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Selenium Toxicity to *Daphnia magna, Hyallela azteca,* and the Fathead Minnow in Hard Water[1]

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This is a report on the comparative toxicity of selenium to three common representatives of the warm-water biota, Daphnia magna, Hyallela azteca, and the fathead minnow (Pimephales promelas).

Selenium is one of several metalloids that could become more widely distributed in the environment through increased mining and burning of fossil fuels. The known biochemical roles and toxicity of selenium in mammals have been reviewed by CERWENKA & COOPER (1961), ROSENFELD & BEATH (1974), SCHROEDER et al. (1970), and FROST (1972), but the effects of selenium in aquatic systems have received attention only recently (ENVIRONMENTAL PROTECTION AGENCY 1973). Selenium levels in excess of 0.5 µg/g (wet weight) have been measured in fish and zooplankton from Lake Michigan (COPELAND et al. 1973) and in excess of 1.0 µg/g in fish collected from Lake Erie (ADAMS & JOHNSON 1977). BEAL (1974) has shown that selenium in Canadian fishes may be higher near more densely populated areas. The toxicity of selenium to several fish species at various life stages has been determined by CARDWELL et al. (1976), NIIMI & LAHAM (1975) and HUCKABEE & GRIFFITH (1974). CARDWELL et al. (1976) noted that fathead minnows were the most sensitive of the six fish species they tested with selenium.

METHODS AND MATERIALS

Test organisms were exposed to selenium with a proportional dilution system (MOUNT & BRUNGS 1967) which delivered duplicates of six concentrations plus a control. Toxicant flow rate to 30-L glass exposure tanks was 100 mL/min which equaled a 90% replacement time of 12 h. A 12-h photoperiod was maintained over the exposure system.

The diluent water was supplied from an independent well at our laboratory. Water characteristics (mg/L except as noted) were: hardness as $CaCO_3$, 329; total alkalinity as $CaCO_3$, 332; ammonia N, 0.42; total Kjeldahl N, 1.17; total C, 74.0; total solids, 324; Fe, 1.0; Pb < 0.03;Cu< 0.05; Zn < 0.01; Se < 1.0 mg/L; pH, 7.3; conductivity as umhos/cm, 615. Dissolved oxygen ranged from 5.3-6.2 (mode 5.7) mg/L and the test temperataure was 25 ± 2 C.

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Selenium stock solutions were made up with sodium selenite (Na_2SeO_3) and sodium selenate (Na_2SeO_4). Stock solutions were added to the exposure system with a mariotte bottle-dipping bird delivery device (MOUNT & BRUNGS 1967). Selenium concentrations in the test water were measured by the colorimetric method of CUMMINS et al. (1965). The variability of this method, as indicated by measurement of six replicates of 0.6 mg/L Se, was less than 1%. Water was sampled at the center of the test tanks twice during 96-h tests and twice a week during longer tests. Sampling error, determined by two sets of quadruplicate samples from different test tanks, was less than 1%. Selenium analysis from all tests showed that measured selenium concentrations were 94 $\pm$ 6% of the nominal amounts added. Nominal values were used for all data calulations and selenium is expressed as mg/L Se, rather than Na_2SeO_3 or $NaSeO_4$.

D. magna, H. azteca, and fathead minnow eggs were obtained from indoor cultures at our laboratory. Fathead minnow fry were collected from an outdoor breeding pond and held indoors for one week prior to testing. All biological test methods generally followed the recommendations of the ENVIRONMENTAL PROTECTION AGENCY (1975).

D. magna were tested in glass boxes (141 x 6.5w x 14d cm, with one side made of 0.5 mm Nitex screen) which were suspended in the 30-L test tanks. To insure adequate water and toxicant exchange, the boxes were gently raised and lowered at least twice each day. Food (brewer's yeast), sufficient to slightly cloud the water, was placed in the boxes after each water exchange. Daphnid mortalities were recorded at 0800, 1300, 1700, and 2100 daily; criterion for death was cessation of all appendage movement. Acute toxicity tests were performed in duplicate with 15 middle-instar daphnids in each box, and then repeated. Two chronic tests were conducted, with selenium concentrations between 0.03 and 0.13 mg/L and 0.07 and 0.28 mg/L, respectively. These tests were run in duplicate starting with ten two-day-old daphnids per box, and continued for two or three weeks. Reproductive counts were made whenever about 50 or more young were present in a box.

H. azteca were tested in the same manner as the daphnids but in boxes that were 121 x 7w x 8d cm. One three-week, one two-week and two 96-h tests were conducted, each in duplicate. Ten adult amphipods were tested in each box. Food and substrate were not provided during the 96-h tests, but algae (Cladophora, 5 g, wet weight) was present in each test box during the longer tests.

Fathead minnow fry were tested in the 30-L tanks. Twenty fish of about 0.03 g, 17 mm, and 25-35 days old were randomly assigned to duplicate selenium concentrations ranging from 0.35 to 1.40 mg/L for 17 days. The fish were fed live brine shrimp daily. Mortalities were recorded at the intervals given above.

Fathead minnow eggs (2 days old) were placed in screened bottom cups suspended in 1.0-L beakers. Twenty-five eggs were tested in each cup and eight duplicate concentrations of selenium (0-40 mg/L) were used. A water temperature of 25 C was maintained by a water bath and air was bubbled beneath the cups until hatching was complete. No food was offered to the larvae. The duration of the experiment was 300 h and the test solutions were renewed at 72, 127, and 168 h.

Test results are expressed in terms of median survival times (MST) and median lethal concentrations (LC50), which were determined according to the methods of LITCHFIELD (1949) and LITCHFIELD & WILCOXIN (1949), respectively. In the daphnia tests, the lethal threshold concentration (LTC) was calculated in the manner suggested by SPRAGUE (1969).

RESULTS

Selenium concentrations above about 0.5 mg/L were lethal to daphnia within 96 h, but at lower concentrations toxicity declined sharply (Figure 1A). In chronic tests, daphnid survival and reproduction were unaffected at all concentrations tested (0.03 to 0.28 mg/L). For example, at the three highest selenium concentrations, 0.16, 0.21, and 0.28 mg/L, the average number of young produced per adult was 55, 40 and 48, respectively, in two weeks compared to only 15 in the controls. These production levels correspond to nearly a thousand individuals produced per box in a two-week period at the highest selenium levels. The lethal threshold calculated from the toxicity data was 0.43 mg/L Se. This LTC was then divided into the maximum acceptable toxicant concentration (MATC), which was considered to be 0.28 mg/L, to obtain an application factor (MOUNT & STEPHAN 1967, EATON 1970) of 0.65 for D. magna.

Selenium concentrations up to 40 mg/L had no effect on the hatchability of fathead minnow eggs (Table 1), but levels above 15 mg/L Se did significantly reduce the egg incubation times. The post-hatch median survival times were reduced at all selenium concentrations.

TABLE 1

Incubation Times and Hatchability of Combined Duplicate Groups of 25 Two-Day Old Fathead Minnow Eggs, and Post-Hatching Median Survival Times (MST) of Resulting Fry, Exposed Under Renewed Static Conditions.

Nominal Se Conc. (mg/L)	Median Incubation Time (h)	Percent Hatch	Fry Post-Hatch MST (h)
Control	96	100	188 (183-193)[a]
1	99	100	120 (105-136)
5	98	100	85 (82-88)
10	97	96	62 (58-67)
15	73	100	33 (30-36)
20	57	100	25 (19-33)
25	57	96	11 (9-14)
30	74	100	14 (11-17)
40	47	100	12(11-13)

[a] 95% confidence interval.

DISCUSSION

Selenium toxicity to the test species is compared in Figure 1D and Table 2. Daphnia was the most sensitive species initially but the least sensitive ultimately. Amphipods were the most sensitive species after 336 h (two weeks) of exposure, but further effects apparently could be expected under continued exposure for both amphipods and fish. The threshold reaction time or induction period of selenium toxicity, as seen at higher selenium levels (Figure 1D), for fish was at least double that measured for invertebrates.

The toxicity observed with amphipods may have in part been due to ingested selenium, since a contaminated food source was available throughout the test. H. azteca browses on the epiphytic communities found on substrates such as Cladophora (HARGRAVE 1970), and it seems likely that these communities would accumulate selenium even if the substrate does not (SANDHOLM et al. 1973).

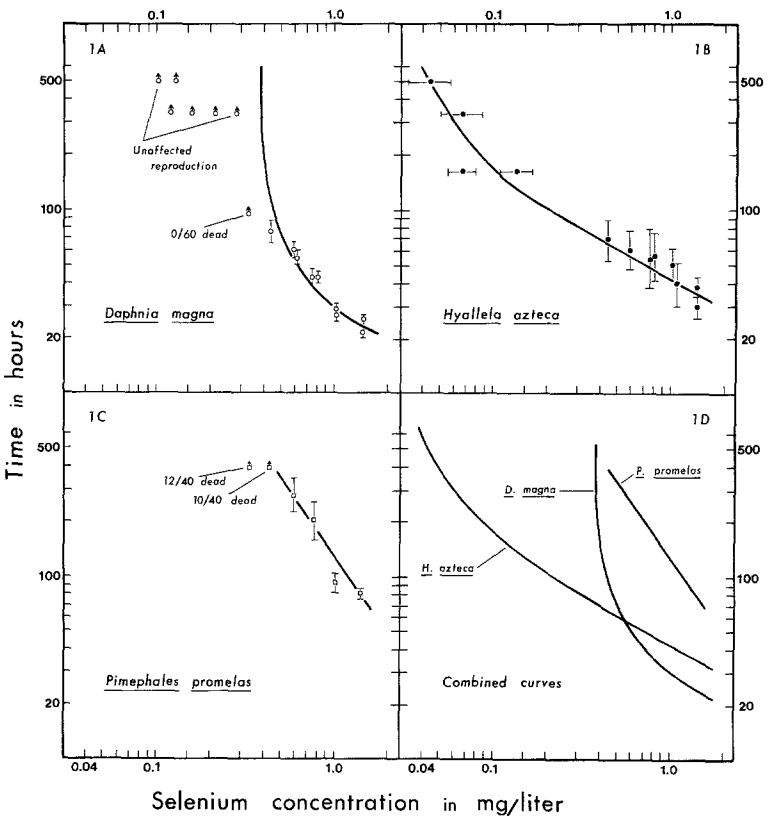

Fig. 1. The relationship between selenium concentration in water and the median survival time for Daphnia magnia, Hyallela azteca, and fathead minnow (Pimephales promelas) fry.

H. azteca were killed by selenium levels above about 0.5 mg/L for 96 h. Continued exposure at lower selenium concentrations produced additional mortalities (Figure 1B). No threshold was apparent from these data although some upward inflection of the toxicity curve is evident at the lower exposure levels. The survival and apparent health of amphipods at the lowest test level (0.03 mg/L) was the same as the controls after three weeks.

Mortalities of fathead minnow fry occurred at all selenium levels tested, from 0.35 to 1.4 mg/L Se (Figure 1C). No lethal threshold was evident after 17 days of exposure. Symptoms of selenium poisoning included abdominal and subocular edema in about 40% of the fish. Similar pathology was reported by NIIMI & LAHAM (1975).

TABLE 2

Selenium Concentrations (mg/L) Lethal to Daphnia magna, Hyallela azteca, and the Fathead Minnow (Pimephales promelas) in Hard Water at Various Time Intervals.

Test Species	48-h LC50	96-h LC50	336-h LC50
D. magna	0.71 (0.64-0.79)[a]	0.43 (0.41-0.45)	0.43 (0.41-0.45)
H. azteca	0.94 (0.72-1.2)	0.34 (0.26-0.45)	0.07 (0.05-0.09)
P. promelas	---- ----	1.0 (0.94-1.2)	0.60 (0.50-0.72)

[a]95% confidence interval.

LC50 values with fathead minnows are lower than those reported by CARDWELL et al. (1976). The 96- and 336-h LC50 values in our study were 1.1 and 0.60 mg/L, whereas the 96- and 220-h LC50 values in their work were 7.3 and 2.9 mg/L. The chemical form of selenium used in the two studies (SeO_2 vs Na_2SeO_3) should have been the same once dissolved in water ($HSeO_3^-$). This disparity may be due to differences in fish age and the duration of study.

The ENVIRONMENTAL PROTECTION AGENCY (1972) has recommended public water supplies contain no more than 0.01 mg/L Se. Our data indicate that in hard waters this human health standard should be adequate to protect Daphnia magna, and possibly Hyallela azteca. To determine if this level would protect the fathead minnow, a complete life cycle study should be performed.

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