Acute and chronic sulfate toxicity to select freshwater organisms in water-only exposures

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Abstract

The objective of this study was to generate acute and chronic sulfate (Na_2SO_4) toxicity data to select freshwater fish and invertebrates that could be used by USEPA to develop national water quality criteria for sulfate and by states and tribes to refine water quality standards for sulfate. Test water was diluted well water (about 100 mg/L as $CaCO_3$ and a pH of about 8.2). Static renewal, acute toxicity tests were conducted with cladoceran (*Ceriodaphnia dubia*; 2-d exposure), midge (*Chironomus dilutus*; 4-d exposure), unionid mussel (pink mucket, *Lampsilis abrupda*; 4-d exposure), and fathead minnow (*Pimephales promelas*; 4-d exposure). Chronic toxicity tests were conducted with the cladoceran (7-d static renewal exposure with survival and reproduction endpoints), the midge (41-d flow through exposure with survival, weight, biomass, and reproduction endpoints), the mussel and fathead minnow (28- to 34-d flow-through exposure with survival, weight, and biomass endpoints). The results of the acute tests indicate that (1) the cladoceran and the mussel were more sensitive to sulfate than the fathead minnow and midge, and (2) the larval fathead minnows were more sensitive than the juvenile fathead minnows, and (3) midge were least sensitive. The results of the chronic tests indicate that (1) the embryonic and newly hatched fathead minnows were extremely sensitive to sulfate (acute to chronic ratios [ACRs] of 34), (2) the cladoceran was relatively insensitive to sulfate, unlike that observed in acute tests (ACR: 2.3), (3) the mussel was more chronically sensitive to sulfate than the cladoceran and midge. The draft Canadian water quality guideline (WQG) for sulfate was below the EC20s for the cladoceran and midge tested in our study, but was above the EC20s for both the fathead minnow and the mussel, indicating the draft Canadian WQG may not adequately protect the fathead minnow or the mussel from chronic sulfate exposure. Further studies are planned to determine the influence of water quality on the chronic toxicity of sulfate to cladocerans and fathead minnows.

Introduction

State water quality standards for sulfate have been developed by Illinois and are being considered by other Midwestern states in the United States (USEPA 2009). These state standards were developed primarily with acute toxicity data. National USEPA ambient water quality criteria (WQC) for sulfate have not been developed. A recent study (Elphick et al. 2010) developed a dataset base for chronic sulfate toxicity data to develop Canadian water quality guidelines. However, further studies are needed to provide a more direct measure of relationships between the acute and chronic toxicity of sulfate to selected species of aquatic organisms to develop the USEPA WQC for sulfate or refine state water quality standards for sulfate. The objective of this study was to generate acute and chronic sulfate toxicity data to select freshwater fish and aquatic invertebrates.

Midge (*Chironomus dilutus*)

Cladoceran (*Ceriodaphnia dubia*)

Fathead minnow (*Pimephales promelas*)

Juvenile Mussel

Materials and Methods

Table 1 summarizes the test conditions for acute and chronic exposures for each of the four species, following standard methods in ASTM (2011a,b,c,d,e). Midge, mussel, and fish chronic tests were conducted in intermittent flow-through diluter systems. The solution of Na_2SO_4 for the highest sulfate concentration was prepared in a 4,000-L polypropylene tank and was delivered to a glass cell associated with the modified diluters (Photo 1).

Photo 1. Intermittent flow-through diluter system

Fathead minnow incubation cup

Table 1. Conditions for conducting acute and chronic water-only toxicity tests with sulfate (adapted from ASTM 2011a,b,c,d,e)

Parameter	Acute				Chronic			
Test species	Cladoceran	Fathead Minnow	Midge	Mussel	Cladoceran	Fathead Minnow	Midge	Mussel
Test type	Static, non-renewal	Static, renewal			Static, renewal	Flow-through		
Test duration	2-day	4-day			7 day when >60% of control animals produce 3 broods	34 days (28 days after day of hatch in controls)	7 days for survival and biomass; up to about 41 days for reproduction	28 (also check survival on days 4, 14, 21)
Temperature °C	25	25	23	23	25	25	23	23
Water volume	30 mL	2 L	30 mL	30 mL	15 mL	1 L	250 mL	200 mL
Water renewal	None	Day 2			Daily	250 mL/chamber every 30 min	125 mL/chamber every 8 hr	125 mL/chamber every 8 hr
Age of test organism	< 24 hr	[1] < 2 days post hatch female [2] about 30 days post hatch	About 7 day old	About 1 month after metamorphosis	< 24 hr	< 24 hr embryos	About 7 day old	About 1 month after metamorphosis
# organisms/chamber	5	10	1	5	1	[1]5; thinned to 10 on Day 3	12	10
# replicate chambers	4	2	10	4	10	4	4- survival and biomass 6-reproduction	4
Chamber cleaning	None	once daily	None	None	None	once daily	Small screen	If necessary
Test water	Well water (about 300 mg/L as CaCO3) diluted with deionized water: about 100 mg/L hardness and 90 mg/L alkalinity as $CaCO_3$.							
Water quality	Dissolved oxygen, pH, conductivity, hardness, alkalinity, ammonia, sulfide, and nitrite/nitrate measured at beginning and end of acute tests and once weekly during chronic tests.							
Data analysis	EC10, EC20, and EC50 estimated by Toxicity Relationship Analysis Program (TRAP; Russell Erickson, USEPA, Duluth MN)							
Endpoint	Survival				Survival (daily) and reproduction	Survival, length, weight, and biomass	7-day survival and weight, percent of emergence, number of egg cases deposited/produced, percent hatching	Survival, length, weight, and biomass
Test acceptability	≥ 90% control survival				> 80% control survival; >15 young/female in 7 days; >90% of surviving control females have 3 broods	≥ 80 % in control chambers	≥ 70 % control survival and ≥ 50% control emergence	≥ 80% control survival

Results and Discussion

Acute toxicity tests:

- Mean control survival of test organisms in all acute tests was >93%, meeting the test acceptability criterion of >90% for control survival (ASTM 2011a,b,d,e; Table 1).
- Cladoceran: The 48-h EC50s for the cladoceran from the four tests were similar, ranging from 2,241 to 2,441 mg SO_4/L (Figure 1).
- Fathead minnow: The 96-h EC50 for newly hatched fathead minnow (4,833 mg SO_4/L) was about 2-fold lower than the EC50 for about 30-d-old juveniles (10,869 mg SO_4/L, only larval fathead minnow EC50 is included in Figure 1).
- Midge: The midge were least acutely sensitive to sulfate with a 96-h EC50 of 5,992 mg SO_4/L (Figure 1).
- Mussel: The 96-h EC50 for mussels from the flow-through test with feeding (2,149 mg SO_4/L) was comparable to the EC50 from the static-renewal with no feeding (2,362 mg SO_4/L).

Results and Discussion (cont.)

Chronic toxicity tests:

- Mean control survival or reproduction of test organisms in all chronic tests met the test acceptability criterion (ASTM 2011a,b,d,e; Table 1). Sublethal endpoints (weight or reproduction) were more sensitive to survival for all four tested species (Figure 1).
- Cladoceran: Three cladoceran tests were conducted. A low number of young (<9) were produced in low to high sulfate concentrations in Test 1 and no EC20 for reproduction could be estimated because the data did not meet the conditions for any regression analysis. The EC20s for reproduction from Tests 2 and 3 were similar between Test 2 (932 mg SO_4/L) and Test 3 (1,064 mg SO_4/L).
- Fathead minnow: Three fathead minnow tests were conducted. The fish tended to hatch earlier in the higher sulfate exposure concentrations and high mortality was observed at or above 800 mg SO_4/L in all three tests by Day 7 of the exposures (Figure 2). The 34-d biomass of fathead minnows were reduced at very low concentrations of sulfate (EC20 ranging from 106 to 185 mg SO_4/L).
- Midge: The 7-d ash-free dry weight of midge larvae (EC20 of 977 SO_4/L) was most sensitive to sulfate compared to survival or to the reproductive endpoints (EC20 ranging from 1,819 to >3,223 SO_4/L).
- Mussel: The dry weight EC20 was 696 mg SO_4/L and the ash-free dry weight EC20 was 640 mg SO_4/L, whereas the EC20 for survival was 1,759 mg SO_4/L.

The draft Canadian WQG (644 mg SO_4/L) calculated for moderately hard water conditions (similar to our test water) using a species sensitivity approach (Elphick et al. 2010) was below the EC20s for the cladoceran and midge tested in our study, but was above the EC20s for fathead minnow and the mussel (Figure 1), indicating the draft Canadian WQG may not adequately protect fathead minnow and the mussel from chronic sulfate exposure. Notably, the fathead minnow test in the study of Elphick et al. (2010) was conducted for only 7 days starting with newly hatched larvae, while our early life-stage tests were conducted for 34 days starting with embryos.

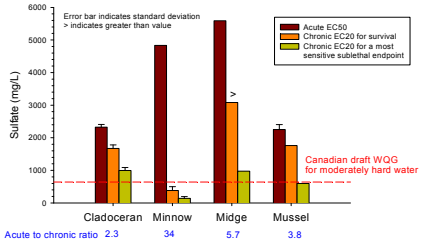

Figure 1. Acute sulfate EC50 and chronic EC20 for cladoceran (*Ceriodaphnia dubia*), fathead minnow (*Pimephales promelas*), midge (*Chironomus dilutus*), and pink mucket (mussel, *Lampsilis abrupda*). Dashed line indicates the Canadian draft water quality guidelines (WQG) for sulfate (following the SSD approach, hardness 80-100 mg/L; Elphick et al. 2011). Acute to chronic ratio was calculated based on acute EC50 for survival and chronic EC20 for the most sensitive endpoint.

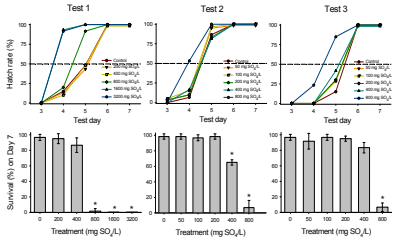

Figure 2. Hatch and survival of fathead minnow (*Pimephales promelas*) during the first 7-d exposure in three 34-d early life-stage sulfate toxicity tests. Asterisk indicates significant reduction relative to the control ($p < 0.05$).

Acute to chronic toxicity relationships:

- Acute to chronic ratio (ACR) for each species was calculated based on acute EC50 and chronic EC20 for the most sensitive sublethal endpoint (Figure 1). The ACR for the fathead minnow was about 6- to 15-fold greater than ACRs for the other three test species.

Conclusions

- Acute tests indicate that the cladoceran and the mussel were more sensitive to sulfate than the fathead minnow and midge.
- Chronic tests indicate that the embryonic and newly hatched fathead minnows were extremely sensitive to sulfate (acute to chronic ratios [ACRs] of 34). In contrast, the cladoceran was relatively insensitive to sulfate in chronic tests, unlike that observed in acute tests (ACR: 2.3). The mussel was more chronically sensitive to sulfate than the cladoceran and midge.
- The draft Canadian water quality guideline for sulfate protects the cladoceran and midge tested in our study, but may not adequately protect the fathead minnow or the mussel tested in our study.
- Sulfate toxicity may be influenced by water hardness and chloride, as seen in previous studies. Further studies are planned to determine the influence of water quality on the chronic toxicity of sulfate to cladocerans and fathead minnows.

Acknowledgements

Funding was provided in part for this study by USEPA Region 5.

We thank everyone in the USGS-Columbia Toxicology Branch and in the Environmental Chemistry Branch for their assistance on this study.

We thank Ed Hammer and other USEPA personnel, and David Soucek for their input on experimental design and interpretation of data.

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Presented at 32nd SETAC NA on November 14, 2011