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EVALUATION OF THE RESIDENT-SPECIES PROCEDURE FOR DEVELOPING SITE-SPECIFIC WATER QUALITY CRITERIA FOR COPPER IN BLAINE CREEK, KENTUCKY

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Abstract – Acute toxicity tests were performed on each of nine different organisms in Blaine Creek (Lawrence County, KY) water to determine 48-h LC50 values for copper (Cu). Blaine Creek is the receiving stream for Kentucky Power Company's Big Sandy Plant fly-ash pond discharge. Selection of the nine tested species and integration of the results were based on the U.S. Environmental Protection Agency's (EPA's) resident-species procedure. The following LC50 (μg Cu/L) values were obtained (hardness 100–120 mg/L as $CaCO_3$): *Daphnia pulex* 37; *Physella* sp. 109; *Isonychia bicolor* 223; *Pimephales promelas* 284; *Stenonema* sp. 453; *Eurycea bislineata* 1,120; *Chironomus riparius* 1,170; *Orconectes* sp. 2,370; *Lepomis macrochirus* 4,300. A final acute value (FAV) of 10.1 μg Cu/L (hardness 50 mg/L as $CaCO_3$) was derived using the Blaine Creek database, which is lower than the EPA's national FAV (18.5 μg/L). The differences in FAVs were due to the effect of database size, not differences in the relative sensitivity of test organisms. Our findings provide caution for dischargers who may choose to use the resident-species procedure for future site-specific water-quality-criteria demonstrations. The resident-species procedure, because of the inherent conservative bias of the calculation process, tends to produce more stringent criteria when used on a site-specific basis. A modified recalculation procedure, in conjunction with site-specific metal bioavailability data, was judged to be the most appropriate method for deriving site-specific Cu criteria for Blaine Creek.

Keywords – Site-specific   Water quality criteria   Copper   Blaine Creek   Toxicity

INTRODUCTION

Approximately 10 years have passed since the first site-specific water quality criteria [1] were developed the U.S. Environmental Protection Agency's (EPA's) "Guidelines for Deriving Site-Specific Water Quality Criteria" [2]. Site-specific procedures [2,3] were developed to recognize the many physical, chemical, and biological factors that affect the toxicity of a substance to aquatic organisms. These factors can result in national or state water quality criteria (WQC) that are either overprotective or underprotective for a specific ecosystem [2,3]. The EPA site-specific criteria derivation procedures are designed to allow consideration of these factors, resulting in the development of site-specific criteria that should more accurately reflect the desired level of protection than national or state WQC. The use of site-specific information to modify national or state WQC has an immediate appeal because it allows an investigator to change the focus from protecting a diverse range of ecosystems to a single receiving system. The EPA site-specific WQC guidelines [2,3] are the principal mechanisms available to dischargers who may want to investigate the appropriateness of statewide or national WQC for a specific receiving system. In this research, site-specific WQC are developed for copper (Cu) in Blaine Creek, Kentucky (Fig. 1).

The EPA site-specific WQC procedures [2,3] are designed to consider two general factors that may make the national criteria inappropriate for a specific ecosystem. Site-specific modification of national or statewide WQC is based on differences in resident-species composition between the national database [4] and the site-specific community and/or the modulating role of site water on the toxicity of a chemical. The three procedures developed to address these factors are the recalculation procedure, which considers species residency; the indicator approach, which determines the site-water effect by testing species in site and reference water; and the resident-species procedure, which addresses both factors by generating an acute toxicity test database using resident species in site water [2,3].

In addition to the biological procedures outlined above, the EPA allows consideration of site-water effects on the bioavailability of heavy metals based on chemical analyses [5]. Although WQC and effluent limits have historically been expressed in terms of total recoverable Cu, it has been widely recognized for some time that Cu toxicity is principally due to soluble forms, such as the free ion Cu^{2+} and some hydroxy and carbonate complexes [4–7]. Precipitated and organically bound forms are generally considered less bioavailable [6,7]. Measurement of dissolved metals is thought

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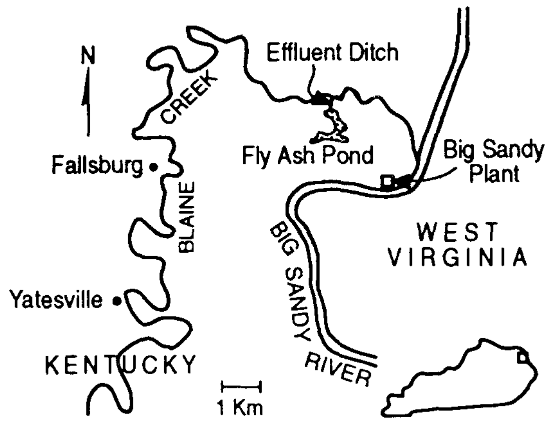

Fig. 1. Location of Blaine Creek and Kentucky Power's Big Sandy Power Plant.

to be a more accurate measurement of the portion of metal responsible for toxicity than either the acid-soluble or total recoverable metal methods [5]. In most water bodies, a significant portion of the metal is typically tied up in unavailable forms, whereas in most toxicity tests the metal initially is in a highly bioavailable form [4,5]. Site waters typically have higher levels of particulate matter and dissolved organics than lab water used for most toxicity tests.

Earlier studies dealing with the development of site-specific WQC [1,8,9] have dealt primarily with illustrating EPA procedures [2,3] and comparing lab results to field information. Although most studies reported in the literature have dealt with heavy metals [1,8,10–12], additional studies have been conducted dealing with ammonia [13,14], chlorine [14], and pentachlorophenol (PCP) [9]. A number of these studies have attempted to correlate lab toxicity results with instream biomonitoring [8,13] or experimental stream data [9]. Carlson and colleagues [8] used the indicator organism procedure to derive site-specific WQC for Cu at a number of sites in the Naugatuck River, Connecticut, and validated their results on the basis of ecological survey information. These researchers concluded that the site-specific criteria were protective of aquatic life, based on the observation that a healthy aquatic community existed where criteria were exceeded only slightly.

The work of Hedtke and Arthur [9] is noteworthy because they compared the results of the resident-species procedure for PCP using single-species lab toxicity tests to findings from large outdoor experimental streams containing the same species. They demonstrated that the order of species sensitivity in the lab and field was the same. Although predictions of field effects based on lab data were similar, some evidence indicated that the site-specific criteria may not have completely prevented adverse effects on all of the aquatic ecosystem. Other than the work of Parkerton and colleagues [10], who evaluated the indicator approach procedure, there has been little critical evaluation of EPA site-specific WQC development procedures [2,4]. These researchers found that use of different reference waters led to significantly different criteria, and that total zinc (Zn) toxicity was significantly reduced when it was allowed to interact with the dilution water for 24 h before adding the test organisms.

In this study, a complete acute toxicity database using nine species that fulfilled all the requirements of the national guidelines procedure [15] was generated for Cu in Blaine Creek (a receiving stream for a treated fly-ash pond effluent in eastern KY). In addition, the concentrations of total and dissolved Cu were determined in the fly-ash pond discharge and in Blaine Creek (after complete mixing of the creek with the effluent) to allow estimation of the amount of bioavailable Cu. The results of the Cu speciation study were subsequently used to adjust the site-specific Cu criteria derived using the EPA recalculation procedure. This approach was used as an alternative method to derive site-specific WQC based on site-water effects. The overall objective of this study was to evaluate EPA procedures to develop site-specific WQC based on our experience with Cu in Blaine Creek. This study was part of a technical demonstration for proposed site-specific Cu and selenium WQC for Blaine Creek.

MATERIALS AND METHODS

Study area

Blaine Creek is a fifth-order tributary to the Big Sandy River, located in eastern Kentucky. The Blaine Creek watershed covers an area of 686 km^2 and lies in the western Allegheny plateau ecoregion, which is characterized as having low to high hills, mixed mesophytic forest, and alfisol-type soils [16]. Blaine Creek receives treated fly-ash pond water from Kentucky Power Company's Big Sandy Power Plant, about 3.2 km upstream of the Big Sandy River confluence (Fig. 1). The Big Sandy Power Plant is coal-fired, and its two units have a combined generating capacity of 1,060 megawatts (MW). Fly ash produced during the coal combustion process is sluiced to a 44-ha settling pond that discharges into Blaine Creek. At the time this study was undertaken, the discharge contributed <10% of the creek's flow. Historically, the discharge has comprised as much as 75% of creek flow during low flow conditions. Blaine Creek was recently impounded by a U.S. Army Corps of Engineers dam located near Yatesville, Kentucky. Because of this flow regulation, the fly-ash discharge would make up no more than 33% of total stream flow under worst-case conditions (stream low flow and effluent maximum design flow).

Long-term instream biomonitoring surveys conducted by Kentucky Power Company have indicated no measurable adverse effects on resident macroinvertebrate and fish communities caused by the fly-ash pond discharge [17,18]. These studies have actually documented that biological communities are enhanced when the instream waste concentration of the fly-ash discharge is highest.

Dilution water

Blaine Creek water was used as dilution water for all tests and was collected 10 km upstream of the discharge, near Fallsburg, Kentucky (Fig. 1), in 19-L polycarbonate carboys and stored at room temperature for a period not exceeding 14 d before use. The water was filtered (1.6 μm) due to high levels of suspended solids during initial water collection trips During the study, Blaine Creek was typified by highly variable flow due to the steep terrain of the watershed, which causes frequent flooding with associated elevated levels of suspended solids Because of the effect that suspended solids have on Cu bioavailability, the decision to filter the water for all tests was made to provide a dilution water with more consistent characteristics

Organism selection

Species used for toxicity testing were selected on the basis of potential residency in the Blaine Creek watershed, fulfillment of the national guidelines selection criteria [15], state requirements, and availability of the test organisms of a suitable age Residency of a species was based on long-term fish and macroinvertebrate biosurvey data from various stream sites [17,18] The only organism that we originally sought but could not obtain in sufficient quantity was the bluntnose minnow (*Pimephales notatus*) The fathead minnow (*P promelas*) was used as a surrogate species Nine different species were tested to fulfill the national guidelines requirements [15] for eight species in selected families and the State of Kentucky's requirement for a species from a Cu-sensitive family (Daphnidae)

Organism collection and holding

The following organisms were obtained from either in-house cultures or commercial sources *Daphnia pulex, P promelas, Lepomis macrochirus, Physella* sp , and *Chironomus riparius* The salamander *Eurycea bislineata*, crayfish *Orconectes* sp , and mayflies *Stenonema* sp and *Isonychia bicolor* were collected from locales where we knew the relative population abundance and condition based on prior collection experience After collection and transportation of organisms to the lab in coolers that had been chilled to the collection temperature, the organisms were allowed to acclimate to Blaine Creek water for a minimum of 48 h Temperature acclimation of test organisms was not a major concern in this study because collection sites remained within 2° of 20°C over the course of the study This period of acclimation was an acceptable balance between allowing the organisms to recover from the stress of handling and the potential problem of reduced health after long-term holding

Toxicity tests

Nonrenewal acute toxicity tests lasted 48 h and used 1 6-μm filtered Blaine Creek water as the dilution water All tests were conducted at 20°C with a 16 8-h light dark photoperiod Standard conditions were three replicate test containers containing 10 organisms each, with five concentrations and a control for each test Method development before performing definitive tests consisted of a series of screening tests with each species to optimize holding conditions and test parameters for nonstandard test species This design was essential for performing valid toxicity tests Testing methods generally followed those outlined by Weber [19] and *Standard Methods for the Examination of Water and Wastewater* [20] The screening tests resulted in selection of optimal test concentrations, which led to narrower confidence intervals (C I s) Samples for total recoverable Cu determinations were taken at the beginning and end of the test and analyzed in accordance with EPA method 200 7 [21] Species specific conditions are described below

Testing of *D pulex* and *P promelas* followed closely the protocols described by Weber [19], except three replicates (instead of two) of 10 organisms each were used and the test duration for both organisms was 48 h *Daphnia pulex* were cultured in filtered Blaine Creek water for at least one month before testing Neonates were <24 h old at test initiation and were tested in 100-ml Pyrex® beakers containing 50 ml test solution Three-week-old *P promelas* from in house cultures was tested in 1 L glass beakers with 750 ml test solution The minnows were reared in dechlorinated tap water (treated New River, VA, water) and transferred to Blaine Creek water one week before testing

Juvenile *L macrochirus* (35 mm length) were obtained from Kurtz Fish Hatchery (Elverson, PA) and were acclimated for one week in Blaine Creek water The average wet weight at the time of testing was 0 51 g per fish Fish were tested in 17 L polycarbonate vessels containing 7 L test solution for 48 h

The two-lined salamander, *E bislineata*, was collected from an unnamed tributary to Little Scary Creek (near Winfield, WV) and transported to the lab in coolers chilled at 20°C Organisms were acclimated before use to Blaine Creek water for 2 d without feeding Salamanders (average length 40 mm) were tested in covered 17 L polycarbonate vessels containing 6 L test solution An equal number of washed stones of similar size (10–20 cm maximum diameter) were placed in each test container to provide refugia

Crayfish (*Orconectes* sp) were collected from Sinking Creek (Newport, VA) and transported in chilled coolers Crayfish were acclimated for 48 h in Blaine Creek water before testing Low DO in screening tests necessitated gently aerating 5 L test solution with Pasteur pipettes in 17-L polycarbonate vessels Crayfish (30–40 mm in length) were used in the tests and were checked for exoskeleton condition before use

Mayflies (sixth- to eighth-instar *Stenonema* sp and *I bicolor*) were collected from Sinking Creek and acclimated for 48 h to Blaine Creek water before use Mayflies were tested in 2-L Nalgene® (Rochester, NY) containers with washed cobble added to provide a substrate Current was provided by a stir bar positioned over a magnetic stir plate in the center of the container Stir-bar speed was determined from trial and error before conducting the definitive tests

Physella sp (<10 mm diameter) were obtained from in house cultures at Virginia Tech (Blacksburg, VA) and acclimated in Blaine Creek water for 48 h before use They were tested in loosely covered 350-ml glass culture dishes filled to within 1 5 cm of the top with test solution to prevent the snails from avoiding the toxicant Snails were considered dead after 48 h if no movement of the foot or antenna was evident in the test solution after being placed in control water for 5 min

Chironomus riparius was obtained from in house cultures at Virginia Tech and acclimated in Blaine creek water for 48 h before use. Second-instar midges were tested in 350-ml glass culture dishes using 250 ml test solution and 10 ml of glass beads (150–300 μm) as an inert substrate.

Water chemistry

DO, pH, and temperature were monitored daily at all concentrations when a sufficient volume was available. Alkalinity and hardness were measured at the beginning and end of the tests in the control and highest level. Water samples (50 ml) were collected at the surface of the containers from all treatment levels at the beginning and end of the tests. The samples were preserved with 150 μl of 50% HNO_3 and shipped to a commercial lab for total recoverable Cu analysis by EPA method 200.7 [21].

Copper speciation

The fraction of dissolved Cu in Blaine Creek water downstream of the fly-ash pond discharge was used as an estimate of instream bioavailable Cu. Analyses of total recoverable and dissolved Cu were performed on samples from the discharge and Blaine Creek during 1990 and 1991. Total recoverable Cu was determined using EPA methods 220.2 or 200.7 [21]. Dissolved Cu was determined by filtration through a 0.45-μm filter at the time of collection, followed by acidification with HNO_3 (0.5%). Analysis of the preserved samples was similar to EPA method 220.2, but without a digestion step. The ratio of total recoverable to dissolved Cu was used to adjust the final criterion value (in terms of total recoverable Cu).

Statistical analysis

The trimmed Spearman-Karber method [22] was used to calculate 48-h median lethal concentrations and corresponding 95% C.I.s. Total recoverable Cu measurements taken at the beginnings of the tests were used for calculation of LC50 values.

Integration of test results was conducted using the EPA final acute value (FAV) equation [15,23]. The FAV equation is an extrapolation procedure that plots the log of the acute values against the cumulative probability of the relative sensitivity of the species in the database to the chemical. The cumulative probability of a species is determined by ranking all acute values in the database and dividing by the total number in the database plus one. The FAV is the concentration that corresponds to a 0.05 cumulative probability level estimated using the best fit line through the four acute values closest to the 0.05 level. At the 0.05 level, 95% of the species in the statistical population that the database represents will be less sensitive, whereas 5% will be more sensitive. This procedure is designed to protect 95% of the species within the entire database. To obtain the criterion maximum concentration (CMC), the FAV is divided by two. For the purposes of this study, we derived a criterion continuous concentration (CCC) by dividing the site-specific FAV by the acute-to-chronic ratio (ACR) of 2.823 from the national criteria document [4].

RESULTS AND DISCUSSION

Acute toxicity tests

Based on the results of the nine definitive acute toxicity tests, *D. pulex* was the most sensitive tested species, with a LC50 of 37 μg/L Cu (Table 1). This agrees with the EPA database [4] that lists species in the family Daphnidae as among the most sensitive. The four most sensitive species in the Blaine Creek database were from a broad range of taxa and included *Physella* sp. (LC50 109 μg/L), *I. bicolor* (LC50 223 μg/L), and *P. promelas* (LC50 284 μg/L).

To compare the LC50 values from the Blaine Creek database directly with those of similar species as reported in the national criteria document [4], values were normalized to a hardness of 50 mg/L as $CaCO_3$, based on the following equation [4]:

$$\text{LC50 at hardness of } 50 = e^Y$$

$$Y = \ln(\text{LC50}) - 0.9422[\ln(\text{test hardness}) - \ln(50)]$$

where 0.9422 is the pooled slope between hardness and LC50 values for all species in the EPA database.

The two databases have a similar ranking of species sensitivity (Table 2). The hardness-adjusted LC50 value for *D. pulex* of 17.3 μg/L in Blaine Creek water is lower than the species mean acute value of 25.42 reported by the EPA. However, the genus mean acute value (GMAV) used to calculate the FAV by the EPA for *Daphnia* was 17.08, which is almost identical to these results. The response of 51.0 μg/L for the snail (*Physella* sp.) is quite similar to that of 39.33 μg/L for *Physa*, which is the most sensitive snail in the EPA database. The acute values for both the fathead minnow (*P. promelas*) and the crayfish (*Orconectes* sp.) are in close agreement in the two databases. No comparisons were possible for the two mayflies and the salamander tested because the EPA database does not contain any similar species.

There appears to be less agreement between the two databases for species that were less sensitive to Cu. Our LC50 value of 547 μg/L for *Chironomus* is appreciably higher than

Table 1. Results of acute toxicity tests with Cu using Blaine Creek water as dilution water

Rank	Species	LC50 (μg/L)	95% Confidence limits
1	*Daphnia pulex* (water flea)	37	35–38
2	*Physella* sp. (snail)	109	100–118
3	*Isonychia bicolor* (mayfly)	223	162–307
4	*Pimephales promelas* (fathead minnow)	284	242–334
5	*Stenonema* sp. (mayfly)	453	372–551
6	*Eurycea bislineata* (salamander)	1,120	872–1,450
7	*Chironomus riparius* (midge)	1,170	946–1,450
8	*Orconectes* sp. (crayfish)	2,370	1,830–3,070
9	*Lepomis macrochirus* (bluegill sunfish)	4,300	3,350–5,520

All tests were conducted at hardness values between 100 and 120 mg/L as $CaCO_3$.

Table 2 Comparison of the Blaine Creek acute database for Cu with that from the EPA [4]

Test species	LC50 (μg/L) Blaine Creek	EPA
Daphnia pulex	17 3	25 42
Daphnia	—	17 08
Physella sp	51 0	—
Physa	—	39 33
Isonychia bicolor	109	—
Pimephales promelas	133	115 5
Pimephales	—	91 29
Stenonema sp	212	—
Eurycea bislineata	524	—
Chironomus	547	76 92
Orconectes	1,110	1,397
Lepomis macrochirus	2,010	1,017

All acute values were standardized to a hardness of 50 mg/L as $CaCO_3$

the GMAV of 76 92 μg/L reported by the EPA This difference may be due to the use of different species of *Chironomus* and/or different test procedures The LC50 value for bluegill sunfish was twice as high as that reported by the EPA and may be due to the effect of site water on the bioavailability of Cu at higher test concentrations A bluish white precipitate was observed at higher test concentrations (>1,000 μg/L nominal), and the quantity appeared to increase with dose, indicating loss of Cu from the test solution The precipitate formed relatively rapidly and was evident at the higher test levels within hours The observation that the bioavailability of a metal changes during the exposure period was also reported by Parkerton and colleagues [10], who found that the toxicity of Zn is reduced by predosing site water 24 h before exposure of test organisms

Water quality parameters were similar for all toxicity tests, typical values for the dilution water were hardness, 100 to 120 mg/L as $CaCO_3$, alkalinity, 50 to 60 mg/L as $CaCO_3$, pH, 7 5 to 7 8 DO levels were within 80% of saturation for all tests Because the fly-ash pond discharge caused a substantial increase in the hardness of the water downstream (i e , 100–173 mg/L as $CaCO_3$ after addition of the effluent at 326 mg/L), the dilution water hardness was lower downstream of the discharge after complete mixing

In conducting the necessary acute toxicity tests, a number of practical decisions affect the relevance of the resident species procedure in accounting for the site-specific factors that may modify chemical toxicity The first decision that affects the results is species selection Although species selection guidelines are relatively broad [15], the actual species selected are based on practical considerations The availability of test organisms usually is a key factor in the selection process Ideally, test organisms should be from the system being studied, but because this can be difficult and have a negative impact on the ecosystem, representative organisms from a variety of sources are used This typically leads to reliance on standard test species as much as possible Although the selected species should represent ecologically important species covering a full range of sensitivities to the chemical of interest, the reliance on standard test organisms can lead to a prevalence toward the use of more sensitive species The more representative the test species are of the ecosystem being protected, the more accurate the resulting criteria will be for protecting aquatic life in a specific ecosystem A site-specific-criteria demonstration should not, by its very design, be subject to overprotective assumptions that often characterize national criteria

In addition to differences in species composition between a site and the national database, the resident species procedure is designed to account for the effect of site water on chemical toxicity The EPA site-specific guidance suggests collection of dilution water from a pristine site for use as diluent [4] In this study, water was collected 10 km upstream of the discharge because of limited river access and concern for nonpoint sources of pollution directly above the discharge Because the effluent substantially changed the chemical characteristics of the creek under low flow conditions (at least historically), the water used for toxicity testing was probably not representative of downstream conditions Collection of water below the discharge, after complete mixing, would more accurately reflect the effect of site water in the area of concern The authors support the recent change in the EPA site-specific guidelines that recommends using water from downstream of any potential pollution sources, after all are well mixed with the receiving system [5] Additional factors that can affect the relevance of the procedure are site-water variability, whether the water is filtered, and water storage conditions

Copper speciation

The geometric mean ratio of dissolved to total recoverable Cu in the discharge from 1990 to 1991 was 0 77 and indicated that approximately three-quarters of the Cu was in bioavailable form (Table 3) There was a distinct difference between the two years During 1990, the geometric mean of the ratios was 0 67 for the discharge, in 1991, Cu was almost completely in the dissolved form with a geometric mean ratio of 0 90 This difference in the relative amount of dissolved Cu may be attributed to climatic factors and decreasing suspended solid levels in the discharge Very wet conditions prevailed in 1990 (causing substantial stormwater runoff into the fly-ash pond), whereas drought conditions were evident in 1991

For regulatory purposes, the samples of concern are from the downstream site after complete mixing of Blaine Creek and the discharge The geometric mean dissolved-to-total-Cu ratio for the 1990 samples (n = 10) was 0 62, for the 1991 samples (n = 10), it was 0 54 When the two studies were pooled, the geometric mean of the ratios for all samples (n = 20) was 0 58 These results suggest that a substantial fraction of the Cu in Blaine Creek below the fly-ash pond discharge is not in a highly bioavailable form Considering the importance of Cu speciation in regulating its toxicity, application of criteria based on total recoverable Cu levels may be overprotective Use of the geometric mean ratio of dissolved to

Table 3. Copper speciation study of Big Sandy Plant fly-ash pond discharge and Blaine Creek samples downstream of discharge (μg/L), 1990 and 1991

	Outfall 001			Blaine Creek, below 001		
Date	Total	Dissolved	Ratio[a]	Total	Dissolved	Ratio[a]
7 Jul 90	13	14	1.0	4	3	0.75
17 Jul 90	23	18	0.78	6	—[b]	—
19 Jul 90	23	18	0.78	6	—[b]	—
24 Jul 90	12	11	0.92	5	3	0.60
31 Jul 90	19	12	0.63	5	4	0.80
7 Aug 90	13	14	1.0	4	3	0.75
14 Aug 90	23	3	0.13	6	5	0.83
21 Aug 90	8	—[b]	—	10	6	0.60
28 Aug 90	31	12	0.39	13	4	0.31
4 Sep 90	15	14	0.93	15	6	0.40
9 Sep 90	—[b]	—[b]	—	4	2	0.50
11 Sep 90	11	8	0.73	5	9	1.0
18 Sep 90	6	6	1.0	4	—[b]	—
24 Oct 91	4	3	0.75	2	1	0.5
29 Oct 91	6	6	1.0	3	3	1.0
5 Nov 91	20	20	1.0	7	5	0.71
12 Nov 91	24	25	1.0	8	7	0.88
18 Nov 91	24	27	1.0	5	5	1.0
25 Nov 91	19	21	1.0	7	5	0.71
5 Dec 91	24	23	0.93	5	1	0.20
9 Dec 91	28	30	1.0	10	2	0.20
16 Dec 91	42	25	0.60	4	2	0.50
23 Dec 91	43	35	0.81	4	2	0.50
Geometric mean	17	14	0.77	6	3	0.58

The ratio is equal to the dissolved Cu levels divided by the total recoverable value. All analyses based on split samples.
[a]For samples in which the dissolved Cu was greater than total Cu, the ratio was set equal to one.
[b]Sample lost due to contamination or not taken.

total recoverable Cu (from the fully mixed downstream location) to modify the national FAV would produce criteria that would provide adequate protection to aquatic life in Blaine Creek.

CRITERIA DERIVATION AND EVALUATION

As discussed earlier, a number of alternative procedures are available to derive WQC. Based on the information available for Blaine Creek, three alternatives to derive site-specific WQC for Cu are possible: (a) recalculation procedure, (b) resident-species procedure, (c) recalculation modified by Cu speciation data. These three alternatives result in distinctly different criteria that are summarized in Table 4. Choice of the most appropriate procedure should be driven by an evaluation of the site-specific factors that are most important in a specific situation and should consider the total sum of data available for a site.

Recalculation procedure

We believe that the first step in determining whether a site-specific WQC study is necessary should be calculating the FAV based on the recalculation procedure. The recalculation procedure entails no lab or field work, as nonresident species are removed from the national criteria database and the FAV is recalculated [2,3]. Because this procedure is designed to correct for differences in species composition between the national database and a specific site, it is an inexpensive method to determine if differences in species composition are a prominent factor for a specific situation. For Blaine Creek, the recalculation procedure involves mainly removing non-indigenous cold-water species such as the northern squaw-

Table 4. Calculation of the continuous maximum criterion (CMC) for Cu using different procedures at a hardness of 50 mg/L (unless otherwise noted, expressed in terms of μg/L Cu)

Criterion derivation procedure	Database size	FAV[a]	CMC	CMC at hardness 173
National	41	18.5	9.23	29.7
Site-specific				
Recalculation	33	19.4	9.68	31.2
Recalculation with metal speciation	33	33.4	16.7	53.8
Resident species	9	10.1	5.1	16.4
Resident species[b]	33	36.3	18.1	58.4

[a]Final acute value.
[b]Example of the role database size has in calculating FAV.

fish from the national database The recalculation procedure ($n = 33$) results in a FAV of 19 4 μg/L at a hardness of 50 μg/L (Table 4) This is slightly higher than the national value (18 5 μg/L), indicating only a slight difference in species sensitivity after nonresident species are excluded The CMC, using the mixed downstream site hardness, would be 31 2 μg Cu/L (Table 4) Although this procedure can address whether differences in species composition are factors, it does not address whether there is a site-water effect Additional work using a procedure that considers site-water effects would be recommended, based on the results of the recalculation procedure

Resident species

After generating LC50s for the nine species in our database, the next step was to integrate this information using the FAV equation The site-specific FAV based on the Blaine Creek database ($n = 9$) was determined to be 22 0 μg/L Cu at the mean test hardness of 112 mg/L as $CaCO_3$, whereas at a hardness of 50 mg/L as $CaCO_3$ the FAV is 10 1 μg/L Cu This is less than two-thirds of the national FAV of 18 5 μg/L at a hardness of 50 mg/L At the downstream hardness of 173 mg/L as $CaCO_3$, the resulting CMC is 16 4 and the CCC is 11 5 μg/L, compared to the statewide values of 29 7 and 21 1 μg/L, respectively This result was unexpected because LC50 values for similar species in both databases were generally within a factor of two (Table 2) and because the lowest acute value used to calculate the FAV was almost identical (Table 5) The results of the calculation pro cess erroneously imply that the Blaine Creek database represents a more sensitive ecosystem than the national database, yet the species-by-species comparison of the two databases does not support this conclusion In addition, the calculated criteria are in conflict with long term instream biosurvey data from Blaine Creek that show the designated aquatic life use being supported [17] Van Hassel and colleagues [18] indicated no statistical correlations between macroinvertebrate taxa richness and measured instream concentrations of several toxic metals, including Cu

An evaluation of the criteria derivation process indicates that the low FAV for Cu in Blaine Creek using the resident-species procedure is a function of the calculation process and does not represent a site-water effect or true differences in species sensitivity The controlling factor appears to be the effect of database size in derivation of the FAV Although the role of database size on the FAV has been mentioned by previous investigators [1,2,23], they failed to recognize the extreme impact it may have on the results of the resident-species procedure The FAV equation is designed so that the fewer available acute values, the more conservative the resulting FAV When the resident-species procedure is used for a chemical for which the national WQC are based on a large number of species, the FAV equation will most likely produce a FAV that is overprotective compared to the national value An overprotective criterion will also occur if the database has a preponderance of sensitive species rather than covering a broad range of species sensitivities

The role of database size in criteria development can be illustrated by considering the hypothetical situation in which the national database consists of only the nine most sensitive species instead of a total of 41 The resulting FAV would be 13 4 μg/L, which is comparable to the value based on the Blaine Creek database (10 1 μg/L) and approximately one-fourth lower than the value of 18 5 μg/L based on a database size of 41 A similar result is evident using the Blaine Creek database Using a database size of 33 (based on the recalculation procedure), the resulting FAV of 36 3 μg/L is more than three times the FAV of 10 1 μg/L based on a database of nine The importance of database size is dependent on how close together the lowest four acute values in the database are, as they determine the slope of the line being used to extrapolate to the FAV The greater the differences between the acute values, the steeper the slope, and the more important database size is in determining the FAV

Two distinctly different values were obtained using the two procedures just discussed (i e , resident-species and recalculation procedures, Table 4) and can be explained by noting the large difference in the cumulative probability values assigned to the most sensitive genus (*Daphnia*) using the different procedures (Table 5) Table 5 compares the lowest four acute values and the corresponding cumulative probabilities used to extrapolate to the FAV (cumulative probability 0 05) for the Blaine Creek database and the EPA database modified by the recalculation procedure It is important to remember that these values are being used for a log GMAV–cumulative probability plot to obtain the FAV Because of the effect of

Table 5 Comparison of lowest four acute values using the resident-species and recalculation procedures for Blaine Creek (all values adjusted to a hardness of 50 mg/L as $CaCO_3$)

Resident species ($n = 9$)			Recalculation ($n = 33$)		
Cumulative probability	Genus	GMAVs[a]	Cumulative probability	Genus	GMAVs
0 10	*Daphnia*	17 3	0 03	*Daphnia*	17 08
0 20	*Physella*	51 0	0 06	*Ceriodaphnia*	18 77
0 30	*Isonychia*	109	0 09	*Gammarus*	25 22
0 40	*Pimephales*	133	0 12	*Plumatella*	37 05

The resident-species values are the four lowest in the Blaine Creek site-specific database The recalculation procedure values are the four lowest in the EPA national database
[a]Genus mean acute values

database size, the cumulative probability assigned *Daphnia* is three times higher (less sensitive) using the resident species compared to the recalculation procedure. Instead of estimating the 0.05 level based on levels that surround it (Table 5) when the database is large, the FAV equation must extrapolate below the lowest acute value to the 0.05 level when database size is <20.

Copper speciation

The results of Cu speciation analyses below the discharge clearly indicate a site-water effect reducing the amount of bioavailable Cu. We used the geometric mean ratio of dissolved to total recoverable Cu to adjust for the effect of site water by multiplying the FAV or CMC from the recalculation procedure by this value. Multiplying the CMC of 31.2 μg/L (at a site hardness of 173 mg/L) by the speciation ratio of 0.58 results in a criterion of 53.8 μg/L, which accounts for both site-water effects and species composition. It should be noted that it would be inappropriate to modify a criterion derived by either the resident- or indicator-species procedures by this method because they already account for site-water effects. Our study represents a novel approach for deriving protective site-specific Cu criteria.

CONCLUSION

The results of the acute toxicity tests for Cu using Blaine Creek water are in general agreement with the values reported by the EPA [4]. However, use of the resident-species procedure for Blaine Creek resulted in a proposed criterion that was overprotective when compared to national WQC for Cu and other site-specific derivation procedures. The overprotective result is principally due to the effect of database size in the calculation process, rather than differences in the relative sensitivity of the test species. The FAV equation is justifiably designed to yield a more conservative criterion when a smaller database is available. For chemicals such as Cu for which a large database already exists, the resident-species procedure has an inherent bias resulting in an unduly conservative value. A conservative bias is also introduced into the resident-species procedure if the range of test species contains a preponderance of sensitive organisms.

The recalculation procedure, as modified on the basis of metal bioavailability, appeared to be the most appropriate site-specific modification of the statewide (national) criterion for Cu in this situation. The resulting criterion was less stringent than the national value, being modified by factors that are known to mitigate the toxicity of Cu in aquatic environments. It should be mentioned that the extensive instream biosurvey database does support a less stringent criterion. In this particular case, a regulatory agency does not have to assume a conservative approach because the weight of evidence (instream biosurvey data, chemical speciation data) supports a less stringent site-specific criterion.

The key to deriving appropriate site-specific WQC is to focus on the chemical and biological factors that are most important at a specific site. Based on this research, use of the resident-species procedure is not recommended unless the national WQC are derived from a small database. Use of the recalculation procedure, metal bioavailability information, the indicator organism approach, or combinations of these appear to be more appropriate and cost-effective techniques to derive site-specific criteria. Regulatory agencies should recognize that novel approaches and method flexibility are acceptable and can result in protective site-specific criteria.

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