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Effect of Extended Sublethal Exposure to Sodium Selenite on *Ceriodaphnia affinis*

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Recent studies of the chronic effects of selenite selenium on freshwater organisms indicate that the "no observed effect level" (NOEL) ranges from 0.36 to 0.06 mg/L (Owsley 1984; Halter et al. 1980; Reading 1979; Goettl and Davies 1977). The reduction of fish populations and observations of cellular abnormalities in fish have been attributed to chronic selenium exposure in reservoirs with selenium concentrations as low as 0.005 mg/L (CP&L 1984a, 1984b; Sorensen et al. 1984; Sorensen et al. 1982). The discrepancies between the NOEL's measured in the laboratory and the possible effect levels observed in the aquatic environment may be due, in part, to the fact the animals tested in the laboratory had not been previously exposed to selenium. The purpose of this study was to determine the effect of extended sublethal exposure to selenite selenium on the acute and chronic toxicity of the material to the Cladocera Daphnidae Ceriodaphnia affinis Lilljeborg 1900.

MATERIALS AND METHODS

Ceriodaphnia affinis were obtained from Dr. Donald Mount of the U. S. Environmental Protection Agency, Environmental Research Laboratory, Duluth, MN and were cultured in our laboratory for 6 months prior to testing. Water for both culturing and testing was obtained by dechlorinating Nashville tap water with 12-24 hours of areation, placing it in 5 gallon glass carboys, augmenting it with 2 mL of a yeast-fish food diet, and allowing it to age for 2 to 4 days. Mean values (±standard deviation) for the dissolved oxygen, pH, and total hardness of the aged dechlorinated Nashville tap water were 8.4 (±0.5) mg/L O_2, 7.9 (±0.3), and 100.8 (±4.6) mg/L $CaCO_3$, respectively.

The yeast-fish food diet consisted of 1.5g yeast (Fleischmann's) and 0.5g ground fish food (Tetra Min, Tetra Werke, W. Germany) suspended in 300 mL aged dechlorinated Nashville tap water. The diet was fed to the culture at a rate of 1.5 mL/L. The culture was maintained at 21.5±3.1°C and provided with a fluorescent light source of approximately 100 footcandles on a 16/8 hour light/dark cycle.

Reagent grade sodium selenite (Na_2SeO_3) (Sigma Chemical

Company, St. Louis, MO, lot #91F-0103) was used in this study. A stock solution of 1000 mg/L Se was made up at the initiation of the tests using double deionized water from a Millipore 4 - Module Milli-Q reagent Grade Water System and filtered through a 0.22 micron filter (Millipore Corporation, Bedford, MA). All test concentrations were prepared on the day of use by volumetric dilution utilizing class A volumetric glassware, the stock solution, and aged dechlorinated Nashville tap water. All selenium concentrations are reported as total selenium. Twenty initial selenium measures conducted with a Perkin-Elmer Model 403 Atomic Spectrophotometer (Perkin-Elmer Instrument Division, Norwalk, CT) using the methods outlined in USEPA (1979) indicated that the nominal concentrations were within ±9% of the measured concentrations. The average selenium concentration (±standard deviation) measured in the aged dechlorinated Nashville tap water was 1.0 (±0.0) µg/L Se.

All toxicity tests were initiated with <24 hour old C. affinis. The test temperatures were 23.0±2.0°C with approximately 50 footcandles of light provided by a 25 watt incadescent light bulb set on a 16/8 hour light/dark cycle.

The acute toxicity tests exposed C. affinis to concentrations of selenite selenium ranging from 0.29 to 3.20 mg/L Se and were conducted in accordance with standard acute toxicity test methods (ASTM 1980). Test animals with previous exposure to selenite selenium were obtained from adults reared in concentrations of the material ranging from 0.018 to 0.36 mg/L Se for 18 to 19 days.

The chronic toxicity tests exposed 4 consecutive generations (P_1, F_1, F_2, F_3) of C. affinis to 4 concentrations of selenite selenium ranging from 0.05 to 0.8 mg/L Se and a control. Each treatment consisted of 50 mL beakers containing 1 female in a 30 mL volume. The animals were transfered to clean beakers with fresh concentrations and fed 0.15 mL of the yeast-fish food diet every other day. Young were counted and removed daily. The tests were continued until 3 broods were produced in the control (Mount and Norberg 1984). This occurred in approximately 8 days. Young obtained from the initial test (P_1) were used in the second test (F_1). F_1 offspring were then used in the F_2 test and F_2 offspring were used in the F_3 test. In the initial test (P_1) 15 animals were used in each treatment. In the remaining tests (F_1, F_2, F_3) the number of animals tested per concentration depended upon the number of young produced in the final brood of the previous test concentration. The end point measured was the average total number of young produced per surviving adult after 3 broods. The Kruskal Wallis test (Sokal and Rohlf 1981) was used to asses the statistical significance of differences among the selenium concentrations. The Dunn's multiple comparison of group means (Hollander and Wolfe 1973) was used to determine which specific

concentrations differed significantly. Differences were considered statistically significant for alpha ≤ 0.05.

RESULTS AND DISCUSSION

Mortality in the acute toxicity test controls was $\leq$10%. The results of the acute toxicity tests are shown in Table 1. These data indicate that C. affinis are less tolerant of acute levels of selenite selenium after previous exposure to sublethal levels of the material. Adams (1976) suggests that selenite selenium toxicity is accumulative.

TABLE 1. - Static acute toxicity of selenite - selenium to <24 hour old Ceriodaphnia affinis showing the effect of previous sublethal exposure to the material.

Type Test	Concentration mg/L Se	95% Confidence Interval	No. of Replicates	Exposure
Not Exposed	24 hr LC_{50} [a]	0.76	0.90-0.64	7
	48 hr LC_{50} [a]	0.60	0.71-0.51	4
Exposed	24 hr LC_{50} [a]	0.39	0.55-0.27	2
	48 hr LC_{50} [b]	0.35	. . .	2

[a] LC_{50} determined by the Litchfield and Wilcoxon abbreviated method (Litchfield and Wilcoxon 1949)

[b] LC_{50} determined by the log-concentration versus percent survival graphical method (USEPA 1978)

Mortality in the chronic toxicity test controls was 27% in the P_1 test, 0% in the F_1 test, 7% in the F_2 test, and 0% in the F_3 test. The average total number of young produced per surviving female in each treatment is shown on Table 2. The P_1 test exhibits a NOEL of 0.2 mg/L Se. This compares favorably to the NOEL's given in the literature for other daphnids not previously exposed to selenite selenium. Owsley (1984) reported a NOEL of 0.36 mg/L Se for C. affinis after 20 days exposure to the material. Halter et al. (1980) reported a NOEL of 0.28 mg/L Se for Daphnia magna after 21 days exposure to the material and Reading (1979) reported a NOEL of 0.20 mg/L Se for D. pulex after 28 days exposure to selenite selenium.

The pattern of significant differences shown in the subsequent

generations (F_1, F_2, and F_3) indicates a gradual decline in the reproductive success of the females exposed to the intermediate concentrations. This decline becomes extreme in the F_2 and F_3 generations. The NOEL of selenite selenium to C. affinis was reduced from 0.2 to 0.1 mg/L Se after 2 generations of exposure. Winner (1976) reported no difference in the reproductive output of 3 generations (P_1, F_1, and F_2) of D. magna when exposed to chronic levels of copper.

TABLE 2. - Average total number of young produced after 3 broods from 4 generations of a group of Ceriodaphnia affinis exposed to chronic levels of selenite - selenium. Values not underscored by a continuous line were found to be significantly different by Dunn's test at the alpha 0.05 level.

	Concentration mg/L Se					
Generation	0.0 (Control)	0.05	0.1	0.2	0.4	0.8
P_1	15.0	a	14.3	10.6	1.0	0.0
F_1	15.8	11.9	10.5	8.7	0.7	
F_2	13.0	18.9	18.1	1.0	0.0	
F_3	15.3	16.3	15.3	0.0		

[a] 0.05 mg/L Se concentration added in F_1 test to replace 0.8 mg/L concentration

The data presented in this study indicate that C. affinis are less tolerant to selenite selenium after extended exposure to sublethal levels of the material. This factor should be taken into consideration in future toxicity testing where the outcome desired is a "safe" cocentration of toxicant.

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