Bull. Environm. Contam. Toxicol. 24, 929-935 (1980)

Effects of Sublethal Concentrations of Selenium on Metabolism and Filtering Rate of *Daphnia pulex*

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Recent attempts to develop adequate water quality criteria for protection of freshwater aquatic life from toxic chemicals has been hampered by the lack of adequate data on sublethal effects (e.g., NATIONAL ACADEMY OF SCIENCES (NAS) 1973, UNITED STATES ENVIRONMENTAL PROTECTION AGENCY (USEPA) 1976, 1979a). Most sublethal toxicity data are on reproduction of fish and Daphnia. These tests are expensive and time consuming, and alternative methods such as filtering rate and oxygen consumption have been proposed to evaluate pollutant stress (SHERR & ARMITAGE 1973, COOLEY 1977).

The purpose of this research was to evaluate the sublethal effects of selenium on Daphnia pulex oxygen consumption and filtering rate. Selenium is a trace element which is receiving increased attention as an environmental pollutant because of its high concentration in coal and other fossil fuels (PILLAY et al. 1969). It is the least plentiful and most toxic of the essential trace elements with a very narrow margin of safety between required and toxic doses (COPELAND 1971, FROST & LISH 1975).

MATERIALS AND METHODS

D. pulex were obtained from our laboratory cultures. They were cultured in 19-L all glass aquaria containing Blacksburg carbon-dechlorinated tap water which was previously filtered through a 50 micron mesh net. Stock cultures were kept at 20 $\pm$ 2°C. A 16L:8D photoperiod and an air-water interface intensity of approximately 1080 lux were provided by cool white fluorescent lights. Stock cultures were fed daily with an ad libitum suspension of Chlamydomonas reinhardi (wild type, minus strain) which were grown in a modified Bold's Basal Medium (BUIKEMA 1970). Algae were centrifuged, washed, and resuspended in dechlorinated tap water before being fed to the Daphnia.

The form of selenium used was reagent grade sodium selenite ($NA_2SeO_3 \cdot 5H_2O$). Stock solutions were made with

0007-4861/80/0024-0929 $01.40

carbon-dechlorinated tap water which had been pre-filtered through a 0.45 micron filter and aerated 12-24 h prior to use. The dilution water received the same treatment.

Water quality characteristics of the filtered dechlorinated tap water were determined according to methods outlined in Standard Methods for Examination of Water and Wastewater (AMERICAN PUBLIC HEALTH ASSOCIATION (APHA) 1975). Mean values (±95% C.I.) for hardness, pH, and dissolved oxygen were 42.0 ± 4.5 mg/L $CaCO_3$, 7.2 ± 0.2, and 8.1 ± 0.4 mg/L, respectively.

Selenium concentrations were determined by hydride generation atomic absorption spectrophotometry (USEPA 1979b) using the sodium borohydride reduction method (FERNANDEZ 1973, CORBIN & BARNARD 1976). The total selenium content of the dilution water was monitored periodically during the study and was always less than 0.01 mg/L. For the tests, a stock solution with a concentration of 0.8 mg/L selenite-Se was diluted to yield 0.6, 0.4, and 0.2 mg/L solutions. The mean (±95% C.I.) of the measured selenium concentration of the stock solution for the oxygen consumption and filtering rate studies, respectively, was 0.85 (± 0.1) and 0.91 (± 0.18) mg/L Se. Results are reported as the concentration of the element selenium rather than the compound sodium selenite.

For the oxygen consumption studies, glass stoppered pyrex bottles of approximately 60-mL capacity, calibrated by weight to the nearest 0.1 mL, were used as respirometers. Preadult Daphnia within a 0.2 mm size class were segregated and not fed for 12 h prior to testing. The animals were pre-rinsed with 0.45 micron filtered dechlorinated tap water. Five to ten Daphnia were placed in each respirometer containing a test solution at 20°C. The bottles were stoppered, checked for air bubbles, and placed in the growth chamber at the same temperature and light regime described above. Three or four replicates at each of the four experimental concentrations (0.2, 0.4, 0.6, and 0.8 mg/L Se), one or two controls, and one or two blanks (filtered water only) were used for each test, depending on the number of Daphnia available. The selenium test solutions, at the concentrations used, did not exert any oxygen demand in 24 h.

After 24 h, the amount of dissolved oxygen in each respirometer was determined with azide modification of the Winkler method (APHA 1975). Aliquots (25 mL) were titrated with 0.005 N sodium thiosulfate solution using a buret calibrated in 0.02 mL. Oxygen concentrations were corrected for dilution by Winkler reagents and for

volume titrated. Oxygen consumed was determined by subtracting the corrected oxygen content of the experimentals from the blank values. Oxygen consumption was then converted to uL O_2/mg dry weight/h by using a length-weight relationship. For animals less than 1.27 mm in length, which included all the animals used in the oxygen consumption studies (Mean length after a 24-h exposure was 0.97 mm), the equation [1] was:

$$W = 0.0025L - 0.0001 \qquad [1]$$

where W is dry weight (mg), and L is length (mm).

For the filtering rate studies preadult Daphnia were exposed to each of the four concentrations of selenium for 24 h. Three 60 mL pyrex bottles for each of the experimental concentrations and two for the controls were filled with 59 mL of the appropriate test solution and seven to ten animals. One mL of algal suspension was then added, diluting each bottle to the appropriate selenium concentration and giving an algal density of 30,000 cells/mL. An algal blank was included at each selenium concentration to serve as an algal control. All bottles were then placed in a growth chamber under the same conditions described above. The mean length for the animals from all tests after 24-h exposures was 0.91 mm.

The log phase Chlamydomonas reinhardi innoculum was prepared by centrifuging, washing, and resuspending the algae in filtered dechlorinated tap water. The algal suspension was then diluted to the appropriate density which was verified with an electronic particle counter (Particle Data, Inc., Elmhurst, IL).

After 24 h, the test was stopped by adding 2-3 drops of 37% formalin to each bottle. Final counts were then made with the electronic particle counter. Filtering rate for each bottle was determined by the equation [2]:

$$F = v \frac{\log_{10}C_o - \log_{10}C_t}{\log_{10}e} \qquad [2]$$

where v is the volume of water per animal, C_o is the final algal concentration in the algal blank for that concentration of selenium, and C_t is the algal concentration after 24 h in each bottle. Data were expressed as filtering rates in mL/animal/day. For both the oxygen consumption and filtering rate studies, the data were analyzed using a one-way factorial analysis of variance (ANOVA) and Duncan's new multiple range test on the Statistical Analysis System (BARR et al. 1976).

RESULTS AND DISCUSSION

Although selenium has not been established as a required trace element for Daphnia, a selenium requirement has been reported for species of mammals, birds, fish, and dinoflagellates (NAS 1976, POSTON et al. 1976, LINDSTROM & RODHE 1978). Selenium in mammals acts as an intermediary between controlled metabolite dehydrogenations in the respiratory chain and is possibly involved in the coupling of oxidative phosphorylations. Selenium-dependent enzymes, including glutathione peroxidase, are involved in the union of hydrogen and oxygen in the final step of the respiratory chain (FROST & LISH 1975). These functions of selenium suggest that oxygen consumption might be a good parameter for monitoring selenium stress.

The results of the oxygen consumption studies are presented in Table 1. There appeared to be a slight but nonsignificant increase in oxygen consumption due to the selenium. There was quite a degree of variation among the replicates. Variability of daphnid oxygen consumption data has been reported for other toxicants as well. SHERR & ARMITAGE (1973) reported great variation among the replicate groups of dichromate exposed Daphnia pulex. Their results are difficult to interpret because of the presence of young in the brood chambers of many of the dichromate exposed animals. GEIGER (1979) exposed D. pulex to water soluble fractions of petroleum and petroleum-related compounds. He reported variable results as well and found no statistically significant differences between exposed and control groups.

TABLE 1. Effects of acute exposure to selenite-selenium on the oxygen consumption of Daphnia pulex. Values were not significantly different ($P > 0.05$).

Se Conc., mg/L	Number of Replicates	Mean Oxygen Consumption µL/mg dry wt/h (SD)	Difference from Control
0.2	6	6.85 (1.56)	0.21
Control	3	6.64 (1.92)	
0.4	7	9.70 (1.76)	0.65
0.6	7	9.15 (2.34)	0.10
0.8	7	9.31 (2.37)	0.26
Control	4	9.05 (3.59)	

The results of the oxygen consumption study indicate no significant impact of selenium on Daphnia. However, the results of a chronic study with D. pulex at the same four selenite-Se concentrations indicated reproductive effects at 0.4, 0.6, and 0.8 mg/L (READING 1979). On the basis of this study, oxygen consumption does not appear to be a good indicator of pollutant stress.

The results for the filtering rate study are presented in Table 2. The values are the result of pooling the replicates from two separate tests. The only statistically significant differences occurred between the groups exposed to 0.2 mg/L Se and those at 0.6 and 0.8 mg/L Se. The 0.2 mg/L Se group had a slightly elevated filtering rate relative to the controls. There was depression in filtering rate at the higher selenium concentrations.

The depressions in filtering rate at the higher concentrations may explain an observation made by READING (1979) in the chronic study. Daphnia feeding on Chlamydomonas usually exhibit a green gut. However, READING reported that the 10 of 19 animals which died had been exposed to 0.8 mg/L Se during the chronic study and did not have the characteristic green gut 24 to 48 h prior to death. A dye study indicated that the gut pH was not affected.

TABLE 2. Effects of acute exposure to selenite-selenium on the filtering rate of Daphnia pulex. Values not joined by a continuous line were found to be significantly different by Duncan's new multiple range test at the 0.05 level.

Se Conc., mg/L	Number of Replicates	Mean Filtering Rate mL/animal/day (SD)
0.2	6	3.15 (1.03)[a]
Control	6	2.78 (1.91)
0.4	6	1.77 (1.28)
0.8	6	1.41 (0.33)
0.6	5	1.19 (0.69)

[a]ANOVA P value = 0.0378

Based on this study, filtering rate appears to be a useful parameter to monitor the sublethal effects of pollutants. READING (1979) reported depressions in reproduction at 0.4, 0.6, and 0.8 mg/L Se, the same three

concentrations at which depressions in filtering rate occurred. Interestingly, READING reported no effects on reproduction at 0.2 mg/L Se where an elevation in filtering rate was observed. This may indicate that filtering rate is more sensitive to selenium stress than reproduction.

The few other studies available also indicate the usefulness of filtering rate as a pollutant monitor. GEIGER (1979) found highly significant effects on the filtering rate of D. pulex due to petroleum and petroleum-related compounds. COOLEY (1977) reported that the filtering rate of D. retrocurva decreased as the exposure time to 5 and 10% pulp mill effluent increased.

The filtering rate test shows promise as a short-term test of sublethal toxicant effects. However, more data on a variety of toxicants are needed before the method can be evaluated adequately.

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