and proven to be consistent and relatively accurate. Although the BLM has been tested for prediction of toxicity to *Mytilus* sp., it has been shown to have a slight bias (Arnold et al., 2005, in press), and the correction of the bias and validation of the corrected model will require time. It is expected that the BLM will eventually replace the DOC-based approach, but until then the need for the DOC-based model remains. The DOC-based model is an improvement over prediction of copper toxicity based on dissolved copper concentrations alone. It is simple to use, and there is familiarity with this type of model because its use is similar to that of the hardness-based model, which has been used for metal criteria development in freshwater since 1984. Last, the DOC-based model is efficient because the cost of measuring DOC is relatively inexpensive compared to the WER procedure. Thus, SSC may be estimated for a fraction of the cost, and all or a portion of the money that is saved could be used to increase the number of data collected either spatially, temporally, or both, which will increase the certainty that the final SSC are representative and protective.

CONCLUSIONS

The DOC-based toxicity and SSC estimation models have now been validated using data from a location that was not used in the development of the original DOC-based toxicity model. The DOC-based model has proven effective by consistently (i.e., $n = 21$ of 21 data in the validation study; $n = 75$ of 75 in the entire data set) predicting the toxicity of copper to *Mytilus* sp. within a factor of ± 2 over a relatively wide range of DOC concentrations (0.3–12 mg C/L). Estimations of chronic copper saltwater criteria using the DOC-based model are statistically similar to those derived using the WER approach.

Further application of the DOC-based model in conjunction with the development of SSC based on the WER method will help to further judge the approach. However, there is now sufficient data to support that the prediction of toxicity of copper to *Mytilus* sp. is repeatable, and there is potential for the use of the DOC-based models in the development of site-specific copper criteria.

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