SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 20, No. 5, pp. 1037–1045, 2001

Printed in the USA
0730-7268/01 $9.00 + .00

# EFFECTS OF SULFATE ON THE ACUTE TOXICITY OF SELENATE TO FRESHWATER ORGANISMS

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(*Received* 9 *March* 2000; *Accepted* 18 *September* 2000)

**Abstract**—This study evaluated the relationship between ambient sulfate concentrations and acute selenate toxicity to freshwater aquatic life. Previous studies indicated that increasing sulfate concentrations reduced selenate bioconcentration and toxicity. However, these studies generally were not conducted in a manner that was conducive to their use in deriving a water quality criterion. We compiled results from previous studies and generated additional data to help define a selenate–sulfate relationship for acute toxicity. Selenate toxicity was determined in standardized test waters with varying sulfate concentrations using *Ceriodaphnia dubia, Gammarus pseudolimnaeus, Hyalella azteca,* and *Pimephales promelas* as the test organisms. Analysis of test results indicated that a significant relationship does exist between acute selenate toxicity and ambient sulfate concentrations. Data from these tests and previous studies were combined to develop a statistical relationship sufficiently robust to derive a sulfate-dependent water quality criterion for selenate. The relationship is similar to those commonly derived between divalent metals and hardness to adjust water quality criteria.

**Keywords**—Selenate Sulfate Acute toxicity Water quality criteria

## INTRODUCTION

The acute toxicity of selenium to aquatic organisms has been widely studied. Since the 1970s, specific selenium forms, such as selenate, selenite, and various organo-selenium compounds, have been recognized as having differing toxicities and that selenate is the least toxic selenium form on an acute basis [1–3]. In addition to differences in relative toxicity, ambient sulfate concentrations also have been recognized to reduce selenate bioconcentration and toxicity. This was first observed for plants [4], yeast [5], and the alga *Chlorella vulgaris* [6]. Additional studies with other algal species, bacteria, and fungi have subsequently supported these initial observations [7–10].

More recently, several key studies have been conducted on multicellular organisms evaluating selenate–sulfate interactions. Hansen et al. [11] evaluated sulfate effects on selenate bioconcentration in *Chironomus decorus* and *Daphnia magna.* They demonstrated that both species had reduced selenate bioconcentration with increasing sulfate concentrations. Analysis of their data also indicated different selenate–sulfate interactions between the two species, with *D. magna* exhibiting a much more rapid decrease in selenium bioconcentration relative to increasing sulfate concentrations.

Maier et al. [12], in studies with *D. magna,* demonstrated significant decreases in selenate toxicity over 48 h by increasing sulfate concentrations from 10 to 163 mg/L. Mortality of *D. magna* dropped from 94% to 7% when exposed to a previously established median lethal concentration (LC50) for selenate of 2.8 mg/L. They also demonstrated that this relationship was not observed for selenite or seleno-DL-methionine, which had variable or no response to increasing sulfate concentrations, respectively. Ogle and Knight [13] performed a similar study with *D. magna* and again showed that increasing sulfate concentrations reduced selenate bioconcentration and toxicity. They also reviewed the literature and found that analysis of data pooled from six independent studies revealed a general relationship between sulfate concentrations and selenate LC50s, although this relationship was somewhat obscured by differences in experimental design and study quality.

Finally, Forsythe and Klaine [14] examined the effects of sulfate on brine shrimp (*Artemia* sp.) in the context of the extreme sulfate concentrations that occur in agricultural drainage evaporation ponds of the San Joaquin Valley (CA, USA). A 96-h LC50 for selenate of 1.43 mg/L was estimated in artificial seawater with a sulfate concentration of 50 mg/L. This compares with an LC50 of 81.97 mg/L when shrimp were exposed in a dilution water with sulfate at 14,000 mg/L, which was representative of some of the more saline evaporation ponds.

The mechanism for inhibition of selenate bioavailability by sulfate seems to be related to direct competition at the cell uptake site. Selenate and sulfate are structurally similar group VI oxyanions of the form $XO_4$. Experiments with bacteria indicate that selenate and sulfate have a common membrane carrier and that active transport by this carrier is the only means by which selenate may enter a cell [15,16]. Hansen et al. [11] provided a more thorough review of these mechanisms, including a discussion on whether one or two membrane carriers, with different assimilation efficiencies, provide for sulfate and

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selenate uptake into a cell. They hypothesized that the potential for one or two membrane carriers may explain why different selenate–sulfate dose–response relationships were observed for *D. magna* and *C. decorus* in their study.

Based on existing data, ambient sulfate concentrations clearly play a significant role in controlling selenate bioavailability, bioconcentration, and subsequent toxicity. This relationship has implications for deriving selenate water quality criteria. The guidelines for deriving water quality criteria provide methods for quantifying how a water quality parameter influences a chemical's bioavailability, via least squares regression and analysis of covariance techniques. This regression can then be incorporated into a final equation for calculating a site-specific water quality criterion [17]. This type of relationship has been well defined between several divalent metals and hardness [18–22], and is used in deriving site-specific water quality criteria.

Until now, much of the existing data supporting a selenate–sulfate relationship were unusable for deriving water quality criteria because either they were based on unicellular organisms or they evaluated bioconcentration rather than toxicity. The objectives of our study were to summarize existing acute toxicity data on selenate for which ambient sulfate concentrations were also available, to supplement these data with additional data using standard fish (*Pimephales promelas*) and invertebrate (*Ceriodaphnia dubia*, *Gammarus pseudolimnaeus*, and *Hyalella azteca*) test species, and to apply the methods described in Stephan et al. [17] to the entire data set to derive a sulfate-dependent final acute equation for selenate.

## MATERIALS AND METHODS

### *General*

In addition to the studies described below, data on the acute toxicity of selenate were compiled from the most recent U.S. Environmental Protection Agency (U.S. EPA) water quality criteria document for selenium and several publications summarizing recent studies [1–3]. All studies for which dilution water sulfate concentrations were available were used for deriving a final criterion.

### *Testing facilities*

Toxicity tests were conducted at two laboratories in the United States. The toxicology laboratory of the Great Lakes Environmental Center (GLEC) in Traverse City (MI, USA) conducted testing on *G. pseudolimnaeus*, *H. azteca*, and *P. promelas*. Parametrix's toxicology laboratory in Kirkland (WA, USA) conducted testing on *C. dubia* and *P. promelas*. Supporting analytical chemistry measuring selenium test concentrations were performed at TransEnviro Analytical Services in Warrensville Heights (OH, USA) for toxicity testing conducted at GLEC. Supporting analytical chemistry for testing conducted at Parametrix was performed by Sound Analytical Services (Tacoma, WA, USA) and at Kennecott Environmental Laboratory (Magna, UT, USA). Selenium speciation analyses associated with the GLEC studies were performed by Brooks Rand (Seattle, WA, USA).

### *Test substance*

Reagent grade sodium selenate ($Na_2SeO_4$), obtained from Sigma Chemical (St. Louis, MO, USA), was used to conduct studies performed at Parametrix. Hydrated reagent-grade selenate ($Na_2SeO_4 \cdot 10H_2O$) obtained from Aldrich Chemical (Milwaukee, WI, USA) was used for tests conducted at GLEC. Following receipt, the test substance was stored in the dark at room temperature. Several different stock solutions were used over the course of this study, depending on anticipated test concentrations. Reagent-grade anhydrous magnesium sulfate ($MgSO_4$), also obtained from Sigma Chemical, was used to manipulate sulfate concentrations in the various dilution waters used for testing performed at Parametrix. Dilution waters for testing performed at GLEC were modified using sodium sulfate ($NaSO_4$) obtained from EM Science (Gibbstown, NJ, USA).

### *Experimental design*

All tests were conducted according to U.S. EPA or American Society for Testing and Materials guidelines, or both [23–25]. The different requirements of the various species tested necessitated slightly different experimental designs. For all tests, temperature, pH, and dissolved oxygen were measured at test initiation and every 24 h until test termination. All amphipod and fish tests were 96 h in duration and all daphnid tests were 48 h in duration, in accordance with U.S. EPA guidelines [25]. The tests conducted at GLEC were performed under flow-through conditions, whereas those at Parametrix were static nonrenewal tests. The flow-through testing was conducted using a modified Benoit continuous flow minidiluter system. For all studies, test organisms were not fed during the selenate exposure and mortality, defined as immobility under gentle prodding, was monitored every 24 h.

*Ceriodaphnia dubia.* Tests with *C. dubia* were conducted according to standard U.S. EPA guidelines for conducting 48-h acute toxicity tests [25]. Test organisms were obtained from laboratory stock cultures and were $<$24 h old at test initiation. The experimental design for each test consisted of four replicate test chambers (30-ml polypropylene cups) for each of five selenate concentrations and a control. Each test chamber contained five daphnid neonates. Test temperature was maintained at 20 $\pm$ 1°C inside a temperature-controlled environmental chamber. The tests were conducted under static nonrenewal conditions and organisms were not fed. Neonate survival was monitored at 24 and 48 h, with mortality defined as immobility under gentle prodding. The base dilution water was synthetic moderately soft water as described by U.S. EPA [25] with a hardness of 52 mg/L as $CaCO_3$. This dilution water was augmented with varying concentrations of magnesium sulfate ($MgSO_4$) to produce the desired sulfate concentrations. Acute toxicities were determined in dilution waters with sulfate (nominal) at 25, 50, 100, 200, 400, and 800 mg/L. Two tests were conducted at each sulfate concentration to provide information on variability.

*Gammarus pseudolimnaeus.* Adult amphipods were obtained from Environmental Consulting and Testing (Superior, WI, USA) for testing. The experimental design used four replicate test chambers (10 cm wide $\times$ 14 cm high $\times$ 25 cm deep) for each of five test concentrations and a control. Each test chamber contained five amphipods. The tests were conducted at 18 $\pm$ 2°C using a combination of emersion heaters in the dilution water headbox and chilled water pumped into the headbox. The dilution water was dechlorinated Lake Michigan, USA, water with a hardness of 139 mg/L as $CaCO_3$ and sulfate at 25 mg/L. The dilution water was augmented with sodium sulfate to obtain the desired sulfate concentrations. Acute selenate toxicity was determined in waters with $SO_4^{2-}$ at 25, 100, 400, and 800 mg/L.

*Hyalella azteca.* Testing followed standard American Society for Testing and Materials guidelines for conducting acute

Table 1. Water quality conditions during toxicity tests

| | Parametrix | | Great Lakes Environmental Center | | |
|---|---|---|---|---|---|
| | *Ceriodaphnia dubia* | *Pimephales promelas* | *Pimephales promelas* | *Hyalella azteca* | *Gammarus pseudolimnaeus* |
| Temperature (°C) | 19–21 | 19–21 | 23.0–25.9 | 16.5–20.1 | 16.0–19.8 |
| Dissolved oxygen (mg/L) | 6.9–8.9 | 6.9–8.9 | 6.3–8.7 | 8.1–9.6 | 8.1–9.6 |
| pH | 7.0–8.1 | 7.0–8.1 | 7.8–8.6 | 7.4–8.2 | 7.7–8.3 |

toxicity tests [23]. Test organisms were obtained from Environmental Consulting and Testing (Superior, WI, USA) and were 17 to 22 d old at test initiation. The experimental design consisted of four replicate test chambers (250-ml beakers) for each of five selenate concentrations and a control. The dilution water was a synthetic, moderately hard water (143 mg/L hardness as $CaCO_3$ and sulfate at 25 mg/L). Each test chamber contained five juvenile amphipods. Test temperature was maintained at 17 ± 2°C using a combination of submersed heaters and chilled water in the dilutor headbox. The same dilution waters described for *G. pseudolimnaeus* were also tested with *H. azteca.*

*Pimephales promelas.* Tests performed by Parametrix with *P. promelas* were conducted according to standard U.S. EPA guidelines for conducting 96-h acute toxicity tests [25]. Test organisms were obtained from Aquatic Biosystems (Fort Collins, CO, USA) and were 7 d old at test initiation. The experimental design for each test consisted of four replicate test chambers (1-L borosilicate glass beakers) for each of five selenate concentrations and a control. Each test chamber contained five fathead minnow larvae. Test temperature was maintained at 20 ± 1°C inside a temperature-controlled environmental chamber. Temperature, pH, and dissolved oxygen were measured at test initiation and every 24 h until test termination. The tests were conducted under static conditions with a test solution renewal at 48 h. Test organisms were fed *Artemia* nauplii just before test solution renewal. Larval survival was monitored every 24 h, with mortality defined as immobility under gentle prodding. The same dilution waters described for *C. dubia* were tested with *P. promelas,* except dilution water with sulfate at 25 mg/L was not tested.

Tests on *P. promelas* performed by GLEC were conducted in accordance with American Society for Testing and Materials procedures [23]. Test organisms were obtained from in-house cultures and were 3 to 5 d old at test initiation. The experimental design used four replicate test chambers (10 cm wide by 14 cm high by 25 cm deep) for each of five test concentrations and a control. The tests were conducted at 25 ± 2°C using a combination of emersion heaters in the dilution water headbox and chilled water pumped into the headbox. The dilution water was dechlorinated Lake Michigan water with a hardness of 139 mg/L as $CaCO_3$ and sulfate at 25 mg/L. The dilution water was augmented with sodium sulfate to obtain the desired sulfate concentrations. Acute toxicity was determined in waters with $SO_4^{2-}$ at 25, 100, 400, and 1,000 mg/L.

### *Chemical analysis*

Total selenium concentrations were measured at test initiation and termination for each concentration and control. Total selenium was determined by graphite furnace atomic absorption spectrometry (Varian Zeeman 400, Varian, Walnut Creek, CA, USA) according to U.S. EPA method 7740 [26] for all *C. dubia* tests and by inductively coupled plasma and inductively coupled plasma–mass spectroscopy using U.S. EPA methods 200.7 and 200.8 for all *P. promelas, H. azteca,* and *G. pseudolimnaeus* tests. Matrix spikes, duplicate samples, and method blanks were evaluated with each batch of samples analyzed. Sulfate concentrations in the dilution waters were measured at the beginning of each test using a colorimetric technique (U.S. EPA method 300.0).

### *Statistical analysis*

Toxicity data generated in this study were analyzed statistically by probit analysis using the computer software program TOXIS, Version 2.4 [27]. Endpoints evaluated were the 48- and 96-h LC50 (and its 95% confidence limits), as appropriate.

These new data were then pooled with existing acute toxicity data presented by the U.S. EPA [3] and Canton [2]. Sulfate concentrations for these studies were obtained by either reviewing the published studies or by directly contacting the original researcher. Data points in the original U.S. EPA water quality criteria data set or the work of Canton [2] for which sulfate concentrations were not measured were excluded from further analysis [17].

The sulfate regression model was developed using analysis of covariance. A model was developed using the statistical software SPSS® for Windows, Version 6.1 [28]. To verify the model's accuracy for this purpose, the acute toxicity data from the ambient water quality criteria document [20] for lead were entered and the existing hardness-dependent criterion for lead was confirmed.

## RESULTS

### *Toxicity tests*

*Water quality and analytical chemistry.* Water quality conditions for all tests are summarized in Table 1. Test temperature, dissolved oxygen, and pH all met test acceptability requirements, although several minor (1°C) deviations in test temperature occurred in the tests with *H. azteca* and *G. pseudolimnaeus.* Mean measured total recoverable selenium concentrations for each study were comparable to nominal concentrations. Measured concentrations across all tests remained stable for the duration of testing. All statistical analyses were performed based on the mean of the measured test concentrations for both selenium and sulfate.

*Biological results.* The 48-h LC50s for *C. dubia* ranged from 580 to 9,311 μg/L selenium based on measured test concentrations, whereas 96-h LC50s for *P. promelas* ranged from 6,210 to 42,100 μg/L (Table 2). The 96-h LC50s for *G. pseudolimnaeus* ranged from 1,180 to 3,710 μg/L, and the LC50s for *H. azteca* ranged from 1,350 to 3,580 μg/L (Table 3). All tests demonstrated a strong dose–response relationship, with ≤10% mortality in control groups.

Data from the new studies, and from existing studies (Table 4) listed by the U.S. EPA [3] and Canton [2], were compiled

Table 2. Acute toxicity of selenate at varying sulfate concentrations for *Ceriodaphnia dubia* and *Pimephales promelas*

| *C. dubia* | | *P. promelas* | |
|---|---|---|---|
| Sulfate (mg/L) | 48-h LC50 (μg/L) (95% CI) | Sulfate (mg/L) | 96-h LC50 (μg/L) (95% CI) |
| 31 | 1,078 (920–1,266) | 24 | 6,210 (5,420–7,120) |
| 38 | 580 (507–664) | 50 | 12,332 (10,482–14,159) |
| 52 | 1,967 (1,728–2,241) | 65 | 12,539 (10,985–14,331) |
| 55 | 1,864 (1,642–2,109) | 117 | 15,261 (12,643–18,032) |
| 98 | 1,822 (1,569–2,109) | 127 | 16,586 (15,021–18,128) |
| 98 | 1,728 (1,483–1,991) | 160 | 10,800 (9,340–12,420) |
| 213 | 1,453 (1,322–1,606) | 212 | 25,665 (22,990–28,651) |
| 217 | 2,812 (2,392–3,345) | 230 | 30,063 (26,667–33,507) |
| 378 | 5,553 (4,808–6,355) | 403 | 18,361 (16,308–20,061) |
| 378 | 5,481 (5,040–6,037) | 474 | 18,000 (14,940–21,630) |
| 926 | 9,157 (7,921–10,407) | 555 | 23,781 (21,252–26,699) |
| 1205 | 9,311 (7,495–10,504) | 906 | 42,100 (34,500–51,390) |
| | | 978 | 35,164 (31,319–39,726) |
| | | 1013 | 32,171 (28,998–35,907) |

for statistical analysis according to Stephan et al. [17]. Inclusion or exclusion of a specific study in the data set followed U.S. EPA guidelines. Several studies previously included by U.S. EPA were excluded in this analysis because either no information was available on sulfate concentrations or test concentrations were not measured and new data for the same species using measured test concentrations had become available [17].

*Statistical analysis*

The sulfate-dependent toxicity relationship used for this model is limited to the sulfate levels tested, that is, the toxicologic data available are limited to sulfate concentrations from 12 to 1,205 mg/L. Natural log transformation was used to normalize all data. In a stepwise manner, analysis of covariance was used to test if data from an individual species could be pooled with other species. The regression lines were positively sloped, showing a relationship between ambient sulfate and acute toxicity. *Pimephales promelas, C. dubia, D. magna,* and *G. pseudolimnaeus* had the most data and showed strong relationships between ambient sulfate concentrations and the resulting selenate LC50 (Figs. 1 to 4). Several other species (*Daphnia pulex, Chironomus riparius,* and *Xyrauchen texanus*) had positive and significant relationships, although only two data points were available for each of these species. Finally, *H. azteca* had a positive but not statistically significant relationship between ambient sulfate concentrations and acute selenate toxicity, eliminating it from further analysis (Fig 5). If the slope for an individual species was significantly different ($p < 0.05$) from slopes for other species, it was not pooled. A total of eight species were evaluated, with six (*D. magna, D. pulex, C. dubia, C. riparius, P. promelas,* and *X. texanus*) meeting this criterion ($p = 0.201$). A significant ($p < 0.001$) selenate–sulfate relationship was identified for these species with an $r^2$ of 0.93. Data for *H. azteca* and *G. pseudolimnaeus* had significantly different slopes than the other species and were excluded.

An analysis of covariance was used to estimate the criterion maximum concentrations (CMCs), in accordance with Stephen et al. [17]. The analysis of covariance was performed with the natural logarithm of LC50s as the dependent variable, species as the treatment or grouping variable, and the natural logarithm of sulfate as the independent variable. The model was fit to the data in Tables 2, 3, and 4 for the six species shown to be suitable for pooling.

The pooled slope ($S$) was used to normalize the acute toxicity data (LC50) consistent with methods described in Stephan et al. [17]

$$\text{sulfate-normalized LC50}_Z = \exp\{\ln(\text{LC50})\text{C}S \times [\ln(\text{SO}_4)S \ln(Z)]\}$$

where LC50 = individual acute toxicity datum, $SO_4$ = the sulfate concentration associated with the individual test, $LC50_Z$ = LC50 data point normalized to a selected sulfate concentration of $Z$, and $S$ = pooled slope = 0.58916.

This equation was applied to normalize selenate toxicity data to a sulfate concentration of 100 mg/L. Species mean acute values at sulfate at 100 mg/L were then calculated as geometric means of the normalized LC50s. Genus mean acute values were then calculated as geometric means of the species mean acute values. These values in turn were used to calculate a final acute value and criterion maximum concentration at a sulfate concentration of 100 mg/L ($CMC_{100}$) in accordance with Stephan et al. [17]. The CMC intercept was then calculated by

$$\ln(\text{CMC intercept}) = \ln(\text{CMC}_{100})S0.58916 \times \ln(100) = 4.032$$

Table 3. Acute toxicity of selenate at varying sulfate concentrations for *Gammarus pseudolimnaeus* and *Hyalella azteca*

| *G. pseudolimnaeus* | | *H. azteca* | |
|---|---|---|---|
| Sulfate (mg/L) | 96-h LC50 (μg/L) (95% CI) | Sulfate (mg/L) | 96-h LC50 (μg/L) (95% CI) |
| 25 | 1,180 (1,050–1,320) | 40 | 2,480 (1,810–3,390) |
| 125 | 2,870 (2,090–3,940) | 125 | 1,350 (990–1,850) |
| 367 | 3,710 (3,000–4,590) | 367 | 1,540 (720–3,290) |
| 635 | 3,270 (2,550–4,180) | 822 | 3,580 (2,390–5,370) |

Table 4. Summary of selenate acute toxicity data for freshwater organisms

| Species | LC50 (μg/L Se) | $SO_4$ (mg/L) | Reference |
|---|---|---|---|
| *Aplexa hypnorum* | 193,000 | 12 | [37] |
| *Ceriodaphnia dubia*[a] | 1,078 | 31 | This study |
| *Ceriodaphnia dubia*[a] | 580 | 38 | This study |
| *Ceriodaphnia dubia*[a] | 1,967 | 52 | This study |
| *Ceriodaphnia dubia*[a] | 1,864 | 55 | This study |
| *Ceriodaphnia dubia*[a] | 1,822 | 98 | This study |
| *Ceriodaphnia dubia*[a] | 1,728 | 98 | This study |
| *Ceriodaphnia dubia*[a] | 1,453 | 213 | This study |
| *Ceriodaphnia dubia*[a] | 2,812 | 217 | This study |
| *Ceriodaphnia dubia*[a] | 5,553 | 378 | This study |
| *Ceriodaphnia dubia*[a] | 5,481 | 378 | This study |
| *Ceriodaphnia dubia*[a] | 9,157 | 926 | This study |
| *Ceriodaphnia dubia*[a] | 9,311 | 1,205 | This study |
| *Chironomus decorus* | 23,700 | 27 | [11] |
| *Chironomus riparius*[a] | 10,500 | 41 | [38] |
| *Chironomus riparius*[a] | 16,200 | 68 | [38] |
| *Daphnia magna*[a] | 570 | 12 | [37] |
| *Daphnia magna*[a] | 1,010 | 22 | [39] |
| *Daphnia magna*[a] | 2,560 | 41 | [38] |
| *Daphnia magna*[a] | 2,840 | 82 | [12] |
| *Daphnia magna*[a] | 4,070 | 68 | [38] |
| *Daphnia magna*[a] | 5,300 | 163 | [40] |
| *Daphnia pulex*[a] | 8,126 | 38 | This study |
| *Daphnia pulex*[a] | 10,123 | 54 | This study |
| *Daphnia pulicaria* | 246 | 22 | [39] |
| *Gammarus pseudolimnaeus* | 75 | 12 | [37] |
| *Gammarus pseudolimnaeus* | 57 | 12 | [3] |
| *Gammarus pseudolimnaeus* | 2,400 | 10 | This study |
| *Gammarus pseudolimnaeus* | 1,180 | 25 | This study |
| *Gammarus pseudolimnaeus* | 2,870 | 125 | This study |
| *Gammarus pseudolimnaeus* | 3,710 | 367 | This study |
| *Gammarus pseudolimnaeus* | 3,270 | 635 | This study |
| *Gammarus lacustris* | 3,100 | 120 | This study |
| *Gila elegans* | 26,400 | 174 | [41] |
| *Hyalella azteca* | 1,868 | 13 | [30] |
| *Hyalella azteca* | 2,480 | 40 | This study |
| *Hyalella azteca* | 1,424 | 55 | This study |
| *Hyalella azteca* | 1,350 | 125 | This study |
| *Hyalella azteca* | 1,540 | 367 | This study |
| *Hyalella azteca* | 3,580 | 822 | This study |
| *Hydra* sp. | 7,300 | 12 | [37] |
| *Ictalurus punctatus* | 66,000 | 12 | [37] |
| *Lepomis macrochirus* | 63,000 | 12 | [37] |
| *Nephelopsis obscura* | 442,000 | 12 | [37] |
| *Oncorhynchus kisutch* | 32,500 | 185 | [42] |
| *Oncorhynchus mykiss* | 24,000 | 12 | [37] |
| *Oncorhynchus tshawytscha* | 100,000 | 185 | [42] |
| *Oncorhynchus tshawytscha* | 121,000 | 185 | [42] |
| *Paratanytarsus parthenogenecticus* | 20,000 | 12 | [37] |
| *Pimephales promelas*[a] | 2,300 | 12 | [37] |
| *Pimephales promelas*[a] | 6,210 | 24 | This study |
| *Pimephales promelas*[a] | 12,332 | 50 | This study |
| *Pimephales promelas*[a] | 12,539 | 65 | This study |
| *Pimephales promelas*[a] | 15,261 | 117 | This study |
| *Pimephales promelas*[a] | 16,586 | 127 | This study |
| *Pimephales promelas*[a] | 10,800 | 160 | This study |
| *Pimephales promelas*[a] | 25,665 | 212 | This study |
| *Pimephales promelas*[a] | 30,063 | 230 | This study |
| *Pimephales promelas*[a] | 18,361 | 403 | This study |
| *Pimephales promelas*[a] | 18,000 | 474 | This study |
| *Pimephales promelas*[a] | 23,781 | 555 | This study |
| *Pimephales promelas*[a] | 35,164 | 978 | This study |
| *Pimephales promelas*[a] | 42,100 | 906 | This study |
| *Pimephales promelas*[a] | 32,171 | 1,013 | This study |
| *Ptychocheilus lucius* | 24,600 | 174 | [41] |
| *Thymallus arcticus* | 100,000 | 41[b] | [43] |
| *Xyrauchen texanus*[a] | 15,900 | 89 | [44] |
| *Xyrauchen texanus*[a] | 48,000 | 164 | [45] |

[a] Used in calculation of sulfate-dependent criterion.
[b] Estimated based on reconstituted soft water [25].

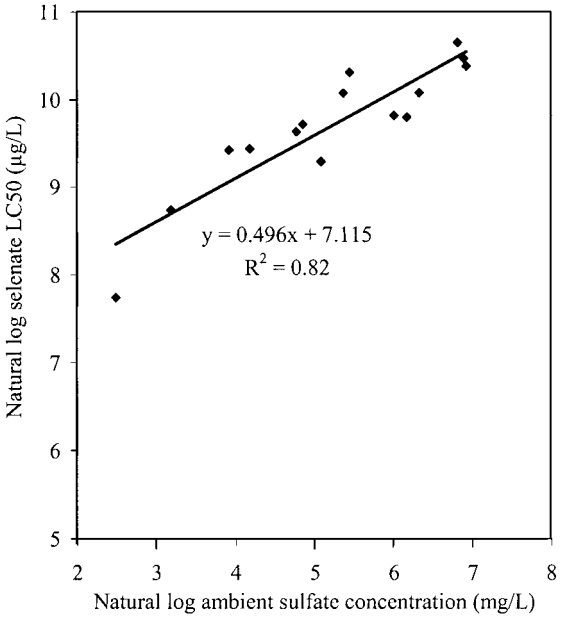


Fig. 1. Relationship between ambient sulfate concentration and acute selenate toxicity for *Pimephales promelas.*

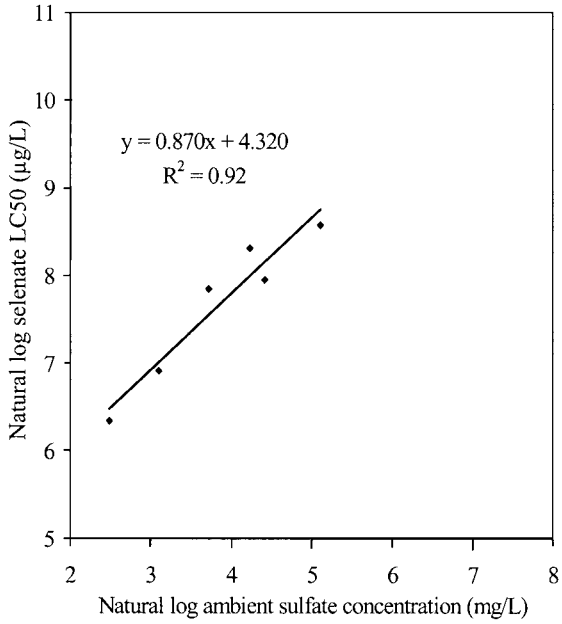


Fig. 3. Relationship between ambient sulfate concentration and acute selenate toxicity for *Daphnia magna.*

The final equation for calculating a criterion maximum concentration is then

$$CMC = \exp\{[0.58916 \times \ln(SO_4 \text{ concn. in receiving water})] + (4.032)\}$$

## DISCUSSION

The current U.S. EPA acute water quality criterion (CMC) of 20 µg/L does not discriminate between different selenium forms such as selenate and selenite. This criterion is based on a reverse application of the acute–chronic ratio to the chronic water quality criterion of 5 µg/L [2,3]. The U.S. EPA currently is considering deriving separate criteria for selenate and selenite [29]. Using the standard U.S. EPA guidelines for deriving water quality criteria [17], a CMC of 12.8 µg/L is calculated using the data set published in the water quality criteria document [3]. Canton [2] updated this data set with all tests conducted after 1987 and recalculated a CMC of 404 µg/L selenate.

We have used the data set presented by Canton [2] and the additional data presented here to derive a final sulfate-dependent criterion. Table 5 shows the acute selenate criterion associated with a range of ambient sulfate concentrations. Ambient sulfate concentrations ranging from 25 to 800 mg/L would result in CMCs ranging from 375 to 2,893 µg/L. The

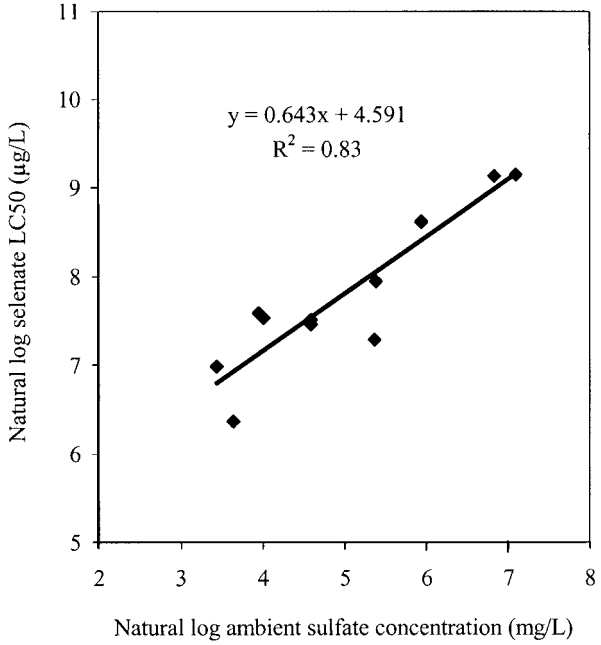


Fig. 2. Relationship between ambient sulfate concentration and acute selenate toxicity for *Ceriodaphnia dubia.*

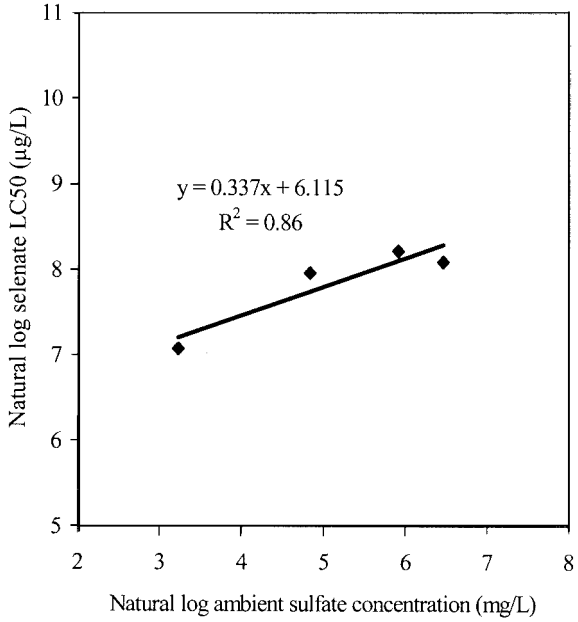


Fig. 4. Relationship between ambient sulfate concentration and acute selenate toxicity for *Gammarus pseudolimnaeus.*

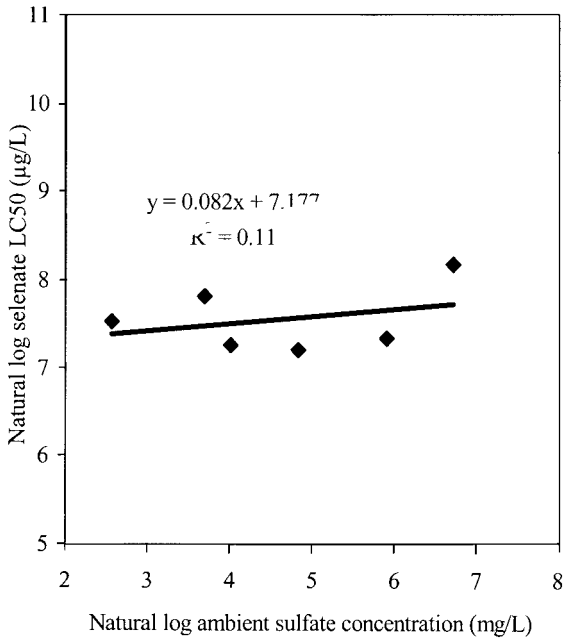


Fig. 5. Relationship between ambient sulfate concentration and acute selenate toxicity for *Hyalella azteca.*

CMC of 404 µg/L derived by Canton [2] would occur at a sulfate concentration of 28.3 mg/L.

Of the eight species having at least two toxicity values at different ambient sulfate concentrations, *G. pseudolimnaeus* and *H. azteca* had significantly different slopes from the other species and the selenate–sulfate relationship for *H. azteca* was insignificant. Two reasons are possible for the shallow slope for *G. pseudolimnaeus* and the insignificant slope for *H. azteca*. First, these organisms possibly have alternative membrane transport mechanisms for sulfate and consequently selenate that result in a different relationship (or even lack of relationship) between acute selenate toxicity and ambient sulfate concentrations. It is worth noting that these species are closely related (both are talitroidean amphipods) and can occur in similar habitats. Consequently, broadly similar ionoregulatory mechanisms might be expected that result in a similar selenate–sulfate relationship, and one that differs from other organisms.

Alternatively, or in combination with the phylogenetic hypothesis, the weak relationship between selenate and sulfate for these two species possibly is an artifact of variable test results and combining data from varying experimental designs. For example, of the six data points for *H. azteca,* the lowest sulfate concentrations tested were 13 mg/L [30] and 40 mg/L (this study). The study of Brasher and Ogle [30] used 60-d-old amphipods, whereas the data point for $SO_4$ at 40 mg/L from this study was the last test conducted with *H. azteca* using organisms nearly 30 d older than those used in some of the earlier tests. Age-related sensitivity to selenate quite possibly influenced the results from these two tests when compared to the other data points. If these two data points are removed, the resulting significant regression has a slope of 0.31 ($r^2 = 0.64$), quite similar to the *G. pseudolimnaeus* slope of 0.34. With the existing data, adequately testing these hypotheses is not possible and so further testing with amphipods is recommended.

Table 5. Sulfate-dependent criterion maximum concentrations (CMCs) for selenate

| Ambient sulfate concn. (mg/L) | Selenate CMC ((µg/L) |
|---|---|
| 25 | 375 |
| 50 | 565 |
| 65 | 659 |
| 100 | 850 |
| 200 | 1,278 |
| 400 | 1,923 |
| 800 | 2,893 |

A comparison of the selenate–sulfate equation with those derived for divalent metals and hardness reveals the slope of the selenate–sulfate relationship to be shallower. The pooled slopes for cadmium, copper, lead, nickel, and zinc regressed against hardness range from 0.85 to 1.27 [18–22], whereas the pooled slope for the selenate–sulfate relationship is only 0.59, indicating that the competitive interaction between sulfate and selenate is generally less than that observed for hardness and the divalent metals. The shallower slope for selenate and sulfate should not be misinterpreted to mean the relationship is any less significant than those observed for divalent metals and hardness because the relationship is highly significant ($p < 0.001$) with relatively little variability ($r^2 = 0.93$).

Conceptually, both the revised selenate criterion described by Canton [2] and the sulfate-dependent criterion described in this paper would allow for higher selenate concentrations to be released to receiving waters. However, increased release of selenium in an acute mixing zone can only occur to the extent that compliance with a chronic mixing zone is still achieved. For selenium, the chronic water quality criterion of 5 µg/L will limit the allowable increase in selenium discharge in many cases. Only in situations where a large difference exists in degree of dilution between acute and chronic mixing zones will the CMC govern the permissible discharge concentration. For example, at an ambient sulfate concentration of 200 mg/L, a selenium discharge concentration within an acute mixing zone of 1,278 µg/L would be permitted. However, if the ratio between acute and chronic mixing zones is only 20:1, a maximum concentration of 100 µg/L in the acute mixing zone would be necessary in order to achieve compliance with the 5-µg/L criterion at the edge of the chronic mixing zone. Hence, although dischargers will be allowed to increase selenium releases, in most cases this increase will be less than indicated by the sulfate-dependent criterion.

Although a sulfate-dependent acute criterion for selenate seems appropriate, extending this relationship to a chronic selenate criterion does not seem appropriate for several reasons. The chronic water quality criterion is based on observed reproductive effects to centrarchids in Belews Lake (NC, USA) [3,31]. These effects seem largely due to dietary exposure, rather than aqueous exposure as for acute toxicity, in which inorganic selenium (predominantly selenite) was biotransformed to organo-selenium compounds and moved up the food chain [32]. Selenate was similarly transformed in the evaporation ponds of the San Joaquin Valley, resulting in reproductive effects in birds [33].

Consideration of selenium biochemistry together with limited laboratory studies indicates that sulfate does not inhibit the bioavailability of the organo-selenium compounds principally responsible for chronic environmental effects [12]. This is also supported by data from the San Joaquin Valley evaporation ponds, in which significant selenium bioaccumulation

and subsequent effects on birds were observed despite average sulfate concentrations of approximately 12,000 mg/L in these ponds [34]. Consequently, because diet rather than water is the principal exposure pathway for chronic selenium toxicity, the selenate–sulfate interaction does not seem to play a significant role and the relationship developed for acute toxicity should not be applied in a chronic context.

Finally, some researchers have cautioned that the total loading and final environmental fate of selenium released to a water body needs consideration before raising the acute water quality criterion or deriving a site-specific water quality criterion [35,36]. They argue that although discharging higher selenate levels may be safe with respect to acute toxicity or site-specific conditions, eventually the increased selenium load will be transported to an environment where it will enter the food chain and cause chronic toxicity. This argument merits consideration, but it too has to be interpreted in light of site-specific conditions and potential for downstream dilution. Higher acute selenium criteria might best be implemented in the context of a watershed or total maximum daily load context to prevent increasing watershed selenium to levels that have the potential to cause chronic toxicity.

*Acknowledgement*—We acknowledge Allegheny Power, American Electric Power, Electric Power Research Institute, Kennecott Utah Copper, Ohio Valley Electric, Parametrix, the Utility Water Act Group, and Virginia Power for sponsoring this study, and Tadd Berger and Debbie Fetherston for assisting in manuscript preparation.

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