Acute Toxicity of Sodium Selenate to Two Daphnids and Three Amphipods

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ABSTRACT: This study evaluated the acute toxicity of sodium selenate to two daphnid and three gammarid amphipod species. The daphnids, *Ceriodaphnia dubia* and *Daphnia pulex*, were evaluated in 48-hour static tests and the amphipods, *Gammarus pseudolimnaeus*, *Gammarus lacustris*, and *Hyalella azteca*, were evaluated in 96-hour static and flow-through tests. Tests resulted in mean LC_{50}'s of 1.92, 9.12, 1.82, 3.05, and 1.95 mg/L selenium for *C. dubia*, *D. pulex*, *G. pseudolimnaeus*, *G. lacustris*, and *H. azteca*, respectively. The LC_{50}'s for the *G. pseudolimnaeus* tests are more than 30-fold higher than previously reported LC_{50}'s for the same or similar species. The explanation for these differing results appears to be partially, but not entirely, explained by differences in ambient pH between the new studies and previous ones. Depending on how the new data are included in U.S. EPA's selenium freshwater quality criterion data set, the selenate acute water quality criterion (i.e., Criterion Maximum Concentration) increases from 12.8 to as high as 583 μg/L selenium. Environ Toxicol 16: 142–150, 2001

Keywords: selenium; selenate; daphnids; amphipods; water quality criteria

INTRODUCTION

The acute toxicity of selenium to aquatic organisms has been widely studied. Since the 1970s, it has been recognized that specific selenium forms such as selenate, selenite, and various organo-selenium compounds have differing toxicities (Adams, 1976; U.S. EPA, 1987a). In general, acute toxicity data for a wide range of freshwater organisms indicate that selenite is more toxic than selenate (Table I). For selenate, species mean acute values (SMAVs) range from 65 to 442,000 μg/L. Typical of most metals, crustaceans are the most sensitive group with *Gammarus pseudolimnaeus*, *Hyalella azteca*, and *Daphnia magna* being the most sensitive species tested. These are followed by fish species being intermittent in sensitivity and other invertebrates (e.g., the

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TABLE I. Comparison of acute selenate and selenite toxicity (U.S. EPA 1987a)

Taxa	Selenate LC_{50} ($\mu g/L$)	Selenite LC_{50} ($\mu g/L$)
Leech, *Nephelopsis obscura*	442,000	203,000
Snail, *Aplexa hypnorum*	193,000	34,910
Channel catfish, *Ictalurus punctatus*	66,000	13,600
Bluegill, *Lepomis macrochirus*	63,000	28,500
Rainbow Trout, *Onchorynchus mykiss*	47,000	10,490
Hydra, *Hydra* sp.	7300	1700
Fathead minnow, *Pimephales promelas*	5500	1601
Cladoceran, *Daphnia magna*	1450	856
Amphipod, *Hyalella azteca*	760	340
Amphipod, *Gammarus pseudolimnaeus*	65[a]	2704
Criterion Maximum Concentration	12.8	185.9

[a] The value shown for *G. pseudolimnaeus* represents the only data point where selenate is more toxic than selenite.

snail *Aplexa hypnorum* and the leech *Nephelopsis obscura*) being the least sensitive (U.S. EPA, 1987a).

Despite the considerable amount of data indicating that selenite is the more toxic than selenate, U.S. EPA's acute water quality criterion for selenate, 12.8 $\mu g/L$, is considerably lower than the selenite criterion of 185.9 $\mu g/L$ (U.S. EPA, 1987a). Several factors contribute to this inconsistency in relative sensitivity including the limited data for acute selenate toxicity ($n = 11$), the apparent extreme sensitivity of *Gammarus pseudolimnaeus* to selenate as compared with selenite (Table I), and the data distribution for the four most sensitive species tested with selenate. Specifically, selenate toxicity values for the four most sensitive species range from 65 $\mu g/L$ for the amphipod, *G. pseudolimnaeus*, to 5500 $\mu g/L$ for the fathead minnow, *Pimephales promelas* (U.S. EPA, 1987a).

Our study had two objectives: (1) to re-examine the sensitivity of gammarid amphipods to selenate and (2) to reduce uncertainty in the selenate data by testing additional sensitive species. To accomplish this, we undertook acute toxicity studies of selenate with three amphipods, *Gammarus pseudolimnaeus*, *Gammarus lacustris*, and *Hyalella azteca*, and two daphnids, *Ceriodaphnia dubia* and *Daphnia pulex*. Daphnids were selected in addition to the amphipods because of their demonstrated sensitivity to selenate by other researchers (Boyum, 1984; Brooke *et al*., 1985; Dunbar *et al*., 1983).

METHODS AND MATERIALS

Testing Facilities

Testing was conducted at three laboratories in the United States. Parametrix's toxicology laboratory in Kirkland, Washington, conducted testing on *G. lacustris*, *H. azteca*, *D. pulex*, and *C. dubia*. Great Lakes Environmental Center's (GLEC) laboratory in Traverse City, Michigan, conducted testing on *G. pseudolimnaeus* and *H. azteca*. ABC Laboratories in Columbia, Missouri, conducted testing on *G. pseudolimnaeus*. The use of three laboratories resulted in slight differences in dilution waters and test methods for the various studies but reasonably consistent results, as described below.

Test Material

Reagent grade sodium selenate, Na_2SeO_4 (CAS #13410-01-0), obtained from Sigma Chemical Company (St. Louis, Missouri) was used for studies conducted at Parametrix and ABC Laboratories. A hydrated reagent grade salt of sodium selenate, $Na_2SeO_4 \cdot 10H_2O$ (CAS #30844-7), was used for testing at GLEC. All stock solutions were prepared in deionized water just before initiation of testing.

Experimental Design

All tests were conducted according to U.S. EPA or ASTM guidelines (ASTM, 1993; U.S. EPA 1975, 1987b, 1993). The different requirements of the various species tested necessitated slightly different experimental designs; these are described below. For all tests, temperature, pH, and dissolved oxygen were measured at test initiation and every 24 hours until test termination. All amphipod tests were 96 hours in duration and all daphnid tests were 48 hours in duration, in accordance with U.S. EPA guidelines (Stephan *et al*., 1985). The tests conducted at GLEC were performed under flow-through conditions while those at Parametrix and ABC Laboratories were static non-renewal. The flow-through testing was conducted using a modified Benoit continu-

ous flow mini-diluter system. For all studies, test organisms were not fed during the selenate exposure. Mortality, defined as immobility under gentle prodding, was monitored every 24 hours.

Gammarus pseudolimnaeus Testing

Test organisms were obtained from Osage Catfisheries (Osage Beach, Missouri) and Environmental Consulting and Testing (Superior, Wisconsin) for testing by ABC Laboratories and GLEC, respectively. ABC Laboratories tested juvenile organisms (5 to 10 mm in length) while GLEC tested adults.

The ABC Laboratories study used four replicate test chambers (2-L Pyrex glass beakers) for each of the five selenate concentrations and a control. The dilution water was composed of well water and demineralized well water mixed to a final hardness of 140 mg/L as $CaCO_3$ with 10 mg/L sulfate. Each test chamber contained 10 juvenile amphipods. The test was conducted in a temperature controlled water bath maintained at 18 ± 1°C.

The GLEC study used four replicate 2-liter test chambers (10 cm wide by 14 cm high by 25 cm deep) for each of the five selenate concentrations and a control. The dilution water was dechlorinated water from Lake Michigan with a hardness of 139 mg/L as $CaCO_3$ and 25 mg/L sulfate. Each test chamber contained five amphipods. The test was conducted at 18 ± 2°C using a combination of emersion heaters in the dilution water headbox and chilled water pumped into the headbox.

Gammarus lacustris Testing

The *G. lacustris* test experimental design was modified from that described above for *G. pseudolimnaeus*. Test organisms, obtained from Lincoln Bait (Cushing, Minnesota), were sub-adults (8 to 12 mm in length and not sexually mature) at test initiation. Preliminary testing demonstrated that *G. lacustris* was extremely prone to cannibalism, resulting in poor survival across all concentrations. To control for this, test chambers consisted of 20-L high-density polyethylene containers, each containing 8 L of test solution. Ten amphipods were placed in each test chamber in separate 250-mL borosilicate glass beakers covered with Nitex screen to prevent direct interaction of test organisms. The experimental design used four replicate test chambers for each of the five selenate concentrations and a control. The dilution water was a synthetic, moderately hard water (116 mg/L hardness as $CaCO_3$ and 120 mg/L sulfate). Test temperature was maintained at 15 ± 1°C inside a temperature controlled environmental chamber.

Hyalella azteca Testing

Like *G. pseudolimnaeus*, testing of *H. azteca* was performed at two laboratories. Testing at Parametrix followed standard U.S. EPA guidelines for conducting acute toxicity tests (U.S. EPA, 1993). Test organisms, obtained from laboratory stock cultures, were 7 to 10 days 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 soft water (52 mg/L hardness as $CaCO_3$ and 55 mg/L sulfate). A 2 to 3 mm layer of silica sand was placed in the bottom of each test beaker to provide substrate for the amphipods. This sand was chemically characterized to confirm that no organic carbon was associated with it. Each test chamber contained 10 juvenile amphipods. Test temperature was maintained at 20 ± 1°C inside a temperature controlled environmental chamber.

Testing at GLEC followed standard ASTM guidelines for conducting acute toxicity tests (ASTM, 1993). Test organisms, obtained from Environmental Consulting and Testing (Superior, Wisconsin) and were 17 to 22 days old at test initiation. The experimental design consisted of four replicate test chambers (250 mL beakers) for each of the five selenate concentrations and a control. The dilution water was a synthetic, moderately hard water (143 mg/L hardness as $CaCO_3$ and 25 mg/L sulfate). 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.

Daphnid Testing

Daphnid tests followed standard U.S. EPA guidelines for conducting acute toxicity tests (U.S. EPA, 1993). Two tests were performed with each species. Test organisms were obtained from laboratory stock cultures and were less than 24 hours old at test initiation. The experimental design for each test consisted of four replicate test chambers (30 mL polypropylene cups) for each of the five selenate concentrations and a control. The dilution water was a synthetic, moderately soft water (52 mg/L hardness as $CaCO_3$). Dilution water sulfate concentrations ranged from 31 to 54 mg/L. Each test chamber contained five daphnid neonates. Test temperature was maintained at 20 ± 1°C inside a temperature controlled environmental chamber.

Chemical Analysis

Total recoverable selenium concentrations were measured at test initiation and termination for each concentration and control in all studies. Studies conducted at GLEC also measured test concentrations at 48 hours.

Total selenium was determined by graphite furnace atomic absorption spectrometry (Varian Zeeman 400 Palo Alto, California) according to U.S. EPA method 7740 (U.S. EPA, 1995) for studies conducted at Parametrix. Studies performed at ABC Laboratories and GLEC analyzed selenium concentrations by inductively coupled plasma (ICP) or inductively coupled plasma/mass spectrometry (ICP/MS) using U.S. EPA method 200.7 or 200.8 (U.S. EPA, 1995). Detection limits varied based on the nominal test concentrations being evaluated but were always at least a factor of 10 lower than the lowest concentration tested, excluding the control.

Under the oxic and alkaline test conditions used in these studies, selenium introduced as sodium selenate is expected to remain in the selenate form (Turner *et al*., 1981). Selenium speciation of low, middle, and high test concentrations for toxicity testing performed at GLEC confirmed these expectations. Samples analyzed using the hydride generation technique of Cutter (Cutter, 1986) demonstrated that more than 97% of total selenium was in the selenate form.

Statistical Analysis

Statistical endpoints (LC_{50}) were calculated by probit analysis (Finney, 1971) using the computer software program TOXIS, version 2.4 (EcoAnalysis, Ojai, California) or Trimmed Spearman Karber (Hamilton *et al*., 1977). Endpoints included the 48 or 96 hour LC_{50} (and its 95% confidence limits) as appropriate for the experimental design. Statistical endpoints were calculated using mean measured selenium concentrations in each of the tests.

RESULTS

Water Quality and Selenium Concentrations

Water quality conditions for all tests are summarized in Table II. Test temperature, dissolved oxygen, and pH all met test acceptability requirements as defined by U.S. EPA (1993). Mean measured total recoverable selenium concentrations for each study are presented in Table III. Measured concentrations across all tests remained stable for the duration of testing.

Toxicity Test Results

The dose response relationships and LC_{50}'s for each test are presented in Table III. Control survival ranged from 90–100% with good dose response relationships observed for all tests. The LC_{50}'s for the *G. pseudolimnaeus* tests were 2469 and 1180 μg/L, while the *G. lacustris* test resulted in an LC_{50} of 3054 μg/L. The *H. azteca tests* resulted in LC_{50}'s of 2480 and 1428 μg/L.

Testing for the two daphnids indicates distinctly different sensitivities for the two species. The two *Ceriodaphnia dubia* tests resulted in LC_{50}'s of 1864 and 1969 μg/L. In comparison, the two *Daphnia pulex* tests resulted in LC_{50}'s four to five times higher at 8111 and 10,123 μg/L selenium.

DISCUSSION

The acute toxicity of selenate to *G. pseudolimnaeus* was evaluated previously by Brooke and co-workers (1985) and Brooke (1987) in two separate studies, where similar LC_{50}'s of 75 and 57 μg/L selenium were reported. These data significantly affect the current selenate water quality criterion and are inconsistent with the rest of the data in terms of comparative species sensitivity to selenate and selenite (Table I). Data generated in our study for this species and the closely related *G. lacustris* resulted in much higher LC_{50}'s, thus supporting the rest of the data that demonstrate selenate is less toxic than selenite.

A comparative review of previous and present studies provides some insight on why results differ substantially. Most experimental design factors that might influence test results (temperature, test organism age, hardness, sulfate) were quite similar and are summarized in Table IV. The only identifiable difference in

TABLE II. Summary of water quality conditions for toxicity test performed as part of the present study

Test	Temperature (°C)	pH	Dissolved Oxygen (mg/L)
G. pseudolimnaeus (ABC)	18 ± 0	7.6 ± 0.3	8.3 ± 0.1
G. pseudolimnaeus (GLEC)	17.8 ± 1.8	7.8 ± 0.2	8.8 ± 0.2
G. lacustris (PMX)	15 ± 0	7.6 ± 0.1	9.8 ± 0.2
H. azteca (PMX)	20 ± 0	7.4 ± 0.1	8.3 ± 0.1
H. azteca (GLEC)	18.2 ± 1.9	7.8 ± 0.4	9.1 ± 0.9
D. pulex ($n = 2$) *(PMX)*	20 ± 0	7.7 ± 0.2	8.2 ± 0.6
C. dubia ($n = 2$) *(PMX)*	20 ± 0	7.8 ± 0.1	8.4 ± 0.3

TABLE III. Summary of toxicity test results from present studies

	G. pseudolimnaeus		*G. lacustris*	*H. azteca*		*D. pulex* # 1	*D. pulex* # 2	*C. dubia* # 1	*C. dubia* # 2
Test Conc. (μg/L Se)	ABC (Se[a]/%S[b])	GLEC (Se/%S)	PMX (Se/%S)	GLEC (Se/%S)	PMX (Se/%S)	PMX (Se/%S)	PMX (Se/%S)	PMX (Se/%S)	PMX (Se/%S)
Control	ND/100	1.0/100	ND/93	6.0/95	ND/93	ND/90	ND/95	ND/95	ND/100
1	492/100	219/75	750/85	202/94	462/95	1200/100	4000/95	423/100	450/100
2	1080/83	518/80	1000/90	433/100	801/83	2150/100	6650/95	689/95	800/100
3	2255/50	802/85	2150/63	1057/72	1320/50	4050/100	11,050/0	1135/80	1450/85
4	4745/15	1940/5.0	3450/45	4023/0	2855/10	7650/80	18,350/0	1891/70	2400/15
5	9340/13	4027/0	5800/15	6977/47	5550/0	14,950/0	NT/NT	3145/0	3900/0
LC_{50}	2469	1180	3054	2480	1428	10,123	8111	1969	1864
(95%C.I.)	(2037–2994)	(1050–1320)	(2530–3693)	(1810–3390)	(1188–1675)	(9140–11,212)	(7567–8694)	(1730–2242)	(1642–2109)

S = survival; NT = not tested; ND = not detected.
[a] Mean of measured test concentrations. Values reported as μg/L.
[b] Mean survival of four replicates.

terms of experimental design is that studies conducted by Brooke and colleagues were both performed at a lower pH than the more recent studies. The more recent studies were conducted at an ambient pH of 7.6–7.8 while the Brooke and colleagues studies were conducted at pH 6.8–7.0.

We investigated the influence of differences in pH between experimental designs on test results using the computer model Geochemist Workbench (Bethke, 1995). Using this model, we estimated the chemical speciation at equilibrium of a 1 mg/L selenate solution spiked into standard U.S. EPA (U.S. EPA, 1993) dilution water with a hardness of 100 mg/L under oxic conditions (Eh = 450 mV). Selenium speciation was estimated between pH 6.8 and 7.8, which represents the range between the Brooke studies and the current studies (Table IV). As shown in Table V, the pH difference between the two sets of studies potentially has significant effect on selenium speciation. It was hypothesized that the present studies, conducted under more alkaline test conditions, were testing only the selenate ion while the Brooke *et al*. studies were testing primarily biselenate (61%) with some selenite (24%) and selenate (15%). Two additional studies were performed to further examine this issue.

First, additional tests were conducted in order to confirm the effects of pH on acute selenium toxicity. *Ceriodaphnia dubia* and *Hyalella azteca* were exposed to nominal concentrations of selenate at a pH of 6.7, resulting in LC_{50}'s of 450 and 741 μg/L. This compares with LC_{50}'s of 1917 (SMAV) and 1428 μg/L for these two species when tested at pH 7.8 in the same laboratory. While these additional data do support the hypothesis that selenate is more toxic at a lower ambient pH, the difference in toxicity was only a factor of 4 for *C. dubia* and 2 for *H. azteca*. This compares with the approximately 32-fold difference in toxicity observed when *G. pseudolimnaeus* was tested at pH 6.8–7.0 versus 7.6–7.8. Consequently, it is unlikely that differences in pH between the *G. pseudolimnaeus* studies fully explain observed differences in test results.

The second study examined the kinetics of selenium speciation as it pertains to the toxicity test results being evaluated. Selenium speciation kinetics are known to be relatively slow for some reactions, for example, the oxidation of selenite to selenate (Cutter and Bruland, 1984; Scott, 1991). Consequently, although the speciation model predicts significant changes in selenium speciation between pH 7.8 and 6.8, that rate of this reaction may be too slow to influence a 96-hour toxicity test. To evaluate the kinetics of the predicted pH-related changes in selenium speciation, four water samples, from the same source as the dilution for the toxicity studies conducted at Parametrix, were spiked with 1 mg/L total selenium as selenate. Two of the

TABLE IV. Summary of gammarid amphipod test conditions

Test Parameter	Brooke *et al.* (1985)	Brooke (1987)	GLEC	ABC Laboratories	Parametrix
Test Species	*G. pseudolimnaeus*	*G. pseudolimnaeus*	*G. pseudolimnaeus*	*G. pseudolimnaeus*	*G. lacustris*
Test Condition	Static	Static	Flow-through	Static	Static
LC_{50} (μg/L)	75	57	1180	2469	3054
Test Duration (hrs)	96	96	96	96	96
Test Organism Size/Age	5–7 mm Sub-Adults	7–11 mm Adults	NR Adults	5–10 mm Sub-Adults	8–12 mm Sub-Adults
Feeding	Unfed	Unfed	Unfed	Unfed	Unfed
Temperature (°C)	16	22	18	18	15
Dissolved Oxygen (mg/L)	7.5	6.8	8.8	8.3	9.8
pH	6.8	7.0	7.8	7.6	7.6
Sulfate (mg/L)	12	NR	25	10	120
Hardness (mg/L as $CaCO_3$)	148	51	139	140	116

NR = not reported.

TABLE V. Modeled selenium species as percent of system at selected conditions (1 mg/L SeO_4^{2-}, 100 mg/L hardness)

Species	pH = 6.8, Eh = 450 mV	pH = 7.8, Eh = 450 mV
SeO_4	15%	100%
$HSeO_3$	61%	< 0.1%
SeO_3	24%	< 0.1%
$HSeO_4$	< 0.1%	< 0.1%
H_2SeO_3	< 0.1%	< 0.1%
Hse	< 0.1%	< 0.1%
H_2Se	< 0.1%	< 0.1%
Total	100%	100%

samples were adjusted to pH 6.8 using 2N HCl and the other two were maintained at the initial pH of 8.2. All four samples were placed in an environmental chamber maintained at 20 ± 1°C. After 24 hours, one sample from each pH regime was cooled to 4°C to inhibit further changes in speciation and stored for analysis. After 96 hours, the other two samples were cooled and all samples were analyzed for selenium speciation using ion chromatography hydride generation atomic fluorescence spectrometry (Wallschläger and Bloom, 1999).

Results of this follow-up study indicated that all the selenium was still in the selenate form, regardless of the pH at which it was maintained. Considering these results, it is unlikely selenium speciation between the Brooke *et al.* tests and the new tests presented in this paper differed substantially due to the slow kinetics of pH-dependent selenium speciation. However, selenate toxicity does appear to be at least partially pH-dependent. The extent of this effect appears to be organism specific as demonstrated by the *C. dubia* and *H. azteca* results from this study. The considerably more significant effect observed for *G. pseudolimnaeus* creates uncertainty as to whether pH is the sole factor causing differences in observed LC_{50}'s. Additional testing with this species is needed to resolve this question. It is possible that other unidentified factors may be influencing test results. Sulfate is the only water quality parameter that has been demonstrated to influence selenate bioavailabilty, with increasing ambient sulfate concentrations reducing selenate bioconcentration and toxicity (Hansen *et al.*, 1993; Ogle and Knight, 1996; Williams *et al.*, 1994). Sulfate concentrations were comparable (10–25 mg/L) for all *G. pseudolimnaeus* studies, eliminating it as a possible modifying factor. In contrast, the *G. lacustris* study with selenate performed at Parametrix was conducted at a significantly higher sulfate concentration (120 mg/L) which may account for its slightly higher LC_{50} compared to *G. pseudolimnaeus*.

The *H. azteca* tests conducted in the present study resulted in LC_{50}'s of 2480 and 1428 μg/L selenium (Table III). These values are two to three times higher than the only *H. azteca* value in the current selenium water quality criteria (WQC) document of 760 μg/L (Adams, 1976). The values we obtained for *H. azteca* are consistent with recent study results by Brasher and Ogle (1993) (LC_{50} of 1868 μg/L). Since *H. azteca* is the second most sensitive species tested with selenate (U.S. EPA, 1987a), a revised species mean acute value (SMAV) including these new data will significantly change the current acute water quality criterion for selenate.

An examination of the available toxicity data for *Daphnia* sp. indicates its sensitivity is highly variable (Table VI). Of the species tested, the most sensitive is *Daphnia pulicaria* ($EC_{50} = 246$ $\mu g/L$) and the least sensitive is *Daphnia pulex* ($EC_{50} = 9061$ $\mu g/L$). The difference in values is approximately 50-fold. An obvious explanation for the variability in sensitivity in phylogenetically related species is not apparent. Testing of *Daphnia* sp. with other metals and metalloids has resulted in much smaller differential sensitivities (expressed as SMAVs), between 1.4 and 4.6, although in most cases only two species rather than three have been tested (U.S. EPA, 1984a, b, 1986, 1987c). Correction/normalization of the EC_{50} values for sulfate concentration in the test media reduces the overall range of sensitivity to a factor of 25 from 50 (Brix *et al*., in press). This remaining variability is unaccounted for and noteworthy.

The data presented in this paper have significant implications with respect to the acute selenate water quality criterion. Using the standard U.S. EPA guidelines for deriving numeric water quality criteria (Stephan *et al*., 1985), consideration of the data presented in this study indicates the need to raise the water quality criterion from 12.8 to either 451.5 or 583.3 $\mu g/L$, depending on whether the Brooke data are included or excluded from the data set. This suggested increase in the criterion would make the selenate criterion higher than that for selenite and consistent with the majority of the scientific evidence (Table I).

The significant increase in the selenate water quality criterion resulting from addition of these limited additional data is related to two factors that are important to highlight. First, by repeating the *G. pseudolimnaeus* study and conducting testing with *G. lacustris*, the resulting *Gammarus* genus mean acute value (GMAV) has been dramatically raised from 65 to 2283 $\mu g/L$ (not considering the Brooke data). The new LC_{50} values for *H. azteca* and *D. pulex* had similar though less significant effects, raising the GMAVs for *Hyalella* and *Daphnia* substantially.

TABLE VI. Acute toxicity of selenate to daphnids

Species	LC_{50} ($\mu g/L$)	SMAV ($\mu g/L$)	Reference
Daphnia pulex	10,123	9117	This study
Daphnia pulex	8111		This study
Ceriodaphnia dubia	1969	1917	This study
Ceriodaphnia dubia	1864		This study
Daphnia magna	5300	1450	(Dunbar *et al*., 1983)
Daphnia magna	1010		(Boyum, 1984)
Daphnia magna	570		(Brooke *et al*., 1985)
Daphnia pulicaria	246		(Boyum, 1984)

The second, and perhaps not as obvious factor, is the addition of the genus *Ceriodaphnia* to the data set. Intuitively, one would think the addition of this taxon to the data set would lower the water quality criterion due to the sensitivity of *C. dubia*. In fact, inclusion of the new data resulted in an opposite effect. For example, exclusion of *C. dubia* from the data set results in a CMC of 365.4 $\mu g/L$ as compared to 583.3 $\mu g/L$ when *C. dubia* results are included. The reason for this counterintuitive effect relates to the means by which WQC are derived under U.S. EPA guidelines which focus on the distribution of the four most sensitive taxa tested. As reviewed earlier, the GMAVs of the four most sensitive taxa in the current data set range in sensitivity from 65 to 5500 $\mu g/L$. This wide distribution results in a shallow slope (expressed as % species affected), and the subsequent regression to estimate the 95% level of protection has an extremely long tail that drives the criterion down. In contrast, inclusion of the *C. dubia* data into the data set eliminates the relatively insensitive *P. promelas* (GMAV of 5500 $\mu g/L$) from the four most sensitive species. This steepens the resulting slope, resulting in a regression with a significantly shorter tail, raising the criterion accordingly (Fig. 1). Because the current selenate toxicity data set has a limited number of species, the current overestimation of the 95% protection level is exaggerated further. For data sets where more species have been tested (e.g., 20) this effect will not be as great.

The type of data distribution observed for the acute selenate data set, where a limited number of data points skew the distribution and resulting criterion, is not unique. A number of other U.S. EPA criteria have similar data distributions and a limited number of species tested (U.S. EPA, 1984c, 1984d). The end result of a skewed data distribution is a very conservative water quality criterion. Recognizing that the intent of a criterion is to be protective of the water resource and water use, it is appropriate that the CMC be overprotective rather than underprotective when calculated from a limited data set.

Results from this study highlight the need for confirmatory testing when one or several toxicity tests within a data set are found to be driving a water quality criterion. By gathering additional data, the quality of the data set is improved, as well as the confidence in the predicted CMC. Additionally, this study highlights the need to evaluate the range of toxicity values used to calculate the CMC. It is counterintuitive that a value from an insensitive species should result in a lower criterion. The concept that the criterion should be influenced (weighted) by the (four) most sensitive species has merit and is supported by our results. Several other water quality criterion values are affected by a few data points that have not been validated by

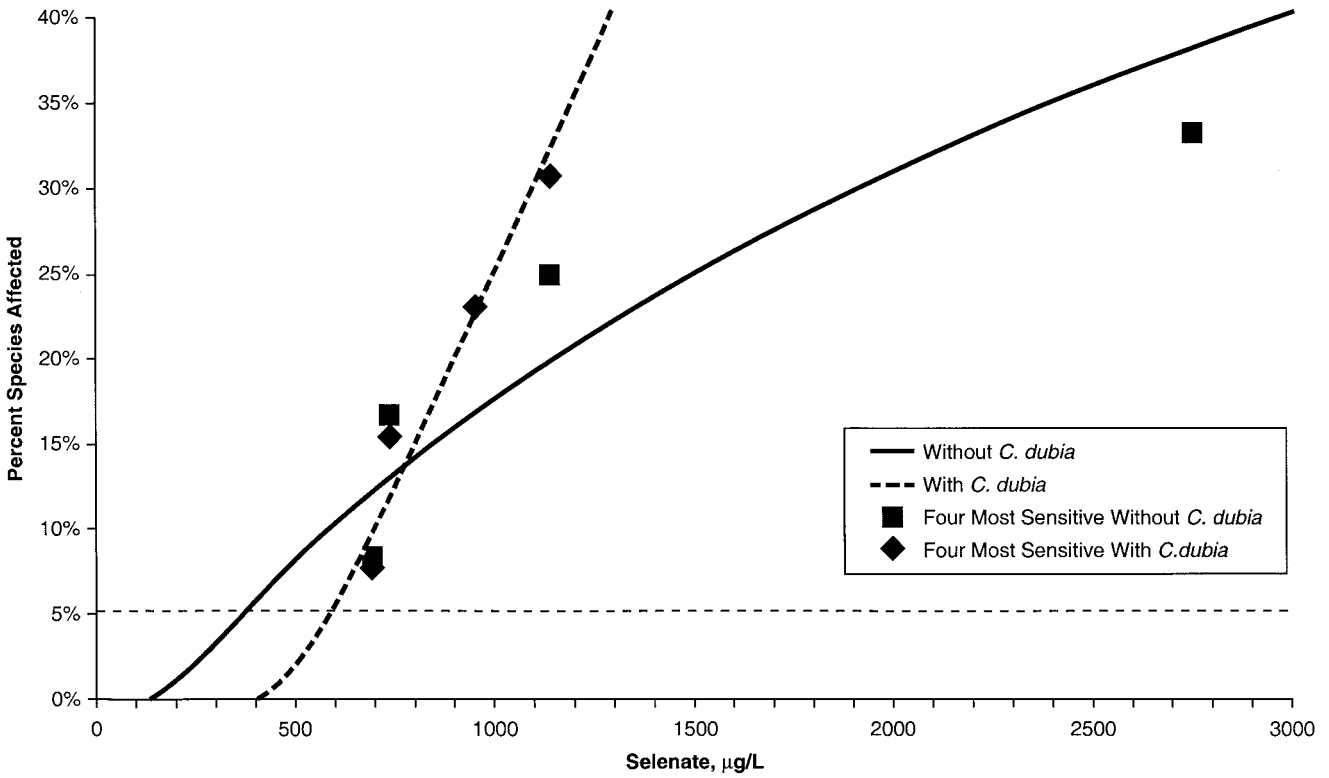

Fig. 1. Acute selenate data set with and without *C. dubia*.

independent testing. While this does not necessarily mean that repeating these key studies will dramatically change the criterion as is the case with selenate, further testing may provide additional information on why a given data outlier exists, as has certainly been the case with selenate.

Specific to the selenate criterion, the results of this study indicate that ambient pH is potentially critical to the regulation of selenate discharges to surface water, perhaps being more important than sulfate as has been discussed by other researchers (Brix *et al.*, in press; Ogle and Knight, 1996). Further investigation into the influence of pH on inorganic selenium speciation and subsequent toxicity is recommended.

CONCLUSIONS

Results from toxicity tests conducted on species sensitive to selenate indicate the current U.S. EPA acute water quality criterion is overprotective. The current criterion is driven by data for the gammarid amphipod *G. pseudolimnaeus*. New testing with this species and the closely related *G. lacustris* indicates the selenate LC_{50} for this genus is approximately 30 times higher than previously reported. The exact reason for these differences is unclear, but it is partially explained by differences in ambient pH between previous and new testing. Inclusion of these new data results in an acute water quality criterion of 583 $\mu g/L$. This study highlights the need to re-evaluate test results that appear to be outliers, particularly when they drive a water quality criterion.

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