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ACUTE AND CHRONIC TOXICITY OF SELENIUM TO ESTUARINE ORGANISMS

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ABSTRACT: Acute toxicity tests were conducted with selenium and five estuarine organisms, and chronic or early life stage tests were conducted with mysid shrimp *(Mysidopsis bahia)* and sheepshead minnows *(Cyprinodon variegatus).* The concentrations of selenium lethal to 50% of the test animals after 96 hours of exposure (96-hour LC50's) ranged from 1.2 mg/l for brown shrimp *(Penaeus aztecus)* to 7.4 mg/l for sheepshead minnows. The maximum acceptable toxicant concentration (MATC) was > 0.14 < 0.32 mg/l for mysid shrimp and > 0.47 < 0.97 mg/l for sheepshead minnows. The application factor limits for mysid shrimp and sheepshead minnows were 0.09-0.21 and 0.06-0.13, respectively.

Selenium is a widely distributed metalloid usually found in combination with heavy metals such as lead, silver, copper, gold, and zinc as selenides. Selenium enters the aquatic environment as run-off of seleniferous soils, by dissemination of fly ash from coal-burning power plants (Crecelius, 1980), or from industrial consumption of selenides which yield selenium as a by-product (Schroeder and Darrow, 1973). In the specific case of an electrolytic zinc plant, selenium, released from the purification of zinc as residual particulates and from the scrubbing of stack gases, was incorporated into the plant effluent (Cardwell, 1979).

Information on the toxicity of selenium to estuarine organisms is very limited. To our knowledge, no tests have been conducted with estuarine fish and relatively few with estuarine invertebrates. The limited data prompted the two projects reported here, the first a "priority pollutants" program sponsored by the U.S. Environmental Protection Agency (Contract 68-01-4646) and the second a study to evaluate the potential effects of selenium in the effluent of ASARCO's electrolytic zinc refinery located in Corpus Christi, TX.

MATERIALS AND METHODS

Test Materials.— The test material for all tests except the mysid shrimp acute and chronic was sodium selenite, 5-hydrate (J. T. Baker Chemical Company). Selenous acid (ALFA Products, Ventron) was the test material in the mysid tests. All stock solutions were prepared by using deionized water as the solvent/carrier. Concentrations of the test material are reported here as milligrams (mg) of selenium per liter (l) of seawater or parts per million.

Test Animals.— All organisms tested were native to the Gulf of Mexico. Brown shrimp *(Penaeus aztecus),* 42-67 millimeters (mm) rostrum-telson length, were purchased from a bait dealer near Ocean Springs, MS. Juvenile blue crabs *(Callinectes sapidus),* 8-13 mm carapace width, and subadult pinfish *(Lagodon rhomboides),* 55-73 mm standard length, were collected from Big Lagoon, an estuary adjacent to Bionomics Marine Research Laboratory (BMRL). The subadult mysid shrimp *(Mysidopsis bahia),* 4-6 mm total length, and juvenile sheepshead minnows *(Cyprinodon variegatus),* 12-16 mm standard length which were used in the acute, flow-through tests were reared at BMRL. Mysid shrimp used to initiate the chronic test were 2 mm total length and were laboratory reared. Sheepshead minnow embryos for the early life stage test were obtained by stripping eggs from adult fish whose egg production was en-

hanced by injections of human chorionic gonadotrophin hormone. The eggs were fertilized by the addition of a sperm suspension made from macerated testes.

Test Water.— The natural seawater used in the studies was pumped from Big Lagoon. The water was filtered through sandfilled fiberglass filters and 10-micrometers (μm) pore size polypropylene core filters. All water used in the static tests was filtered again to 5 μm with a polypropylene core filter.

Test Conditions.— Test methods for the static, acute tests followed those described by The Committee on Methods for Toxicity Tests with Aquatic Organisms (1975). The tests were conducted in 19 l uncovered glass jars which contained 15 l of filtered seawater. Salinity was 30 parts per thousand (‰) and temperature was maintained at 22±1 degrees Celsius (°C). Brown shrimp and pinfish were tested 3 per jar and all treatments were quadruplicated, resulting in 12 animals per treatment. Five blue crabs were tested per jar, 10 per treatment. There was no aeration, nor were test animals fed during the tests.

The mysid shrimp and sheepshead minnow acute and chronic tests were conducted in intermittent-flow systems by using a proportional diluter (Mount and Brungs, 1967) at a dilution rate of 50%. Test methods for the mysid shrimp chronic followed those reported by Nimmo *et al.* (1977). The sheepshead minnow early life stage test methods were those reported by Schimmel *et al.* (1974) and Ward *et al.* (in press).

Statistical Analyses.— The 96-hour LC50's and 95% confidence limits were determined, where possible, by linear regression after converting each test concentration to a logarithm and the corresponding percentage mortality to a probit (Finney, 1971). There was no correction for control mortality.

At the termination of the mysid shrimp chronic test and the sheepshead minnow early life stage test, mortality, hatching success, growth, and number of offspring per female were analyzed by analysis of variance (ANOVA), where appropriate. All percentages were converted by square root arcsin percentage transformations of binomial percentages to angles of equal information in degrees prior to ANOVA. Statistical comparison between the control and each test concentration was made by either Williams' method (Williams, 1971) or Dunnett's procedure (Steel and Torrie, 1960). Differences between the control and test concentrations were considered significant at the 95% confidence level ($P < 0.05$).

Chemical Analyses.— The seawater samples from the mysid shrimp and sheepshead minnow flow-through tests were analyzed for selenium by atomic absorption spectrophotometry by using a Perkin-Elmer Model 305A atomic absorption spectrophotometer equipped with a factory installed deuterium arc background corrector. The analytical method was based on that of Pierce *et al.* (1976) and the Perkin-Elmer Manual, #990-9822.

RESULTS

The acute (96-hour) toxicity of selenium for five estuarine organisms tested was in the low parts per million range (1.2-7.4 mg/l). Brown shrimp and mysid shrimp were the most sensitive, while sheepshead minnows were the least sensitive (Table 1).

Chronic exposure to mean measured selenium concentrations ≥ 0.32 mg/l had a statistically significant effect on survival of the parental (F_0) mysid shrimp. No offspring were produced by surviving mysids in 0.58 mg/l and the number of offspring produced per female was significantly lower in 0.32 mg/l than in the control. All offspring produced in all treatments survived until termination of the

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TABLE 1. Calculated 96-hour LC50's for test animals exposed to selenium in seawater. (The 95% confidence limits are in parentheses.)

Species	Test System	96-hour LC50 (mg/l) Nominal	Measured
Mysidopsis bahia	Flow-through	———	1.5(1.1-2.1)
Penaeus aztecus	Static	1.2(0.8-1.8)	———
Callinectes sapidus	Static	4.6(2.7-7.8)	———
Cyprinodon variegatus	Flow-through	———	7.4(4.5-12)
Lagodon rhomboides	Static	4.4(2.9-6.7)	———

test (Table 2). The maximum acceptable toxicant concentration (MATC) for mysid shrimp was > 0.14 < 0.32 mg/l. The application factor limits, based on the 96-hour LC50 for mysids of 1.5 mg/l, were 0.09-0.21.

Exposure to mean measured selenium concentrations $\leq$ 6.4 mg/l had no significant effect on the hatching success of sheepshead minnow embryos. However, selenium concentrations $\geq$ 0.97 mg/l significantly reduced the survival of juvenile fish. There was no significant effect of selenium on growth of surviving juveniles (Table 3). The estimated MATC of selenium for embryos and juveniles of sheepshead minnows was > 0.47 < 0.97 mg/l. The application factor limits, based upon the 96-hour LC50 of 7.4 mg/l for sheepshead minnows, were 0.06-0.13.

DISCUSSION

The 96-hour LC50's for saltwater invertebrates (1.5 mg/l for mysid shrimp, 1.2 mg/l for brown shrimp, and 4.6 mg/l for blue crabs) were much higher than those reported for freshwater invertebrates, *Hyallela azteca* and *Daphnia magna*, of 0.34 and 0.43 mg/l, respectively (Halter *et al.*, 1980). These LC50 values were similar, however, to that reported for the saltwater Dungeness crab *(Cancer magister)* of 1.04 mg/l (Glickstein, 1978).

Likewise, the 96-hour LC50's of 7.4 and 4.4 mg/l for the saltwater fishes, sheepshead minnows and pinfish, respectively, were comparable to those previously determined for freshwater fish which ranged from 1.0-2.1 mg/l for fathead minnow fry *(Pimephales promelas)* to 28.5 mg/l for juvenile bluegill *(Lepomis macrochirus)* (Cardwell et al., 1976, and Halter *et al.*, 1980).

The mysid shrimp is the only saltwater invertebrate known to be chronic tested with selenium to date. In freshwater chronic tests, *Daphnia magna* were unaffected in selenium concentrations $\leq$ 0.28 mg/l while selenium concentrations $\geq$ 0.5 mg/l were lethal (Halter *et al.*, 1980). The MATC of > 0.28 < 0.5 mg/l was

TABLE 2. Summary of water chemistry and biological data from a chronic exposure of mysid shrimp *(Mysidopsis bahia)* to selenous acid in flowing natural seawater. Salinity was 26 ± 2 ‰ and temperature, 23 ± 1°C.

Nominal conc. (Se++;mg/l)	Mean measured conc. ± S.D.[a] (Se++; mg/l)	Percent of nominal	Percentage survival	Number of offspring per female	Offspring percentage survival
Control	N.D.[b]	———	85	6.7	100
0.037	0.05±0.02	135	85	6.2	100
0.075	0.13±0.05	160	70	6.2	100
0.150	0.14±0.07	93	70	5.2	100
0.300	0.32±0.13	107	50[c]	3.2[c]	100
0.600	0.58±0.24	97	15[c]	0[c]	———

[a] Standard deviation.
[b] Not detectable; <0.005 mg/l.
[c] Significantly less (P <0.05) than the control.

TABLE 3. Summary of water chemistry and biological data collected during an embryo-juvenile exposure of sheepshead minnow *(Cyprinodon variegatus)* to sodium selenite in flowing natural seawater. Salinity was 27±2 ‰ and temperature, 29±1°C.

Nominal conc. (Se++;mg/l)	Mean measured conc. and S.D.[a] (Se++;mg/l)	Percent of nominal	Percentage hatch	Percentage juvenile survival	Growth (mm)
Control	N.D.[b]	———	99	76	12±2
0.375	0.47±0.18	125	89	72	11±2
0.75	0.97±0.36	129	99	58[c]	11±2
1.5	1.9±0.4	127	95	3[c]	_[d]
3.0	3.6±0.6	120	96	0[c]	_[e]
6.0	6.4±0.2	107	91	0[c]	_[e]

[a] Standard deviation.
[b] Not detectable; <0.13 mg/l. There was one sample (day 7) on which the concentration was above the detection limit (0.88 mg/l).
[c] Significantly less ($P < 0.05$) than the control.
[d] Mean not calculated since only two fish survived.
[e] All fish had died.

similar to that determined for mysid shrimp, > 0.14 < 0.32 mg/l. However, because *D. magna* were more acutely sensitive than were mysid shrimp, the application factor of > 0.65 was higher than the 0.09-0.21 application factor limits calculated for mysid shrimp.

Results of the early life stage test with sheepshead minnows were similar to those reported for zebrafish *(Brachydanio rerio)* (Niimi and LeHam, 1975), carp *(Cyprinus carpio)* (Huckabee and Griffith, 1974), and fathead minnows (Halter *et al.*, 1980). In the freshwater fish tests, no effect on hatchibility of embryos was observed in selenium concentrations ≤ 10 mg/l. Mortality of zebrafish larvae was significant in selenium concentrations ≥ 3 mg/l and mortality of fathead minnow fry was significant in concentrations ≥ 1 mg/l.

In a 27-month exposure of rainbow trout *(Salmo gairdneri)* to selenium, the no-effect concentration was >0.06<0.13 mg/l, based on both post swim-up mortality and deformed fish (Goettl and Davies, 1977). This test indicated that rainbow trout were much more sensitive to selenium than were sheepshead minnows or other species of freshwater fish. However, the trout test was conducted in soft water while the sheepshead minnow early life stage test was conducted at a relatively high average salinity of 27‰. As in the case of metal salts, selenium toxicity is probably related to salinity or hardness of the test water, and we would expect the sheepshead minnow to be more susceptible to selenium at a lower average salinity. Interestingly, although the 96-hour LC50 for rainbow trout was higher than that determined for mysid shrimp and sheepshead minnows, trout were much more sensitive to chronic exposure of selenium, resulting in an application factor approximately an order of magnitude lower (0.005-0.011) than that for sheepshead minnows (0.06-0.13).

An application factor of 0.01 recommended by the U.S. Environmental Protection Agency for marine aquatic life (U.S. EPA, 1976) appears to be adequate to protect the estuarine species tested. However, with the possibility of the salinity modifying the toxicity, similar studies performed at lower salinities would be beneficial to insure protection.

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