Environmental Toxicology and Chemistry, Vol. 26, No. 10, pp. 2066–2074, 2007

Printed in the USA
0730-7268/07 $12.00 + .00

Contaminant Sensitivity of Freshwater Mussels

AN EVALUATION OF FRESHWATER MUSSEL TOXICITY DATA IN THE DERIVATION OF WATER QUALITY GUIDANCE AND STANDARDS FOR COPPER

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(*Received* 7 *November* 2006; *Accepted* 20 *April* 2007)

Abstract—The state of Oklahoma has designated several areas as freshwater mussel sanctuaries in an attempt to provide freshwater mussel species a degree of protection and to facilitate their reproduction. We evaluated the protection afforded freshwater mussels by the U.S. Environmental Protection Agency (U.S. EPA) hardness-based 1996 ambient copper water quality criteria, the 2007 U.S. EPA water quality criteria based on the biotic ligand model and the 2005 state of Oklahoma copper water quality standards. Both the criterion maximum concentration and criterion continuous concentration were evaluated. Published acute and chronic copper toxicity data that met American Society for Testing and Materials guidance for test acceptability were obtained for exposures conducted with glochidia or juvenile freshwater mussels. We tabulated toxicity data for glochidia and juveniles to calculate 20 species mean acute values for freshwater mussels. Generally, freshwater mussel species mean acute values were similar to those of the more sensitive species included in the U.S. EPA water quality derivation database. When added to the database of genus mean acute values used in deriving 1996 copper water quality criteria, 14 freshwater mussel genus mean acute values included 10 of the lowest 15 genus mean acute values, with three mussel species having the lowest values. Chronic exposure and sublethal effects freshwater mussel data available for four species and acute to chronic ratios were used to evaluate the criterion continuous concentration. On the basis of the freshwater mussel toxicity data used in this assessment, the hardness-based 1996 U.S. EPA water quality criteria, the 2005 Oklahoma water quality standards, and the 2007 U.S. EPA water quality criteria based on the biotic ligand model might need to be revised to afford protection to freshwater mussels.

Keywords—Mussels Copper Water quality Toxicity

INTRODUCTION

For animals worldwide, described species of freshwater and terrestrial mollusks constitute the second most diverse phylum of animals and have had the greatest number of extinctions [1]. In North America, over 70% of native unionids are federally listed as endangered, threatened, or of special concern [2,3]. In the state of Oklahoma, 52 freshwater mussel species presently occur, three of which are state-listed as endangered and two are species of special concern. Within the watersheds that sustain the highest biodiversity and healthiest populations, species abundances are declining and the biological integrity of numerous subpopulations has been greatly decreased by the loss of individuals [4]. The Oklahoma Department of Wildlife Conservation has designated several areas as freshwater mussel sanctuaries in an attempt to provide freshwater mussel species a degree of protection and to facilitate their reproduction. These areas support a diverse freshwater mussel fauna, including commercially valuable and noncommercial species.

Freshwater mussels provide ecosystem services (e.g., cycling nutrients and energy in streams and lakes) and are important indicators of the quality of surface waters. The continued decline of native freshwater mussels nationwide and in Oklahoma is a concern because freshwater mussels are indicators of water quality conditions including, but not limited to, potential contamination, habitat degradation and alteration of the natural flow regimes of lotic systems, and fish community changes as a result of loss of fish hosts required for glochidia transformation and freshwater mussel dispersal. Declines in freshwater mussel fauna have an effect on the overall diversity of aquatic ecosystems and could also result in cultural and economic losses.

Review of the Oklahoma copper standard and its application in Oklahoma freshwater mussel sanctuaries designated in Title 800, Chapter 15, Section 7-4, of the Oklahoma Administrative Code [5] led to the development of the database for copper toxicity to freshwater mussels and the current evaluation. This paper is one of a series of papers in this issue evaluating the reliability and usefulness of freshwater mussel toxicity tests and the environmental relevance of including results from such tests in developing protective criteria for freshwater ecosystems. To evaluate the protection of freshwater mussels by copper water quality criteria (WQC) and standards, we used an approach similar to Augspurger et al. [6]. We used procedures outlined by the U.S. Environmental Protection Agency (U.S. EPA) [7,8] for developing ambient WQC and by the state of Oklahoma [9,10] for developing water quality standards (WQS) for copper. Data used for this analysis were required to meet the test acceptability requirements identified in Amer-

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ican Society for Testing and Materials (ASTM) E 2455-06 [11] guidelines (i.e., control survival, duration of exposure). The objective of this work was to determine the concentrations of copper protective of freshwater mussels on the basis of data not included in the derivation of the U.S. EPA WQC or Oklahoma WQS.

MATERIAL AND METHODS

To develop the freshwater mussel copper acute and chronic toxicity database, we reviewed journal articles, interim and final reports, and dissertations and theses that included toxicity information for native North American unionid species. Data extracted from these sources included species, life stage tested (glochidia vs juvenile), length of exposure, endpoint (e.g., lethality, growth), hardness of the test water (mg/L as $CaCO_3$), and control survival. For tests conducted with juvenile freshwater mussels, it was also noted whether the transformation was in vivo (on a fish host) or in vitro (artificially). All information was tabulated into a single dataset to allow for various evaluations and calculations.

Water quality criteria developed by the U.S. EPA [7] and water quality standards developed by states and tribes typically use both acute and chronic values to protect aquatic organisms. These two estimated values are referred to as the criterion maximum concentration (CMC) and the criterion continuous concentration (CCC). The CMC is that estimated acute concentration that should not be exceeded in a one-hour averaging period more than once in a three-year period. The CCC is that estimated chronic concentration that should not be exceeded in a 4-d averaging period more than once in a three-year period.

Copper WQC and WQS are derived as equations with varying water hardness. When developing the freshwater mussel databases, acute toxicity data were tabulated and then toxicity endpoints (e.g., effect concentration to 50% of the organisms [EC50], or lethal concentration to 50% of the organisms [LC50]) were normalized to a water hardness of 50 mg/L as $CaCO_3$ with the equation described in Stephan et al. [7]. This approach is consistent with the approach used by U.S. EPA in developing the hardness-based 1996 WQC for copper [8]. Unless otherwise noted, only freshwater mussel data that met test acceptability requirements outlined in ASTM [11] were included in assessments presented in this evaluation. Test acceptability criteria for acute tests with glochidia are typically 24 h in duration (or longer if ecologically relevant for the species) and ≥90% control survival; for juveniles, tests should be 96 h duration with ≥90% control survival [11]. For chronic tests, ASTM [11] recommends the following test acceptability criteria for juveniles: 21 to 28 d duration and >80% control survival. Juvenile freshwater mussels used in testing should be produced in vivo unless tests are conducted to demonstrate that toxicity results are the same for juveniles produced either in vivo or in vitro.

Toxicity tests for glochidia and juveniles vary in duration because of life history and availability. The relatively short duration for toxicity tests with glochidia is based on limited exposure times in the water column [11]. Although the ecological relevance of glochidia tests have been questioned, tests performed with glochidia are particularly useful in cases in which availability of adult freshwater mussels for producing juvenile freshwater mussels are limited or propagation techniques are unknown for a species [11].

Acute data evaluation

After editing the database for consistency with ASTM E 2455-06 [11], the U.S. EPA 1996 copper CMC was evaluated. Species mean acute values (SMAVs) and genus mean acute values (GMAVs) for freshwater mussels were calculated. Freshwater mussel GMAVs were incorporated into the 1996 U.S. EPA copper criteria database [8]. A modified final acute value (FAV_{fm}) that incorporated freshwater mussel GMAVs and a modified CMC_{fm} ($CMC_{fm} = FAV_{fm}/2$) were determined following U.S. EPA guidelines [7,8].

In addition to evaluating the national copper criteria, the protectiveness provided to freshwater mussels by the Oklahoma acute copper WQS was evaluated. The final edited database, consistent with ASTM [11] test acceptability requirements, was further modified to include only Oklahoma freshwater mussel species as identified by Mather [12]. This approach is consistent with method 3 of the Oklahoma Water Resources Board [9]. The 36 Oklahoma GMAVs used in developing the acute copper standard for Oklahoma are not specifically identified; however, the approach Oklahoma used for developing the list is provided in the document *Rationale for Numeric Criteria* [9]. This approach describes the use of a U.S. EPA species list and then subsequent removal of species that do not occur in Oklahoma. An interim CMC is calculated from the modified species list, and the U.S. EPA criterion and interim CMC are then compared. The less stringent of the two numbers is incorporated as the Oklahoma state standard (CMC). Consistent with Stephan et al. [7], if the species distribution has less than 59 GMAVs, then the FAV is determined on the basis of the data for the four most sensitive genera. The Oklahoma Water Resources Board document *Rationale for Numeric Criteria* [9] uses the four lowest GMAVs for determining the copper FAV. We calculated SMAVs and GMAVs with the Oklahoma-specific freshwater mussel database. Table 1 summarizes the freshwater mussel species and associated SMAVs and GMAVs used in evaluating U.S. EPA WQC and the Oklahoma WQS. An Oklahoma (OK)-specific freshwater mussel final acute value (OK-FAV_{fm}) and freshwater mussel CMC (OK-CMC_{fm} = OK-$FAV_{fm}/2$) were determined following the methodology described in the *Rationale for Numeric Criteria* [9].

Chronic data evaluation

To determine the protection provided by the hardness-based 1996 U.S. EPA chronic WQC and the state of Oklahoma WQS, we evaluated all freshwater mussel toxicity data that used longer term exposures. Chronic values (geometric mean of the no observed effect concentration and the lowest observed effect concentration) were calculated using both lethal and sublethal (e.g., growth) freshwater mussel responses.

Because of the limited number of chronic tests available (fewer than eight families), the acute to chronic ratio (ACR) approach [7] was used for developing a CCC incorporating freshwater data for all freshwater mussels (CCC_{fm}) and an Oklahoma-specific freshwater mussel CCC (OK-CCC_{fm}). Acute to chronic ratios were determined for each freshwater mussel species for which both acute and chronic toxicity data were available. The U.S. EPA 1996 [8] copper criteria update and Oklahoma chronic WQS used ACRs for determining the CCC. Acute to chronic ratios used in the U.S. EPA calculation were required to have SMAVs close to the FAV, and we used a similar approach to calculate an ACR for deriving the CCC_{fm} and OK-CCC_{fm}.

Table 1. Freshwater unionid mussel species and associated species mean acute values (SMAVs) and genus mean acute values (GMAVs) used in evaluating U.S. Environmental Protection Agency copper criteria and Oklahoma copper standard. Only those species that met the American Society for Testing and Materials test acceptability requirements for toxicity testing with unionid mussels are listed. Species noted with an asterisk (*) indicate mussels common to Oklahoma (OK). All data were normalized to a water hardness of 50 mg/L as $CaCO_3$

Taxonomic name	Common name	SMAV (μg/L)	GMAV (μg/L) All species	GMAV (μg/L) OK species
*Actinonaias ligamentina**	Mucket	15.8		15.8
Actinonaias pectorosa	Pheasantshell	34.9	23.4	
Alasmidonta heterodon	Dwarf wedgemussel	26.1	26.1	
Epioblasma capsaeformis	Oyster mussel	3.27	3.27	
Lampsilis abrupta	Pink mucket	10.8		
*Lampsilis cardium**	Plain pocketbook	66.3		
Lampsilis fasciola	Wavy-rayed lampmussel	9.14		
*Lampsilis rafinesqueana**	Neosho mucket	10.4		
*Lampsilis siliquoidea**	Fatmucket	10.2	14.7	19.2
Lasmigona subviridis	Green floater	41.6	41.6	
*Leptodea fragilis**	Fragile papershell	28.4		
*Leptodea leptodon**	Scaleshell	6.69	13.8	13.8
*Ligumia subrostrata**	Pond mussel	47.4	47.4	47.4
Medionidus conradicus	Cumberland moccasin shell	15.6	15.6	
*Megalonaias nervosa**	Washboard	56.8	56.8	56.8
*Potamilus ohiensis**	Pink papershell	4.25	4.25	4.25
*Pyganodon grandis**	Giant floater	44.4	44.4	44.4
*Utterbackia imbecillis**	Paper pondshell	22.5	22.5	22.5
Venustaconcha ellipsiformis	Ellipse	3.04	3.04	
*Villosa iris**	Rainbow	16.8	16.8	16.8

RESULTS

Acute WQC/WQS

More than 200 acute observations for glochidia and juveniles were identified from 10 sources [13–21] (M. T. McCann, 1993, Master's thesis, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA). The database included toxicity data for 20 species from 14 genera. On applying the ASTM [11] test acceptability requirements, the number of observations was reduced to 101 (referred to hereafter as the final acute database [FA database]) having 20 species from 14 genera. The primary reasons for eliminating observations included: glochidia tests >24 h, juvenile tests <96 h, and test acceptability control survival criteria were not met.

Species in the subfamily Anondontinae, which includes the genera *Alasmidonta* and *Utterbackia*, have comparatively large glochidia and life histories where glochidia are released into the water. The released glochidia remain viable and are able to successfully attach to a host fish for several days following release. This life history strategy supports the use of longer exposures (>24 h) and is the reason we included three 48-h *U. imbecillis* tests as well as supporting the use of the single *A. heterodon* test. Finally, we also included seven 48-h juvenile tests with *Actinonaias pectorosa* (M. T. McCann, Master's thesis) because longer term (96-h) exposures were not available.

Within the 101-observation FA database were 61 glochidia tests and 40 juvenile tests. Three species had only juvenile tests, nine species had only glochidia tests, and eight species had both glochidia and juvenile tests. The two species with the greatest number of observations were *Villosa iris* (18 observations) and *U. imbecillis* (17 observations). Of the 18 observations for *V. iris*, 15 were glochidia tests and three were juvenile tests, whereas the 17 observations for *U. imbecillis* included results for seven glochidia tests and 10 juvenile tests. Eight species had only one observation (*A. heterdon*, *Lampsilis cardium*, *Leptodea fragilis*, *Leptodea leptodon*, *Ligumia subrostrata*, *Megalonaias nervosa*, *Potamilus ohiensis*, and *Venustaconcha ellipsiformis*). For those eight species with only a single observation, all were glochidia tests except for *L. leptodon*.

Utterbackia imbecillis was the only species for which we found 50% lethal (LC50) or effect (EC50) concentrations for both in vivo- and in vitro-produced juveniles. A total of 22, 96-h juvenile tests [13,15,18] were conducted, with 12 tests of in vitro–produced juveniles and 10 tests of in vivo–produced juveniles. The overall 96-h geometric mean EC50 for *U. imbecillis* was 25.9 μg/L. The geometric mean EC50 for in vivo–produced juveniles was 21.5 μg/L, and the geometric mean for in vitro–produced juveniles was 30.4 μg/L. Within a single author's publication [15], the geometric mean EC50 for in vivo–produced juveniles was 26.2 μg/L (n = 7) and the in vitro geometric mean was 23.7 μg/L (n = 10). Because a clear relationship between in vivo- versus in vitro–produced juveniles could not be identified, we used only toxicity test results from *U. imbecillis* juveniles produced in vivo.

Using the FA database, we calculated SMAVs and then GMAVs for those genera with more than one SMAV. The GMAV equaled the SMAV for those genera with data for only one species. The 14 freshwater mussel GMAVs were incorporated into the list of 43 GMAVs identified in the 1996 U.S. EPA copper criteria [8]. The probability distribution of the 57 GMAVs (14 freshwater mussels plus 43 other species) is provided in Figure 1. Genus mean acute values for freshwater mussels accounted for 10 of the lowest 15 GMAVs, with three mussel species having the lowest GMAVs (Fig. 2). All freshwater mussel GMAVs were in the lower half of the GMAV distribution (Fig. 1). The lowest freshwater mussel GMAV (*Venustaconcha*, GMAV 3.04 μg/L) was about a third of the GMAV of the lowest nonmussel (Cladoceran, GMAV 9.92 μg/L). The three lowest freshwater mussel GMAVs were also lower than the CMC of 7.286 μg/L (at hardness of 50 mg/L $CaCO_3$) determined in the 1996 U.S. EPA copper criteria [8].

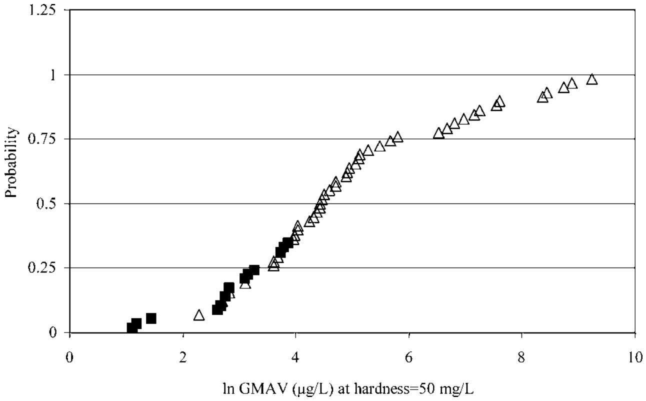

Fig. 1. Probability distribution of the natural log of genus mean acute values (GMAVs) with addition of mussel GMAVs used in evaluating the U.S. Environmental Protection Agency (U.S. EPA) copper water quality criteria. Freshwater mussel species are indicated by (■). All other species indicated by (△).

At a hardness of 50 mg/L, the CMC_{fm} (calculated after adding 14 freshwater mussel GMAVs to the 43 other GMAVs already in the 1996 criteria document) is 2.790 µg/L (Table 2).

To evaluate the Oklahoma copper standard, we edited the FA database to include freshwater mussel species known to occur in Oklahoma (OK-FA database), an approach consistent with method 3 in Oklahoma's *Rationale for Numeric Criteria* [9]. The OK-FA database had 67 observations for 12 species from nine genera. Within the 67-observation OK-FA database, there were 42 glochidia tests and 25 juvenile tests. One species (*L. leptodon*) had only a juvenile test, six species had only glochidia tests, and five species had both glochidia and juvenile tests. As was the case with the FA database, the two species in the OK-FA database with the greatest number of observations were *V. iris* (19 observations) and *U. imbecillis* (17 observations). Six species had only one observation (*L. cardium*, *L. fragilis*, *L. leptodon*, *L. subrostrata*, *M. nervosa*, *P. ohiensis*). For those six species with only a single observation, all were glochidia tests except for *L. leptodon*.

Using the OK-FA freshwater mussel database, we calculated OK-SMAVs and then OK-GMAVs for those unionid genera with more than one OK-SMAV (Table 1). The OK-GMAV equaled the OK-SMAV for those genera with data for only one specie. For the existing Oklahoma copper CMC, the four lowest GMAVs identified [9] were *Daphnia* (17.08), *Ceriodaphnia* (18.77), *Gammarus* (22.09), and *Plumatella* (37.05). Six of the freshwater mussel GMAVs are less than the GMAV for *Plumatella*. Four of the unionid GMAVs (*Potamilus*, *Actinonaias*, *Leptodea*, *Villosa*) are less than the lowest GMAV (*Daphnia*, 17.08) used in calculating the Oklahoma acute copper standard. At a hardness of 50 mg/L, the $OK\text{-}CMC_{fm}$ calculated with the four lowest GMAVs (all mussels) for Oklahoma species (36 OK-GMAVs plus nine Oklahoma freshwater mussel GMAVs) is 5.418 µg/L (Table 2).

Table 2. Evaluation of U.S. Environmental Protection Agency (U.S. EPA) water quality criteria (WQC) and Oklahoma water quality standards (WQS) for copper at hardness = 50 mg/L as $CaCO_3$. Acute to chronic ratios (ACRs) were determined for each freshwater mussel species for which both acute and chronic toxicity were available

WQC/WQS	CMC (µg/L)[a]	ACR	CCC (µg/L)[b]
Hardness-based 1996 U.S. EPA WQC	7.285	2.823	5.161
Unionid recalculated, U.S. EPA WQC	2.790	3.205	1.741
Current Oklahoma WQS	9.989	2.823	7.077
Unionid recalculated, Oklahoma WQS	5.418	3.205	3.381

[a] Equations below are either existing criterion maximum concentration (CMC) (U.S. EPA [8] and Oklahoma [9]) or unionid-modified CMC (noted by subscript fm). The original slope was reserved from U.S. EPA [8].
$CMC = e^{0.9422(\ln \text{hardness})-1.700}$
$CMC_{fm} = e^{0.9422(\ln \text{hardness})-2.660}$
$OK\text{-}CMC = e^{0.9422(\ln \text{hardness})-1.3844}$
$OK\text{-}CMC_{fm} = e^{0.9422(\ln \text{hardness})-1.996}$

[b] Equations below are either existing criterion continuous concentration (CCC) (U.S. EPA [8] and Oklahoma [9]) or unionid-modified CCC (noted by subscript fm). The original slope was reserved from U.S. EPA [8].
$CCC = e^{0.8545(\ln \text{hardness})-1.702}$
$CCC_{fm} = e^{0.8545(\ln \text{hardness})-2.788}$
$OK\text{-}CCC = e^{0.8545(\ln \text{hardness})-1.386}$
$OK\text{-}CCC_{fm} = e^{0.8545(\ln \text{hardness})-2.125}$

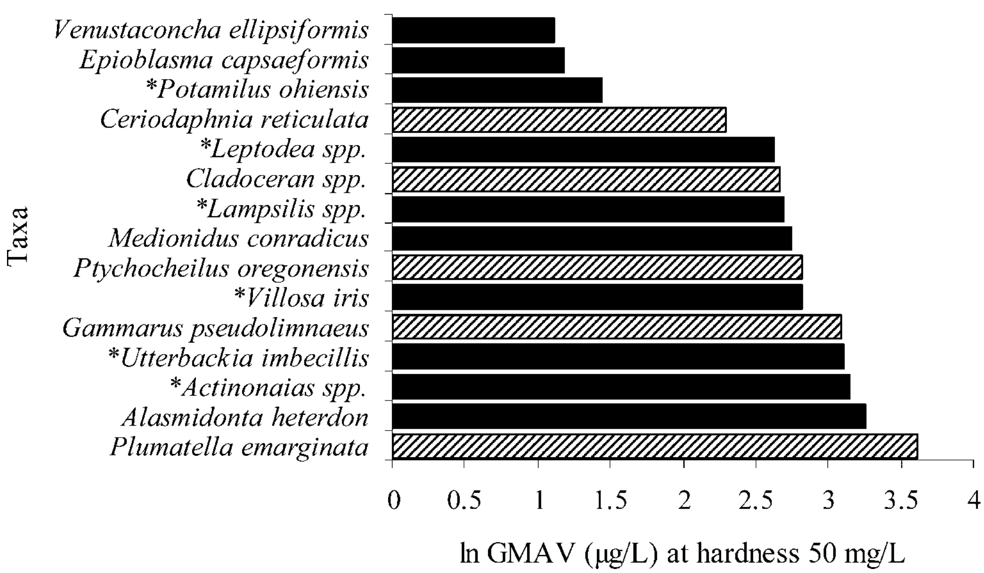

Fig. 2. Natural log of the genus mean acute values (GMAVs) for the 15 most sensitive taxa used in evaluating the U.S. Environmental Protection Agency (U.S. EPA) water quality criteria. Oklahoma freshwater mussels indicated by (*). Freshwater mussel species are indicated by (■). All other species are indicated by hatched bars.

Table 3. Acute to chronic ratios (ACRs) for freshwater mussels were calculated for those tests in which both species mean acute values (SMAVs) and chronic values (ChVs) were determined as part of the same study [7]. Species noted with an asterisk (*) indicate a mussel common to Oklahoma. Chronic values, SMAVs, and ACRs are listed as reported in the original publications (for the hardness tested and not adjusted to a consistent hardness of 50 mg/L as $CaCO_3$)

Taxon	Most sensitive chronic endpoint	ChV (μg/L)	SMAV (μg/L)	ACR	Source
Epioblasma capsaeformis	Growth	4.6	19	4.1	23
*Lampsilis siliquoidea**	Survival and growth	8.5	34	4.0	23
Medionidus conradicus	Cellulolytic activity	15.5	50	3.226[a]	22
*Villosa iris**	Growth	5.1	24	4.7	23

[a] ACR not reported in the original publication [22].

Chronic WQC/WQS

Table 3 summarizes the chronic toxicity test results for those species having both acute and chronic toxicity data available [22,23]. Chronic toxicity endpoints included growth, survival, or cellulolytic activity. Cellulolytic activity is not a primary endpoint identified by ASTM [11]. However, given the few longer term copper exposures with freshwater mussels, we have included all chronic data that met data quality objectives. Only four species had sufficient data to calculate ACRs that ranged from 3.226 to 4.7 (Table 3).

The U.S. EPA copper CCC calculation [8] used only ACRs that had SMAVs close to the FAV. The FAV_{fm} determined when calculating the CMC_{fm} was 5.581 μg/L at a hardness of 50 mg/L $CaCO_3$. For the four freshwater mussel species with chronic values, *Epioblasma capsaeformis* had a hardness-corrected SMAV of 5.774 μg/L, which is close to the FAV_{fm}. The U.S. EPA often calculates ACRs to three significant digits. Wang et al. [23] identified an ACR of 4.1 for *E. capsaeformis*, which we recalculated to three significant digits (4.130). The hardness-normalized SMAVs for other unionids having corresponding chronic effect concentrations were not near the FAV_{fm}, and they were not used. A revised overall ACR of 3.205 was determined by taking the geometric mean of the *E. capsaeformis* ACR and the two ACRs (2.418 and 3.297) used by the U.S. EPA [8]. With the revised ACR of 3.205 and the FAV_{fm} and OK-FAV_{fm}, the CCC_{fm} was calculated to be 1.741 μg/L at 50 mg/L $CaCO_3$, and the OK-CCC_{fm} was calculated to be 3.381 μg/L at 50 mg/L $CaCO_3$ (Table 2).

DISCUSSION

No data for the Asian clam (*Corbicula fluminea*) were included in the database. In North America, the Asian clam is an exotic invasive species and is often plentiful in areas in which other freshwater mussel species or other invertebrates no longer exist. For example, Ingersoll et al. [24] identified five sediments from the Grand Calumet River and Indiana Harbor Canal (Indiana) as too toxic to conduct bioaccumulation tests with the oligochaete *Lumbriculus variegates*. However, *Corbicula* spp. were observed in those sediment samples and were used for assessing bioaccumulation. Those results indicate that *Corbicula* spp. are very tolerant to some contaminants. Furthermore, the reproductive strategy of the Asian clam is significantly different than native freshwater mussels. For these reasons, we determined that copper toxicity data generated for the Asian clam might not be appropriate for considering the protectiveness of water quality criteria and standards for freshwater mussels.

The ASTM standard [11] provides guidance that acute toxicity tests should be conducted for 24 h with glochidia. The standard also notes that a 48-h toxicity test with glochidia might be used for species with a life history in which glochidia are released into the water column and remain viable for days before attaching to a host (in contrast to species that release glochidia in mucus strands or in conglutinates). Many species in the subfamily Anodontinae release glochidia into the water column; therefore, a longer glochidia test is ecologically relevant for these species. Among species for which copper toxicity data were retrieved, we used 48-h exposure results for *Alasmidonta* and *Utterbackia*, which are in the subfamily Anodontinae. Species in the genera *Pyganodon* and *Lasmigona* are also in the subfamily Anodontinae, but we had either 24-h tests only for these (*Pyganodon*) or data only for juveniles (*Lasmigona*).

Warren [15] determined that effect concentrations using artificially transformed (from glochidia) juvenile freshwater mussels were similar or slightly lower than those from tests with juveniles produced in vivo; therefore, results from in vitro tests were protective of natural populations. However, when we evaluated across all the 96-h *U. imbecillis* tests, we determined a greater difference between in vivo and in vitro tests, with in vitro tests having higher effect concentrations. Additional work should be conducted to further evaluate the use of artificially propagated juveniles; however, our results support the ASTM guidance [11] of using only juvenile freshwater mussels transformed with the use of host fish.

Data for native freshwater mussels are rarely included in the derivation of WQC or WQS. The 2007 U.S. EPA update of the ambient WQC for copper [25] did include select freshwater mussels. The 2005 Oklahoma WQS [10] does not include freshwater mussels to derive numerical standards. Instead, the WQS uses a regional list of warm-water organisms developed by the Texas Water Commission and, therefore, relies on surrogate species sensitivity to protect freshwater mussels from exposure to contaminants. Wang et al. [21] compared the sensitivity of commonly tested organisms (cladocerans *Ceriodaphnia dubia* and *Daphnia magna*, amphipods *Hyalella azteca*, fathead minnows *Pimephales promelas*, and rainbow trout *Oncorhynchus mykiss*) and early life stages of freshwater mussels and determined that mussel species are typically more sensitive than these organisms in acute copper or ammonia toxicity tests and might not be suitable as indicators of native freshwater mussel sensitivity. Because Asian clams and commonly tested freshwater organisms might not represent mussel sensitivity to toxicants, it is important that WQC and WQS include freshwater unionid data to better protect native mussel fauna.

The edited FA database and the OK-FA database include GMAVs for freshwater mussel species commonly found in North American and Oklahoma waterbodies. In general the GMAVs for freshwater mussels were among the most sensitive genera. Figure 1 reflects the incorporation of the 14 freshwater mussel GMAVs into the U.S. EPA GMAV database [8]. Figure 2 shows that greater than 50% of the 15 most sensitive species are freshwater mussels. As discussed previously, freshwater mussel species often have GMAVs for copper similar to more sensitive species.

Additional analysis of the copper concentration necessary to protect freshwater mussels from chronic exposures should be conducted. This concern is significant because the freshwater mussel exposures used to determine chronic effects were either 28 [23] or 30 d [22] long, and mussels are long-lived organisms, with some species often living in excess of 30 years. A 28- or 30-d chronic exposure might not adequately represent the concentrations of copper that elicit harmful effects for a long-lived species [23].

Little information is available that documents injury to freshwater mussels from permitted discharges because mussel kills often go unnoticed and the occurrence of glochidia and juvenile freshwater mussels in the environment are difficult to assess. Although not directly linking discharges to the decline of native unionids, Miller and Obermeyer [26] and Miller and Lynott [27] suggest that one of the factors leading to the observed increases in freshwater mussels in the Verdigris River (Oklahoma) is a reduction in contaminants from effluent discharges. Thus, defining effect concentrations for contaminants in a laboratory is an important aspect of deriving protective water quality approaches. The likelihood that these exposures occur in the environment is also important.

The state of Oklahoma has identified several areas as freshwater mussel sanctuaries [5], and mussel harvesting is not allowed in those areas. Freshwater mussel sanctuaries include the first 2 miles of any river entering Oklahoma and the first 2 river miles below any impoundment structures, Tenkiller Lake, the Kiamichi and Illinois Rivers, and three different segments of the Poteau River. To more adequately safeguard freshwater mussels in those areas, additional protective measures should be evaluated, including the effectiveness of state water quality standards. We used the Oklahoma Water Resources Board water quality database [28] to provide insight regarding the likelihood that copper could be a cause for concern in the Oklahoma freshwater mussel sanctuaries. We reviewed all available copper data for the Barren Fork and the Poteau, Illinois, and Kiamichi Rivers. The Barren Fork is considered part of the Illinois River. Copper concentrations were compared with the stream hardness-adjusted Oklahoma state standards (OK-CMC and OK-CCC) and the stream hardness-adjusted OK-CMC_{fm} and OK-CCC_{fm}. For each river, an average hardness was calculated from all available data. Copper concentrations were reported as total copper.

The Barren Fork of the Illinois River in northeast Oklahoma had 15 copper observations, and 14 of those values were less than the minimum reported value (referred to hereafter as less than concentrations). Four of the 14 less than observations were greater than the OK-CMC_{fm} and OK-CCC_{fm}, but less than the current OK-CMC and OK-CCC. Eleven of the 15 values (14 less than plus one measured) were lower than the OK-CCC_{fm}. Of the 33 observations for the Poteau River in southeast Oklahoma, like the Barren Fork, 15 were less than concentrations, with 13 of those 15 greater than the OK-CMC_{fm} and OK-CCC_{fm}. For the 18 recorded copper observations on the Poteau River, 14 observations were less than or equal to the OK-CCC_{fm}, but two of the measured observations were greater than the OK-CMC_{fm}. All 18 observations with recorded copper concentrations were less than the current OK-CMC, and 16 were less than the current OK-CCC. Of the 30 observations for the Illinois River, 27 were less than concentrations. A total of 21 observations (19 less than and two measured) were lower than the OK-CCC_{fm}. One measured observation was less than the current OK-CCC but was greater than the OK-CMC_{fm}. The Kiamichi River in southeast Oklahoma had the greatest number of observations. Of the 54 observations, as was the case for the other three rivers, 27 of the observations were less than values and all those values exceeded the OK-CCC_{fm} and 18 exceeded the OK-CMC_{fm}. Of the 27 reported copper concentrations, 18 exceeded the OK-CCC_{fm}, whereas seven of those 27 exceeded the OK-CMC_{fm} and six exceeded the current OK-CCC.

This overview of the Oklahoma copper data is not meant to provide a definitive answer regarding the imperilment of freshwater mussel populations within Oklahoma sanctuaries from exposure to copper. The available data indicate that the minimum reported value for copper concentrations used in monitoring might not be low enough to ensure protection of Oklahoma freshwater mussels and that there are instances in which stream copper concentrations meet current Oklahoma state standards (OK-CMC or OK-CCC) but are greater than the OK-CMC_{fm} and OK-CCC_{fm} we derived. More extensive monitoring and a recalculation of state copper standards might be necessary to ensure that mussel populations are protected within the Oklahoma freshwater mussel sanctuaries.

Ward et al. [29] evaluated the concentrations of ammonia, chlorine, and copper in three North Carolina streams. They found that exposures to copper were often above the concentrations of concern for the protection of freshwater mussels in all three drainages. Their results show that in site-specific assessments, concentrations of copper in the environment could be a contributing factor to declines in freshwater mussel populations or in preventing their recovery.

The recent copper WQC based on the biotic ligand model (BLM) [25] was released after this study was reviewed by the journal. Although a complete reanalysis of our data was not possible to meet publication timelines, we did conduct a preliminary review of the mussel toxicity data included in the new WQC and a re-evaluation of a subset of our data with the BLM approach outlined in [25]. The new WQC [25] includes acute toxicity data for juveniles of two species of freshwater mussels, *A. pectorosa* and *U. imbecillis*. No glochidia data are included. The U.S. EPA also identifies other data that were considered but not used in the development of the criteria [25]. Toxicity data included in U.S. EPA appendix B [25] included tests with a number of glochidia and juvenile results that we used in the evaluations in our paper. Although no specific reason is provided for not including freshwater mussel data from these additional toxicity tests [25], general reasons provided for excluding data include exposure duration, insufficient water quality data available to apply the BLM, or use of dilution waters not considered appropriate. None of the mussel tests in U.S. EPA appendix B [25] are identified in appendix H of [25] as unused data. Data identified as unused do not meet the basic requirements of test acceptability as outlined by Stephan et al. [7].

In our preliminary evaluation of the BLM, we selected a subset of mussel toxicity data for which we had water quality variables readily available. Wang et al. [21] tested freshwater mussels in well-defined ASTM hard water as dilution water so we were able to easily estimate ion concentrations for use in the BLM in this particular test water. We determined BLM-normalized EC50s for eight genera of freshwater mussels (10 species) and then calculated GMAVs. Because we had toxicity data for *Actinonaias ligamentina*, when calculating the GMAV for *Actinonaias* sp. we used the *A. pectorosa* toxicity data [25] included in the revised U.S. EPA copper criteria. Using Keller's data as referenced by the U.S. EPA [25] and the acute toxicity data from Wang et al. [21], we calculated eight mussel GMAVs and then compared those freshwater mussel GMAVs that were lower than the fourth ranked GMAV identified in the new WQC (Amphipod, *Gammarus pseudolimnaeus*—GMAV 8.57 μg/L [25]). Four mussel GMAVs were less than the fourth ranked U.S. EPA GMAV, including; *L. leptodon* (one juvenile test, GMAV = 8.3 μg/L), *Potomilus ohiensis* (one glochidia test, GMAV = 4.8 μg/L), *E. capsaeformis* (two juvenile tests, GMAV= 3.58 μg/L), and *V. ellipsiformis* (one glochidia test, GMAV = 3.2 μg/L). We inserted the eight mussel GMAVs into the U.S. EPA list of GMAVs [25] and reranked and recalculated the CMC (n = 35; 27 U.S. EPA GMAVs plus eight additional mussel GMAVs). A CMC_{fm} was calculated to be 1.728 μg/L, which is lower than the new BLM-based U.S. EPA WQC of 2.097 μg/L. Using the new U.S. EPA ACR of 3.23 [25], we calculated a CCC_{fm} of 1.070 μg/L (compared with U.S. EPA CCC of 1.3 μg/L). Although this preliminary reevaluation of the data based on the BLM should not substitute for a complete reanalysis, our evaluation indicates that the new U.S. EPA copper WQC based on the BLM needs further evaluation before the approach is applied to waterbodies with populations of freshwater mussels.

The use of the water effects ratio [30] and the BLM [31] to predict bioavailability and toxicity are approaches for deriving acceptable copper concentrations, and the U.S. EPA 2007 revised copper criteria [25] used the BLM in an effort to better predict bioavailability of copper. However, these approaches do not assess the exposure to suspension feeders of copper bound to particulates. Freshwater mussels are suspension feeders, and copper bound to particulate material could serve as a second exposure pathway. For example, Weltens et al. [32] found that ingestion of particulate-bound cadmium causes accumulation and acute toxicity in other filter-feeding organisms. The significance of copper-laden particle ingestion by freshwater mussels should be evaluated to determine whether it is a contributing factor to declines in their populations.

Currently the U.S. Geological Survey (USGS) is conducting acute toxicity tests with juvenile freshwater mussels to determine the relationship between water hardness and dissolved organic carbon on copper toxicity and to evaluate whether similar relationships are observed for mussels compared with other aquatic species (N. Wang, USGS, Columbia, MO, unpublished data). However, these studies will not determine whether copper in the diet or in a particulate form is available to freshwater mussels in longer term exposures. On page 1 of the updated 2007 U.S. EPA copper WQC [25] based on the BLM states that the ''document constitutes EPA's scientific recommendation regarding ambient concentrations of copper, it does not substitute for the Clean Water Act or EPA's regulations, nor is it a regulation itself.'' Given the continuing decline in populations of freshwater mussel species and concerns related to the application of the BLM to estimate the potential effect on suspension-feeding organisms such as mussels, implementation of this new criteria should only be done following a better understanding of protection afforded to freshwater mussels. Specifically, toxicity estimates based on the BLM and dissolved copper might not adequately protect suspension-feeding mussels exposed to metals in the diet.

We used the recalculation procedure identified by the Oklahoma Water Resources Board *Rationale for Numeric Criteria* [9], which is similar to the U.S. EPA's recalculation procedures [33]. This was done to provide a recommendation for freshwater mussel protection following Oklahoma procedures rather than a mechanism that might not be recognized as legitimate by the state regulatory entity. We evaluated Oklahoma copper standards to illustrate that they might not be protective of Oklahoma freshwater mussel fauna. Although these practical concerns are important for our application, they point to a larger issue associated with exclusion of species from datasets when developing water quality standards.

Before truncating a database that meets data quality objectives, it is important to consider what species in that database are intended to represent. Minimum dataset requirements have been specified for WQC development in the United States such that data available for tested species are considered a useful indication of the sensitivities of the numerous untested species [7,34]. The underlying assumption is that if acceptable data are available for a number of taxa from a variety of taxonomic and functional groups, a reasonable level of protection will probably be provided. Important points to consider when identifying what a species in a database represents include its relevance to only that species, to untested species closely related to the tested species (e.g., genus, family, order), to organisms of similar physiological/ecological niches (e.g., benthic suspension feeders), or to other untested species that could be of a similar sensitivity to contaminants (regardless of taxonomic or physiologic considerations). If the database is to represent taxon-level responses, then minimum database size would need to be far greater than are typically available (or required) to provide adequate representation of the entire aquatic community. If the database is to represent a broad range of toxicological responses (including the overwhelming majority of untested species), then eliminating a species not known to occur locally might not be justified without a more extensive examination of the relative sensitivity of species that do occur locally. This information is frequently not known with the limited toxicological data available.

Making the above argument specific to freshwater mussels is instructive, although the issue applies to an entire toxicological database. Taxonomically, in North America, the family Unionidae comprises 49 genera and nearly 300 species [3]. Oklahoma historically had 55 species of freshwater mussels with 52 species known currently. Our Oklahoma-specific copper dataset eliminated data for eight freshwater mussel species not known to occur in the state, and the 12 freshwater mussel species that remain in the Oklahoma database are intended, in this application, to be representative of the other 40 untested species that occur in the state. However, it is unknown how the 40 species would compare in terms of copper sensitivity to the 12 species for which data have been retained and the eight species for which data have been eliminated. In short, our use of the existing regulatory approaches for calculating Oklahoma state standards by eliminating species that do not occur in the state is questionable if the view is taken that species in the larger database represent other untested species and not merely the species itself.

The development of standard procedures for freshwater mussel toxicity testing [11] provides methods sufficient on which to base policy decisions for copper. On the basis of historical and ongoing research concerning the status of freshwater mussels [2–5], and on the basis of our evaluation of the currently available copper toxicity data for freshwater mussels [13–16,18–21] (M. T. McCann, Master's thesis), federal and state agencies should consider the inclusion of freshwater mussel toxicity data in the derivation of water quality criteria and standards. Because toxicity to any life stage of freshwater mussels can affect growth, dispersal, community composition, abundance, diversity, or reproductive viability, toxicity data for freshwater mussels should be included in the databases used to derive national criteria and state standards. Including freshwater mussel toxicity data would provide better protection and help ensure ecosystem diversity is maintained in the future. Furthermore, the U.S. EPA WQC derived from water hardness or derived with the use of BLM and the Oklahoma water quality standards for copper might need to be revised to afford increased protection to freshwater mussels.

Acknowledgement—Financial support for this publication was provided by the University of Oklahoma, Oklahoma Biological Survey, Oklahoma Department of Wildlife Conservation under State Wildlife Grant, T-15-1; U.S. Fish and Wildlife Service; U.S. Geological Survey, Columbia Environmental Research Center; and Oklahoma Department of Environmental Quality. Although information in this paper was developed in part by these organizations, this publication does not necessarily reflect the views of these entities, and no official endorsement should be inferred. This publication has been reviewed in accordance with U.S. Geological Survey and U.S. Fish and Wildlife Service policy. We thank Caryn C. Vaughn (University of Oklahoma), Phil Moershel (Oklahoma Water Resources Board), and Joseph R. Bidwell (Oklahoma State University) for early reviews of this manuscript.

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