ACUTE AND CHRONIC EFFECTS OF SELENIUM ON *Daphnia pulex*

by

Jeffrey Thomas Reading

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APPROVED:

Arthur L. Buikema, Jr., Chairman

John Cairns, Jr.

William H. Yongue, Jr.

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INTRODUCTION

There has been worldwide concern recently over fates and effects of trace elements in the environment and their increasing levels due to human activities. Much of this concern has been prompted by direct threats to human health, such as episodes of "Minamata" and "itai-itai" diseases in Japan due to exposure to mercury and cadmium respectively (Manahan, 1975). As a result of these episodes, potential threats to fish, wildlife and humans by trace elements are being investigated.

One trace element receiving increased attention is selenium. Selenium is the least plentiful and most toxic of the required trace elements with a very narrow margin of safety between required and toxic dose (Copeland, 1971; Frost and Lish, 1975). Located in Group VIA of the periodic table with oxygen and sulfur, selenium is a metalloid, an element with properties intermediate between metals and non-metals. Its chemistry is similar to that of sulfur, but it exhibits important differences in redox characteristics, stability of compounds, and kinetics of reactions (Johnson, 1976). It is widely distributed within the earth's crust in small concentrations ranging from 0.03 to 0.8 ppm (National Academy of Sciences (NAS), 1976), having a total abundance of approximately 7×10^{-5} weight percent (Cooper, Bennett and Croxton, 1974). Natural sources of selenium

include volcanic gases and the weathering of selenium-containing soils and rocks (Lakin, 1973).

Industrial activity and combustion of fossil fuels also release selenium into the environment. The free world production of selenium amounted to 2.9 million pounds in 1970 (NAS, 1976). Industrial uses of selenium include decolorizing of glass, manufacture of photocells, rectifers and xerographic plates, making of pigments for inks and paints, the vulcanizing of rubber, and the production of steels and alloys (Louderback, 1975).

Combustion of fossil fuels represents the most significant man-related source of selenium discharged into the environment. With increased use of coal this source will become even more important in the future. Eighty-six coal samples from 20 states had selenium concentrations ranging from 0.46 to 10.65 ppm with an average value of 3.2 ppm (Pillay et al., 1969). Andren et al. (1975) reported a mass balance for selenium after coal combustion and they found 0.3% in slag and 68% in fly ash. The remaining 31.7% was vaporized and lost to the atmosphere. Fly ashes from 21 states contained from 1.2 to 16.5 ppm, with an average of 8.0 ppm (Gutenmann et al., 1976). Fly ash collected by precipators and discharged into settling basins are a source of selenium contamination in surface waters (Guthrie and Cherry, 1976).

The selenium content of oil is relatively low. Pillay et al. (1969) reported that the selenium content of 40 crude oil samples from ten states ranged from 0.06 to 0.42 ppm with an average value of 0.17 ppm. They estimated the annual release of selenium from coal and oil combustion in the United States at about eight million pounds. Selenium release from coal combustion in the United States and the world was estimated to be respectively 2.5 and 1.5 times greater then that due to natural weathering (Andren et al., 1975).

Selenium is a required trace element. Proteins containing selenium are essential coenzymes of some bacterial and mammalian enzyme systems (Statman, 1974). A selenium requirement has been reported for species of mammals, birds, fish and dénoflagellates (NAS, 1976; Poston et al., 1976; Lindstrom and Rodhe, 1978). Selenium-responsive diseases have been documented in approximately 40 species (Frost and Lish, 1975). Selenium deficiency diseases in livestock, such as white muscle disease, are common in areas where the soils lack this element. Addition of selenite-selenium to these soils to prevent this deficiency is being investigated and this may become an additional source of environmental selenium (Gissel-Nielsen and Gissel-Nielsen, 1973; Allaway, 1975). Although a selenium nutritional requirement in animals has been recognized for years, it is not yet offici-

ally recognized as an essential nutrient in humans (Frost and Lish, 1975). There has been considerable debate over the nutritional requirement for selenium in humans, the situation is complicated by the narrow range between a required and toxic dose. Copeland (1971) suggested dietary required and toxic doses of 0.2 and 5.0 mg/day respectively for humans, which gives a safety factor of only twenty-five fold.

The toxicity of selenium to mammals has been well established (Rosenfeld and Beath, 1964; Maag and Glenn, 1967; Moxon and Olson, 1974; Cooper and Glover, 1974; NAS, 1976), and was reported as early as 1295 by Marco Polo (Copeland, 1971). Several distinct types of selenium poisoning have been reported depending on length and type of exposure. The vascular system in particular, seems to be affected by selenium poisoning (NAS, 1976). Overaccumulation of selenite ions which disrupts SH metabolism, is suggested to be the cause of selenium toxicity (Frost and Lish, 1975).

The United States Environmental Protection Agency (USEPA, 1978c) included selenium and its compounds in its original list of 65 toxic pollutants and is in the process of establishing water quality criteria for the protection of freshwater life. Very little is known about the potential effect of selenium to aquatic life: the limited literature

on this subject consists primarily of acute fish toxicity data and fish body burdens. There are only a few studies concerning the effects of selenium on aquatic invertebrates. In view of the limited information on effects of selenium on freshwater invertebrates, research on Daphnia pulex was conducted. Specifically, research was conducted on acute toxicity; chronic effects on survival, growth and reproduction; and acute sublethal effects on oxygen consumption and filtering rate.

MATERIALS AND METHODS

Selection of Daphnia

Cladocerans are important because of their trophic position in lakes and ponds (Reid and Wood, 1976). Of the cladocerans, both Daphnia magna and Daphnia pulex are recommended organisms for toxicity testing because of their sensitivity to toxicants (USEPA, 1975, 1978b). D. pulex was chosen as the test organism because of its cosmopolitan distribution. D. magna has a very limited geographic range in North America and it is typically a hardwater species (Buikema et al., 1976; Winner, 1976).

Maintenance of Daphnia

Daphnia pulex Leydig (Brooks, 1959) were obtained from cultures which had been maintained continuously in our laboratory for several years; the cultures original stock were obtained from Carolina Biological Supply Company, Burlington, N.C. Daphnia were cultured in 19 liter all glass aquaria containing Blacksburg carbon-dechlorinated tap water previously filtered through a 50 micron mesh net. Stock cultures were kept at 20 $\pm$ 2 C. A 16L:8D photoperiod at an air-water interface intensity of approximately 100 ft-c was provided by cool white fluorescent lights.

Stock cultures were fed daily with an ad libitum suspension of Chlamydomonas reinhardi (wild type, minus strain). This green alga was obtained from Carolina Biological Supply

Company, and was grown in a modified Bolds Basal Medium (Buikema, 1970). Algal cultures were maintained at the same temperature and photoperiod as the Daphnia, but at a higher light intensity of 300-500 ft-c. Before being fed to the Daphnia, the algae were centrifuged, washed and resuspended in dechlorinated tap water.

Selenium Solutions and Dilution Water

The form of selenium used in all phases of the study was reagent grade sodium selenite ($Na_2SeO_3 \cdot 5H_2O$) (Baker Chemicals, Phillipsburg, NJ). Stock solutions were made with Blacksburg carbon-dechlorinated tap water which had been filtered through a Metricel GA-6 0.45 micron filter (Gelman Instrument Co., Ann Arbor, MI) and aerated 12-24 hours prior to use. The dilution water for all tests received the same treatment. All stock solutions were prepared on the day of use.

Water Chemistry and Selenium Determinations

Water quality characteristics of the filtered dechlorinated tap water used are listed in Table 1. All parameters were assessed according to methods outlined in the 14th Edition of Standard Methods (American Public Health Association, 1975). Metal analyses were done using a Perkin-Elmer Model 460 Atomic Absorption Spectrophotometer (Perkin-Elmer Instrument Division, Norwalk, CT). Hardness, pH, and

Table 1. Water quality characteristics of dechlorinated water.

Parameter	No. of Determinations	Mean
Alkalinity, mg/l $CaCO_3$	2	35.5
Conductivity, umhos/cm^2	2	82.5
Dissolved Oxygen, mg/l	17	8.2 (SD = 0.5)
Hardness, mg/l $CaCO_3$	23	45.7 (SD = 5.1)
pH	19	7.4 (SD = 0.3)
Ammonia-N, mg/l	1	0.06
Nitrate-N, mg/l	2	0.10
Orthophosphate, mg/l	2	0.12
Total Phosphate, mg/l	2	0.13
Calcium, mg/l	2	6.0
Copper, mg/l	2	<0.5
Iron, mg/l	2	<0.5
Magnesium, mg/l	2	3.7
Potassium, mg/l	2	1.5
Sodium, mg/l	2	7.5
Zinc, mg/l	2	<0.5

dissolved oxygen were monitored routinely during all tests; during the chronic study they were monitored weekly. Generally there were no significant changes in these parameters during the tests and dissolved oxygen never fell below 7.0 mg/l. Twice during the study a more complete characterization of the water was made, including nutrient and metal analyses.

Selenium concentrations were determined by hydride generation atomic absorption spectrophotometry (USEPA, 1979) using the sodium borohydrate reduction method (Fernandez, 1973; Corbin and Barnard, 1976) with an argon-hydrogen-entrained air flame. The atomic absorption spectrophotometer was equipped with a hollow cathode lamp, a three slot burner head, a deuterium background corrector, and a pen recorder. To verify selenite concentrations of the test solutions, the method for inorganic selenium analysis was used. For determination of selenium levels in the dilution water, the sample was acid digested prior to analysis to yield total selenium (USEPA, 1979).

The total selenium content of the dilution water was monitored periodically during the study and was always less than 0.01 mg/l Se. Selenite-selenium concentrations were not measured during the Gambusia and Physa acute toxicity tests; the LC50 and EC50 values, respectively, were based on nominal concentrations. Because the difference between

nominal and measured concentration of selenite-selenium in all other parts of the study varied an average of 3 percent these nominal concentrations were considered to be accurate. For the Daphnia acute toxicity tests, the LC50 was based on measured concentrations.

For the chronic, oxygen consumption, and filtering rate studies, only the stock solution with a nominal concentration of 0.8 mg/l selenite-Se was measured. This solution was used for the 0.8 mg/l test solution and by simple dilution yielded the other three test solutions. The mean and 95 percent confidence intervals of the measured selenium concentrations for the chronic, oxygen consumption and filtering rate studies respectively, were 0.81 $\pm$ 0.05, 0.85 $\pm$ 0.1 and 0.91 $\pm$ 0.18 mg/l. The geometric mean of the quotients of measured to nominal selenium concentrations for the entire study was 1.03. The results of all studies are reported as the concentration of the element selenium rather than the compound sodium selenite.

The dechlorinated tap water was passed through a 0.45 micron filter prior to use to reduce microbial levels. This was done to reduce microbial formation of organic selenium compounds (e.g. methylated selenides) which are votatile (Fleming and Alexander, 1972; Chau et al., 1976; Doran and Alexander, 1977). Many selenium test solutions had a definite odor, which may have been due to a volatile selenium

compound. To test if volatilization was occurring, equal portions of a 0.8 mg/l selenite-Se stock solution were poured into beakers and sampled after 0, 24, 48, and 96 hours. Subsequent analysis showed no differences in measured concentration among the four portions. However, volatilization of selenium during tests cannot be ruled out because test animals and associated bacteria were not placed in the test solutions.

Acute Toxicity Tests With Daphnia

Neonate Daphnia, 24 ± 12 hours old, were used in all studies except the dye study. Static acute toxicity tests, without aeration, feeding, or renewal were conducted in 500 ml glass beakers containing 300 ml of test solution and five Daphnia. A logarithmic dilution series, consisting of five concentrations and a control, were used for all tests, and several replicates were tested. The animals were assigned randomly to each dilution, and test containers were randomly placed in Scherer CEL 4-4 growth chambers (Kysor Industrial Corp., Marshall, MI). Test containers were loosely covered with plastic wrap to retard evaporation and were checked at 8, 24 and 48 hours. Toxicity tests were conducted for 48 hr because starvation influences longer tests (USEPA, 1975; Buikema et al., in press).

The endpoint of the test was death. Death was defined as the cessation of the movement of all appendages as well

as no heartbeat. Verification of death was performed under a dissecting scope. The median lethal concentration (LC50) was estimated using Finneys probit analysis procedure on the Statistical Analysis System (SAS) (Barr et al., 1976); analysis was carried out on the log base 10 of the test concentration (Stephan, 1977).

Chronic Study With Daphnia

The sublethal effects of 0.2, 0.4, 0.6 and 0.8 mg/l selenite-Se on the survival, growth and reproduction of Daphnia were monitored for 28 days. Each experimental group and the control consisted of twenty Daphnia maintained individually in glass baby food jars with 50 ml of test solution. The test began with animals 24 $\pm$ 12 hr old. Temperature and light regimes were the same as for the stock cultures.

Animals were checked daily for mortality, body length, exuvia, number of eggs, number of live young, number of dead young with and without caudal spines, number of deteriorated eggs (those eggs which fail to develop into live or dead young), and partial and full abortions. Body length was determined with an ocular micrometer and animals were measured from the top of the head to the base of the caudal spine. Young were removed, test containers were cleaned, test solutions were renewed and the animals were fed daily.

The animals were fed 1 ml of algal suspension at a density of 50,000 cells per milliliter in the test container. Algal densities were verified with an Electrozone/Celloscope Model 112 electronic particle counter (Particle Data, Inc., Elmhurst, IL).

Due to their low frequency of occurrence during the study, dead young with and without caudal spines, deteriorated eggs, and partial and full abortions were not statistically analyzed individually. Dead young with and without caudal spines and deteriorated eggs were pooled, and the percentage of the total number of eggs they comprised calculated. This parameter, percent dead young was statistically analyzed. Partial and full abortions were tabulated and only trends will be discussed.

The control data for length (both preadult and adult), number of eggs, and number of live young were tested for normality using the Kolmogorov-Smirnov test, on SAS (Helwig, 1977). Because the hypothesis of normality was not rejected the vast majority of the time at the 0.05 alpha level for the three parameters, parametric analyses were conducted. The percent dead young parameter also approximated a normal distribution (S.K. Lee, per. comm.) and parametric analyses were used.

For preadult instars, length was analyzed on a molt by molt basis using a one-way factorial analysis of variance

(ANOVA) and Duncan's new multiple range test on SAS (Barr et al., 1976). Molt 0 is the instar when the test began; molt 1 is the instar after the first molt to occur during the test; etc. The statistical analysis on adult length and reproduction was done on a brood by brood basis, since all the animals were in a physiologically equivalent state at the time of their first brood and had been exposed to selenium for a minimum of three complete instars. The ANOVA and Duncan's tests were also used to analyze the adult length and reproductive parameters. Reproduction was monitored through nine instars and adult length through ten instars. The final adult length measurement was made after the ninth brood was released and the animals had molted.

Toxicity Tests With Other Organisms

Acute toxicity tests were conducted with two other organisms, the mosquitofish (Gambusia affinis), and the aquatic snail (Physa sp.), both of which were obtained from Carolina Biological Supply Company. General toxicity test procedures according to Standard Methods (APHA, 1975) were followed. Gambusia were maintained in 450 l flow-through tanks in Blacksburg carbon dechlorinated tap water under the same temperature and light conditions as the Daphnia. The fish were fed three times weekly with finely ground trout chow (Ralston Purina Co., St. Louis, MO). Ninety-six hour

static toxicity tests without feeding were conducted in 5 l glass aquaria containing five fish in 4 l of test solution. A logarithmic dilution series of five selenium concentrations and a control were used in each test, and several replicates were tested. Fish were checked after 8, 24, 48, 72 and 96 hours. Death was defined as cessation of all movement including ventilation. An LC50 also was calculated by probit analysis.

Physa were maintained in 5 l glass aquaria containing two liters of dechlorinated tap water under the same temperature and light regimes as the Daphnia. The snails were fed lettuce and Elodea sp.. Ninety-six hour toxicity tests without feeding were conducted with five snails in 500 ml glass beakers containing 300 ml of test solution. A logarithmic dilution series and a control similar to that for fish was used. The median effective concentrations (EC50) was calculated by probit analysis. The criterion used in determining the EC50 was failure of the snail to display any movement upon probing with a blunt glass rod. Tests were checked after 8, 24, 48, 72 and 96 hours.

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 hr 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 selenite-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 hours.

After 24 hours the amount of dissolved oxygen in each respirometer was determined with the azide modification of the Winkler method (APHA, 1975). Twenty-five milliliter alquiots 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. Absolute change in oxygen content between the corrected blank or mean of the blanks and each of the test respirometers was calculated by equation (1):

$$\text{mg } O_2 = \frac{\text{Corrected } (O_2)_{\text{Blank}} - \text{Corrected } (O_2)_{\text{Test}}}{\text{Volume of bottle}} \qquad (1)$$

From this, respiration rates in ul O_2/animal/hour were calculated.

After titration the Daphnia in each respirometer were removed and measured and mean body length in each respirometer was determined. Using the length-weight relationship (Figure 1), the mean body weight was determined and oxygen consumption was recalculated as ul O_2/mg dry weight/hour. The mean length for the animals from all the tests at the end of the 24 hr exposure period was 0.97 mm.

The length-weight relationship was determined by measuring 60 Daphnia and placing them in 0.2 mm size classes. The Daphnia in each size class were placed on tared aluminum pans and dried at 55 C for 36 hours. Dry weights were then determined by substitution weighing on a Cahn Model 4700 Automatic Eletrobalance (Cahn Instruments, Cerritos, CA). Using the SAS method for least squares regression (Barr et al., 1976), the two regression lines in Figure 1 were arrived at. For lengths less than 1.27 mm, the equation (2) was:

$$W = 0.0025L - 0.0001 \qquad (2)$$

where W is dry weight in milligrams, and L is length in

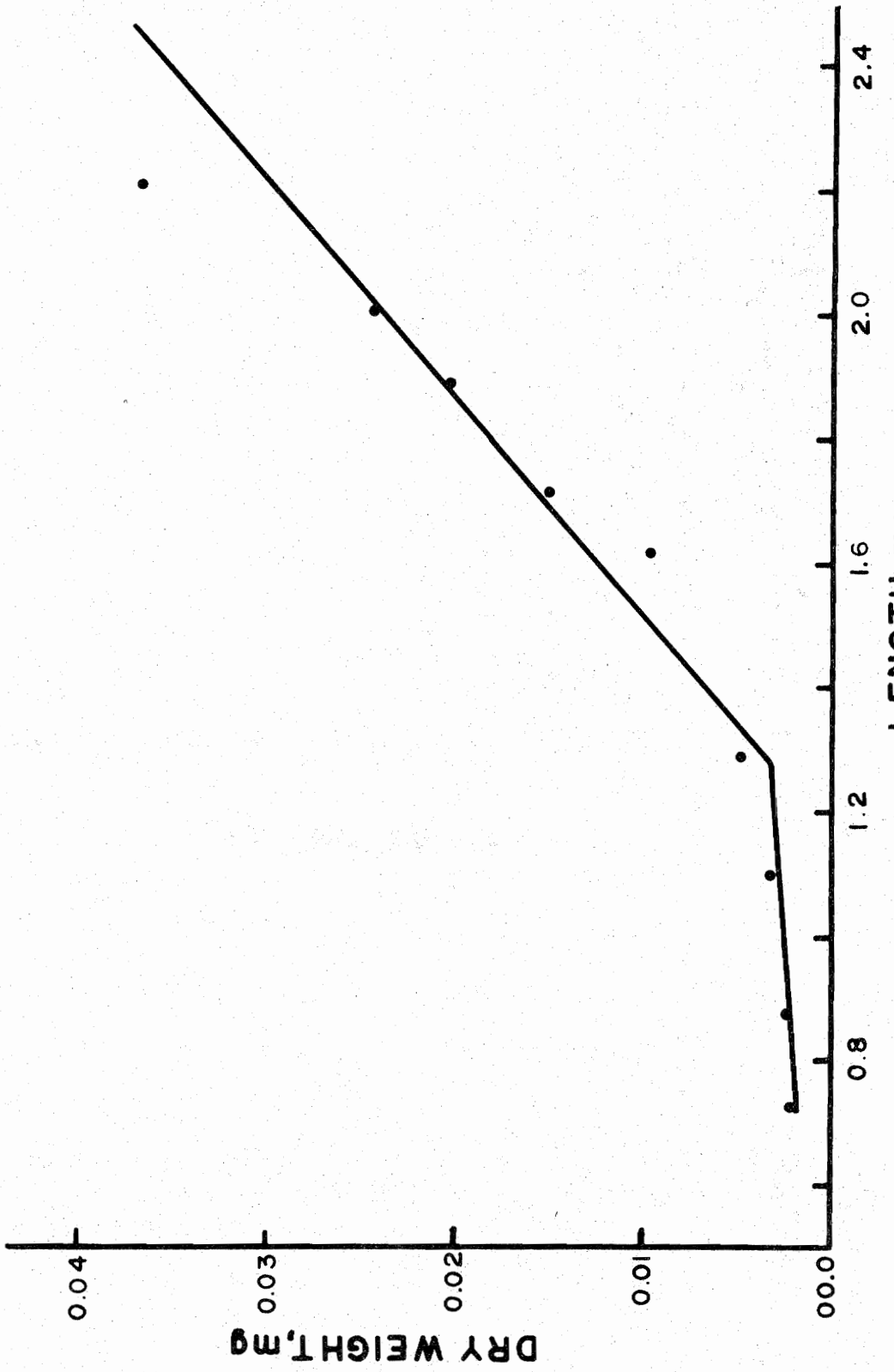

Figure 1. Daphnia pulex length-weight relationship.

millimeters. For lengths greater than 1.27 mm, the equation (3) was:

$$W = 0.0287L - 0.0335 \qquad (3)$$

Statistical analysis on the oxygen consumption data was done by a one-way factorial ANOVA and Duncan's new multiple range test.

Filtering Rate Studies

As in the oxygen consumption studies, preadult Daphnia were exposed to each of four concentrations of selenium for 24 hours. 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 milliliter 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 hr exposure 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 the electronic particle counter.

After 24 hours the test was stopped by adding 2-3 drops of 37 percent formalin to each bottle. Final counts were then made with the electronic particle counter. Filtering rate for each bottle was determined by the equation (4):

$$F = v \frac{\log_{10} C_o - \log_{10} C_t}{\log_{10} e} \qquad (4)$$

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 the algal concentration after 24 hr in each bottle. Data were expressed as filtering rates in ml/animal/day. The data were analyzed using a one-way factorial ANOVA and Duncan's new multiple range test.

Gut pH Study

To get an indication of an effect of selenium on digestion a dye study was conducted to determine if gut pH changed in Daphnia exposed to solutions of 0.6 and 0.8 mg/l Se. Each experimental group and the controls consisted of ten Daphnia of mixed ages. The animals were exposed for 48 hours without feeding in 500 ml glass beakers containing 300 ml of solution.

After 48 hours the animals were allowed to feed on a mixture of neutral red stain and yeast (Hasler, 1935; Buikema and Sherberger, 1977). The animals were then ob-

served under a dissecting microscope for differences in pH gradient of the gut. Neutral red dye is red below pH 6.0, rose at pH 7.0, orange at pH 8.0, and yellow at pH 9.0.

RESULTS AND DISCUSSION

Acute Toxicity

The results of the acute toxicity tests with Daphnia, Gambusia and Physa are presented in Table 2. In probit analysis, the relationship between mortality and log concentration is assumed to be a linear one (Stephan, 1977). The acute results for all three organisms exposed to selenium had Chi-square P values greater then 0.3, indicating a good fit of the data to the probit model.

Daphnia was the most sensitive of the two invertebrate species tested. The greater sensitivity of Daphnia species has been reported in the literature for other chemicals. For example, Buikema et al. (1976) compared the acute sensitivity of Daphnia pulex, D. magna and thirteen other invertebrate species to an artificial refinery effluent and found that the two Daphnia species were the most sensitive. However, Halter et al. (in press) reported that immature Hyallela azeteca were more sensitive to sodium selenite than D. magna after 48 hours exposure. But the amphipods may have ingested selenium from a contaminated food source. Daphnia also were more sensitive than Gambusia to selenium. This was not surprising because Gambusia are resistant to a variety of pollutants (Giesy et al., 1977; Darwazeh and Mulla, 1974). Depending on the compound and the species

Table 2. Static acute toxicity of selenite-selenium to Daphnia pulex, Gambusia affinis, and Physa sp.

Organism	Type	Concentration, mg/l Se (95% C.I.)	No. of Conc.	No. of Replicates
Daphnia pulex 24 ± 12 hrs	48 hr LC50	3.87[a] (3.50 - 4.46)	5	5
Gambusia affinis 28.4 cm	96 hr LC50	12.56[b] (10.66 - 14.58)	5	2
Physa sp.	96 hr EC50	24.08[b] (10.83 - 30.28)	10	2

[a]measured concentrations

[b]nominal concentrations

involved, Daphnia acutely may be more, equal or less sensitive than fish (Buikema et al., 1976; Macek et al., 1976a, 1976b). Adams (1976) concluded that Daphnia were no more sensitive to selenium than fish.

The literature on the toxicity of selenium compounds to fish and aquatic invertebrates is summarized in Table 3. Data for sodium selenite, sodium selenate, selenium dioxide and selenious acid are included. Selenious acid and selenium dioxide are equivalent since selenium dioxide forms selenious acid when dissolved in water (Elkin and Margrave, 1968). Selenious acid is a weak dibasic acid which dissociates predominantly into the biselenite ion in waters between pH 3.5 and 9 (NAS, 1976). The order of toxicity, from highest to lowest, is selenite ion, biselenite ion and selenate ion (Table 3). Although the toxicities of the selenite and biselenite ions are usually similar.

The LC50 and EC50 estimates for Gambusia and Physa of 12.6 and 24.1 mg/l selenite-Se, respectively, are high when compared to other acute toxicity values (Table 3). The known resistance of Gambusia makes the relatively high 96 hr LC50 estimate seems reasonable. There are no comparable selenium toxicity values for Physa in the literature; however, Physa appears to be less sensitive to pollutants than other species (Arthur and Leonard, 1970; Wier and Walter, 1976).

Table 3. Summary of other studies on the toxic effects of inorganic selenium compounds to fish and aquatic invertebrates. Abbreviations for bioassay methods: S = static, R = static with renewal, F = flow-through.

Compound Group Organism	Observed Effect	Effective Conc. mg/l Se	Method	pH	Temp C	Hard. mg/l	Ref
I. SODIUM SELENITE							
A. Invertebrates							
Amphipod (immature), *Hyallela azeteca*	48 hr LC50	0.94	F	7.3	25	329	(20)
	96 hr LC50	0.34	F	7.3	25	329	(20)
	14 day LC50	0.07	F	7.3	25	329	(20)
Ciliate protozoan, *Tetrahymena pryriformis*	Blocked growth	3 - 20	-	-	-	-	(3)
Cladoceran, *Daphnia magna*	48 hr LC50	0.71	F	7.3	25	329	(20)
	14 day LC50	0.43	F	7.3	25	329	(20)
	MATC	0.28	F	7.3	25	329	(20)
	Mortality	>0.1	-	-	-	-	(11)
	24 hr LC50	4.8	S	7.65	21	286	(5)
Cladoceran, *Daphnia* sp.	48 hr EC50	2.5	S	7.5	23	-	(4)
Pacific oyster embryo, *Crassostrea gigas*	48 hr EC50	>10	S	8.1	20	-	(9)
B. Fish							
Bluegill, *Lepomis macrochirus*	48 day LC50	0.40	F	7.8	15.7	318	(1)
Coho salmon fry, *Oncorhynchus kisutch*	48 day LC50	0.16	F	7.8	14.9	325	(1)
Fathead minnows, *Pimephales promelas*	96 hr LC50	10.90	S	7.4	13	312	(1)
	96 hr LC50	6.70	S	8.2	20	303	(1)
	96 hr LC50	2.80	S	8.3	25	292	(1)
	96 hr LC50	1.00	F	7.3	25	329	(20)
	14 day LC50	0.60	F	7.3	25	329	(20)
	48 day LC50	1.08	F	7.8	15.6	338	(1)
Fathead minnow eggs, *Pimephales promelas*	Reduce incubation time	≥15	S	-	25	-	(1)
	Reduce post-hatch median survival time	≥ 1	S	-	25	-	(1)
Goldfish, *Carassius auratus*	Death in 18 to 46 days	2.0	R	-	-	-	(7)
	Death in 4 to 10 days	5.0	R	-	-	-	(7)

Table 3. Continued

Compound Group / Organism	Observed Effect	Effective Conc. mg/l Se	Method	pH	Temp C	Hard. mg/l	Ref
Rainbow trout, *Salmo gairdneri*	96 hr LC50	4.35	S	8.4	14.9	330	(1)
	120 hr LC50	2.73	F	7.4	14.6	325	(1)
	48 day LC50	0.50	F	7.4	14.6	325	(1)
	96 day LC50	0.28	F	7.4	14.6	325	(1)
	Non-lethal after 4 weeks	<0.1	R	-	15	-	(8)
Rainbow trout fry, *Salmo gairdneri*	21 day LC50	0.46	F	7.3	17.4	334	(1)
Zebrafish larva, *Brachydanio rerio*	96 hr LC50	10.5	R	7.0	26	45-50	(15)
II. SODIUM SELENATE							
A. Invertebrates							
Amphipod (immature), *Hyallela azeteca*	96 hr LC50	0.76	F	7.4	15.9	337	(1)
B. Fish							
Channel catfish, *Ictalurus punctatus*	Fry hatched from exposed eggs with increased percentage albinism	0.0005-0.25	R	7.7	24	90	(19)
Fathead minnow, *Pimephales promelas*	96 hr LC50	11.76	S	8.3	15	323	(1)
	48 day LC50	2.00	F	7.4	16.5	338	(1)
Goldfish embryo-larva, *Carassius auratus*	7 day LC50	8.78	R	7.4	22	195	(2)
Rainbow trout embryo-larva, *Salmo gairdneri*	28 day LC50	4.18	R	7.4	13	104	(2)
Zebrafish larva, *Brachydanio rerio*	96 hr LC50	34.3	R	7.0	26	45-50	(15)
III. SELENIUM DIOXIDE							
A. Invertebrates							
Crab larva, *Cancer magister*	48 hr LC50	5.09	S	8.1	15	-	(9)
	96 hr LC50	1.04	S	8.1	15	-	(9)
Pacific oyster embryo, *Crassostrea gigas*	48 hr EC50	>10	S	8.1	20	-	(9)

Table 3. Continued

Compound Group Organism	Observed Effect	Effective Conc. mg/l Se	Method	pH	Temp C	Hard. mg/l	Ref
B. Fish							
Bluegill juveniles, *Lepomis macrochirus*	96 hr LC50	28.5	F	7.7	24.9	150	(6)
	336 hr LC50	12.5	F	7.7	24.9	150	(6)
Brook trout, *Salvelinus fontinalis*	96 hr LC50	10.2	F	7.8	15.5	148	(6)
Carp eggs, *Cyprinus carpio*	No effect on hatching	1 - 5	R	7.3	26	-	(10)
Channel catfish juveniles, *Ictalurus punctatus*	96 hr LC50	13	F	7.9	24.9	140	(6)
Creek chub, *Semotilus atromaculatus*	Death in 48 hours	>12	S	-	24.5	50	(12)
Fathead minnow fry, *Pimephales promelas*	96 hr LC50	2.1	F	7.8	24.7	151	(6)
Fathead minnow juveniles, *Pimephales promelas*	96 hr LC50	5.2	F	7.8	24.7	151	(6)
Flagfish juveniles, *Jordanella floridae*	96 hr LC50	6.5	F	7.9	24.5	152	(6)
Goldfish, *Carassius auratus*	LC50 (48 hr exposure and 7 days of holding in dilution water)	12.0	S	6.0-6.9	23	50	(18)
	Behavioral impairment	0.25	S	6.0-6.9	23	50	(18)
Goldfish juveniles, *Carassius auratus*	96 hr LC50	26.0	F	7.6	25.4	148	(6)
	336 hr LC50	6.3	F	7.6	25.4	148	(6)
Zebrafish embryos, *Brachydanio rerio*	No effects on hatching	0.5-10.0	R	7.0	26	45-50	(14)
	Significant post-hatch mortality	>3	R	7.0	26	45-50	(14)
Zebrafish larva, *Brachydanio rerio*	96 hr LC50	14.3	R	7.0	26	45-50	(15)

Table 3. Continued

Compound Group / Organism	Observed Effect	Effective Conc. mg/l Se	Method	pH	Temp C	Hard. mg/l	Ref
IV. SELENIOUS ACID							
A. Invertebrates							
Asiatic clams, *Corbicula*	Death in 8 days	10	-	-	-	-	(16)
Cladoceran, *Daphnia magna*	48 hr EC50	1.2	S	-	-	-	(13)
	Life cycle test limits	0.19-0.30	-	-	-	-	(13)
B. Fish							
Fathead minnow juvenile, *Pimephales promelas*	96 hr LC50	0.94	F	-	-	-	(13)
Fathead minnow embryo-larva, *Pimephales promelas*	Embryo-larva test limits	0.15-0.30	-	-	-	-	(13)
V. UNSPECIFIED SELENIUM COMPOUND							
A. Invertebrates							
Cladoceran, *Daphnia magna*	48 hr LC50	0.43	S	-	-	-	(17)
Mysid shrimp, *Mysidopsis bahia*	96 hr LC50	0.6	S	-	-	-	(17)
	Life cycle test limits	0.127-0.143	-	-	-	-	(17)
B. Fish							
Sheepshead minnow, *Cyprinodon variegatus*	96 hr LC50	6.7	S	-	-	-	(17)

References:
(1) Adams, 1976. (2) Birge, 1978. (3) Bovee, 1978. (4) Bringmann and Kuhn, 1959. (5) Bringmann and Kuhn, 1977. (6) Cardwell et al., 1976. (7) Ellis et al., 1937. (8) Gissel-Nielsen and Gissel-Nielsen, 1978. (9) Glickstein, 1978. (10) Huckabee and Griffith, 1974. (11) Kasymov and Pyatakova, 1976. (12) Kim et al., 1977. (13) Kimball, unpbl. (14) Niimi and LaHam, 1975. (15) Niimi and LaHam, 1976. (16) Sayurayma, 1960. (17) USEPA, 1978a. (18) Weir and Hine, 1970. (19) Westerman and Birge, 1978. (20) Halter et al., in press.

The 48 hr LC50 for D. pulex from this study is similar to the 48 hr EC50 for Daphnia sp. using selenite-Se (Bringmann and Kuhn, 1959). No 24 hr LC50 estimate can be made for D. pulex (this study), but the data indicates it would be greater then 4 mg/l selenite-Se, a value similar to the value reported for D. magna (Bringmann and Kuhn, 1977). The 48 hr LC50 of 0.71 mg/l selenite-Se reported by Halter et al. (in press) for D. magna is lower than the other reported LC50 values with Daphnia using sodium selenite. However, it was the only study conducted under flow-through conditions with feeding. The mortality level of >0.1 mg/l selenite-Se for D. magna reported by Kasymov and Pyatakova (1976) is lower than any concentration tested in this study, their test methodology could not be evaluated. Kimball (unpublished) reported a 48 hr EC50 of 1.2 mg/l Se for D. magna using selenious acid, USEPA (1978a) reported a 48 hr LC50 for D. magna as 0.43 mg/l Se under static conditions, this value is inconsistant with the other static acute toxicity data, as well as the chronic mortality data from this study. The selenium compound used by the USEPA was not reported, and the concentrations were not measured, thus the USEPA results are suspect in light of the other data.

The only other acute toxicity data on invertebrates with sodium selenite are: the 96 hr LC50 for Hyallela azeteca of 0.34 mg/l Se (Halter et al., in press); the 48

hr EC50 for oyster embryos of greater then 10 mg/l Se (Glickstein, 1978); and a study by Bovee (1978) in which concentrations between 3 and 20 mg/l Se inhibited growth of the protozoan Tetrahymena. As seen in Table 3, there is even less data on aquatic invertebrates for the other selenium compounds.

Several factors including temperature, hardness, and pH are known to modify the toxicity of some compounds (Sprague, 1970). Hardness appears to have no effect on the toxicity of selenium, while temperature has a definite effect (Table 3). Adams (1976) reported 96 hr LC50's for fathead minnows at 13, 20 and 25 C as 10.90, 6.70 and 2.80 mg/l selenite-Se, respectively. Due to the narrow range of the pH values it is difficult to draw conclusions from the literature about pH. However selenium should be more toxic at a lower pH, since selenite is formed under acid conditions (NAS, 1976).

Effects of Chronic Exposure on Mortality

Due to their sensitivity and relatively short life cycles, Daphnia species are popular organisms for studying chronic effects of toxicants on survival, growth, and reproduction (Macek et al., 1976a, 1976b; Biesinger and Christensen, 1972; Winner, 1976; Geiger, 1979). The effects of chronic exposure to selenite-selenium on the survival of Daphnia pulex from this study are presented in Table 4.

Table 4. Mortality in Daphnia pulex due to chronic exposure to selenite-selenium.

Concentration mg/l Se	N	Day 5		Day 28	
		No. Dead	% Dead	No. Dead	% Dead
Control	17	0	0	3	18
0.2	20	1	5	6	30
0.4	19	1	5	3	16
0.6	20	0	0	1	5
0.8	19	5	26	10	53

There was no appreciable mortality during the first five days of the chronic study except at 0.8 mg/l selenite-Se. These results support the selection of the test concentrations and the soundness of the chronic study. At day 28, when the chronic study was terminated, only the groups exposed to 0.2 and 0.8 mg/l selenite-Se had a greater percentage dead than the control group (12 and 35 percent higher respectively). The group exposed to 0.6 mg/l selenite-Se exhibited 13 percent less mortality than the control group. No valid statistical analysis could be done on the mortality data because of the low frequency of occurrence of mortalities during the study. The mortalities at 0.8 mg/l selenite-Se are unquestionably significantly higher biologically than the control group. The mortalities at 0.2 mg/l selenite-Se, however, are probably not biologically significant based on the mortalities at 0.4 and 0.6 mg/l selenite-Se, and the fact that one death in the 0.2 mg/l group as well as one death in the control group could be directly attributed to fungal infections. A biomodal toxicity effect for selenium cannot be ruled out; but there are few reports of toxic effects at or below 0.4 mg/l selenite-Se (Table 3).

Halter et al. (in press) reported a 14 day LC50 of 0.43 mg/l selenite-Se for D. magna, while in this study after 28 days, the approximate LC50 for D. pulex is 0.8

mg/l selenite-Se. These data indicate that D. magna is more sensitive; however, Halter et al. (in press) conducted the test under flow-through conditions. They fed the animals brewer's yeast only which is a poor food supply for Daphnia.

Five animals from the study were deleted from all data analysis, three in the control group, and one in each of the groups at 0.4 and 0.8 mg/l selenite-Se. In the control group, two of the three animals deleted died due to handling, and the third was a non-fertile female which survived the full 28 days. The other two animals deleted from analysis were males. Because they were difficult to identify as neonates, the males were inadvertently included in the study.

Non-fertile females appear to be a normal occurrence, and have been reported in other studies (Anderson, 1932). The growth of the non-fertile female in the control group was not retarded: its length at the end of 28 days was 2.76 mm compared to the mean length for the control group of 2.54 mm. The ovaries of the non-fertile female did not appear to have developed.

The males both survived the full 28 days of exposure. None were observed during any other phase of the research. No ephippia were observed during the chronic study or in any of the culture tanks during the research. It is felt

that the males encountered represented a normal low frequency of occurrence in Daphnia populations and did not indicate a stressed situation in the cultures.

With the exception of three deaths, including the two due to fungal infections, all the mortalities during the study occurred during the brood release-molting sequence or during the preadult instars. This pattern has been observed with other toxicants, Lee and Buikema (1979) reported that D. pulex tolerance to chromate toxicity decreased significantly during molting. Immature stages of a life cycle have been found to be generally more sensitive to toxicant exposure than adult stages (Buikema and Benfield, 1979).

Interestingly, in the group exposed to 0.8 mg/l selenite-Se, all ten of the mortalities occurred before the animals reached their first adult instar. In the three which died between days eight and eleven, it appeared that the selenium stress delayed reproductive maturity.

Effects of Chronic Exposure on Growth

Body length was used as the measure of growth. The results for preadult and adult length are presented in Figures 2 and 3, and Tables A1 and A2 (Appendix), respectively. Statistical differences relative to the control group are presented in Table 5.

One difficulty in using neonate daphnids is that many

Table 5. Summary of the effects of chronic exposure to selenite-selenium on the growth and reproduction of Daphnia pulex.[a]

Parameter	Selenium Concentration 0.2 mg/l	0.4 mg/l	0.6 mg/l	0.8 mg/l
Preadult Length	-	-	-	M2(-),M3(-)
Adult Length	-	B9(+)[b]	B9(+)	B9(+)
Total No. Eggs	-	-	B6(+)	B6(+)
No. Live Young	-	B1(-),B3(+)	B1(-),B2(-)	B1(-),B2(-), B6(+)
% Dead Young	-	B1(+)	B1(+)	B1(+),B2(+), B8(+)

[a]Differences relative to the control group from the brood by brood analysis as determined by Duncan's new multiple range test at the 0.05 level.

[b]M = preadult molt number
B = brood number
(+)= stimulatory
(-)= inhibitory

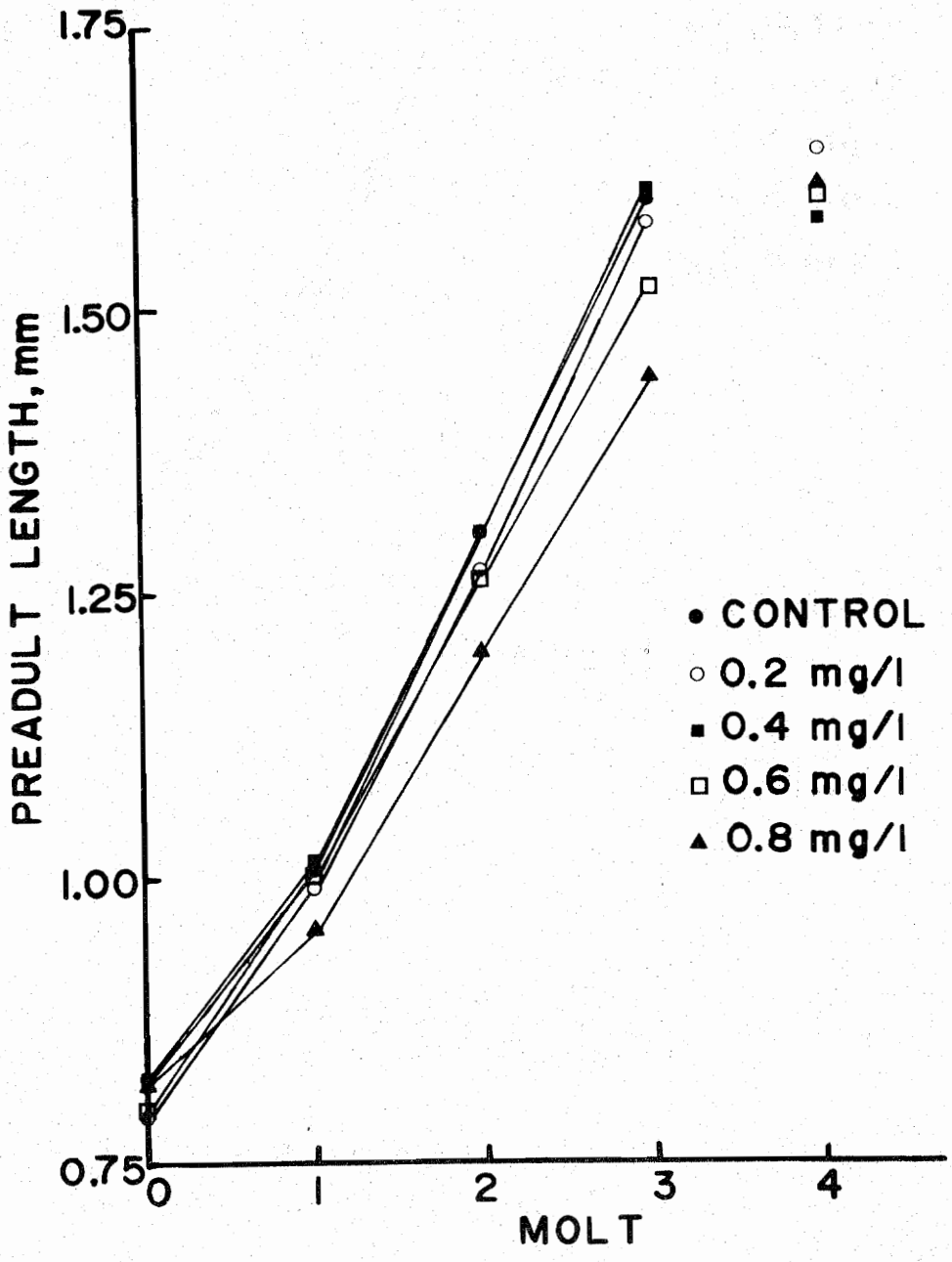

Figure 2. Preadult lengths by molt of Daphnia pulex during chronic exposure to selenite-selenium.

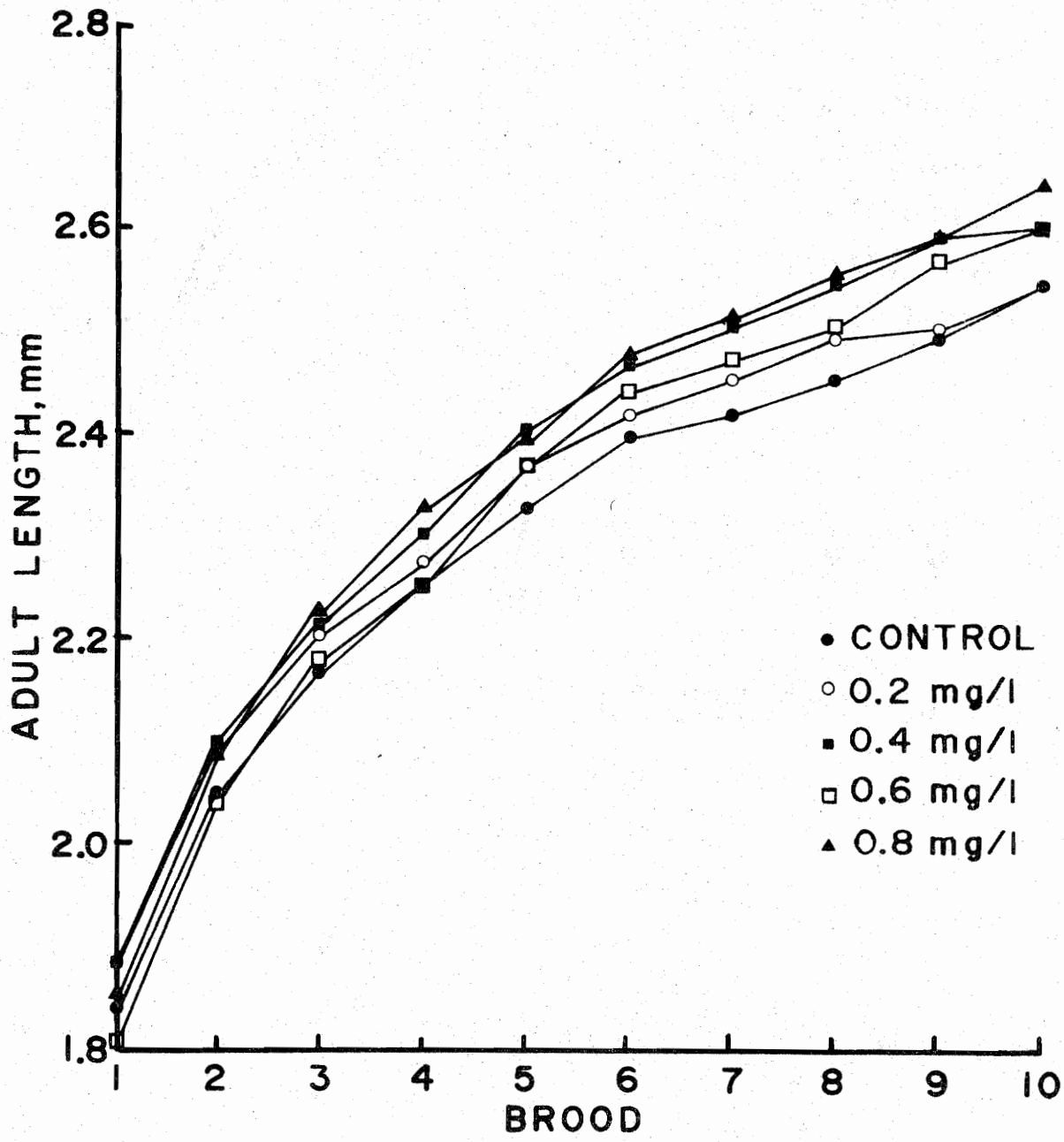

Figure 3. Adult lengths by brood of Daphnia pulex during chronic exposure to selenite-selenium.

may have already molted once within the first 24 $\pm$ 12 hours. Note that for molt 4 in Figure 2 and Table A1 (Appendix), there were only nine animals; the remainder apparently had already molted once before the test began. There was no reason to attribute this to selenium induced early reproductive maturity because there were no control values for molt 4. As a result the data for molt 0 through molt 3 contain animals in two different instars; because there was no significant differences between the groups at molt 0, any differences that appeared in the later molts were attributed to effects of the selenium.

During molt 1 the ANOVA indicated no significant differences, however, the Duncan's test shows the 0.8 and 0.4 mg/l selenite-Se groups to be significantly different from one another. Selenium significantly depressed growth at the higher concentrations during molts 2 and 3. The differences disappeared after molt 4, probably due to the small sample size.

For adult length rather then depressing growth, it appeared that the selenium had a slight stimulatory effect on length. During broods 3, 4, and 6 - 10, the group exposed to 0.8 mg/l selenite-Se had the highest mean length and the control group the lowest. Only during brood 9 however, was the stimulatory effect statistically significant.

There are no comparable studies in the literature on the effects of selenium exposure on growth. The only study on growth effects is that by Bovee (1978), in which selenium was found to inhibit growth of the protozoan Tetrahymena pyriformis.

Effects of Chronic Exposure on Reproduction

Selenium stress had no apparent effect on the number of preadult instars. All but one animal had four preadult molts which is typical for D. pulex (Anderson et al., 1937; Green, 1956; Buikema, 1973). Stress has been known to interupt this sequence; Buikema et al. (1978) reported that a short-term 10 C thermal stress resulted in D. pulex becoming reproductively mature one instar early.

One animal in the group exposed to 0.6 mg/l selenite-Se molted seven times before becoming reproductively mature on day 23; this appeared to be due to a growth impairment. On day 23 the animal was only 1.67 mm long, close to the length of the brood 1 animals (Figure 3); it did not become gravid until it reached this size. Because this occurred with only one animal, it is difficult to conclude whether it was due to the selenium, or an unrelated physiological malfunction. This individual was included in all analyses: its brood 1 data was analyzed along with all the brood 1 data.

Exposure to 0.8 mg/l selenite-Se seemed to delay

reproductive maturity in three animals; each of which died on days eight, nine and eleven, without having reached reproductive maturity. The length of the intermolt period was affected in these animals; all three were observed to molt only three or four times during the study.

The results of the various reproductive parameters are presented in Figures 4 and 5, and Tables 6 through 8 and A3 through A5 (Appendix). Statistical differences relative to the controls again are presented in Table 5. Reproductive impairment has been reported to be a very sensitive sublethal response for both fish and invertebrates (Sprague, 1971; Buikema and Benfield, 1979). This seemed to be the case in this study.

The results for both number of eggs produced are presented in Table A3 (Appendix). No clearcut pattern was evident, although the groups at the two highest concentrations had a slightly greater number of eggs in the later broods. Statistically significant differences occurred during broods 2, 6, and 7; but in broods 2 and 7 they were not different from the controls. Only in brood 6 is there an apparent trend: the groups at 0.6 and 0.8 mg/l selenite-Se produced higher number of eggs than the groups exposed to 0.2 mg/l selenite-Se and the controls. Generally, there seems to be very little impact of selenium stress on egg numbers.

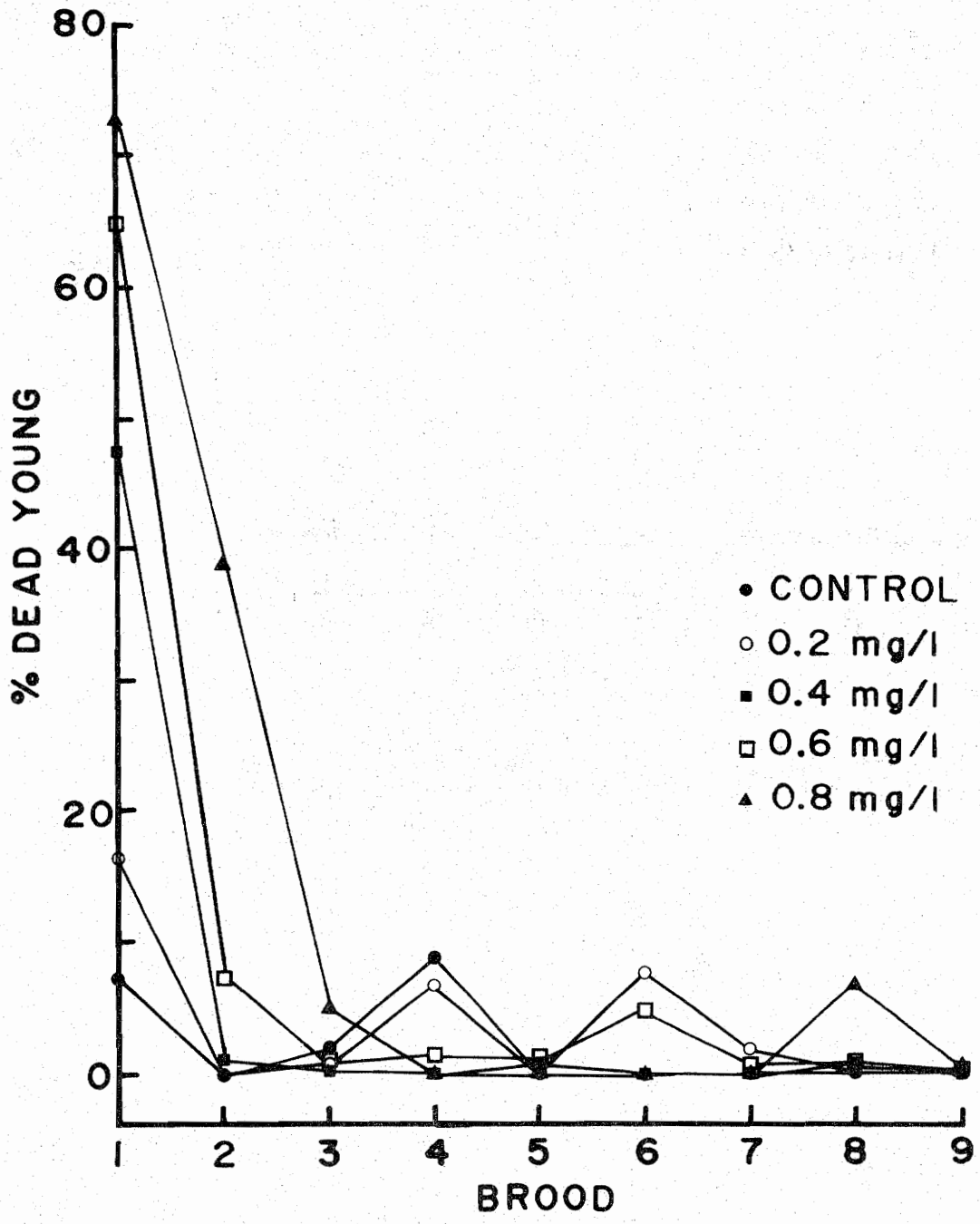

Figure 4. Percent dead young by brood of Daphnia pulex during chronic exposure to selenite-selenium.

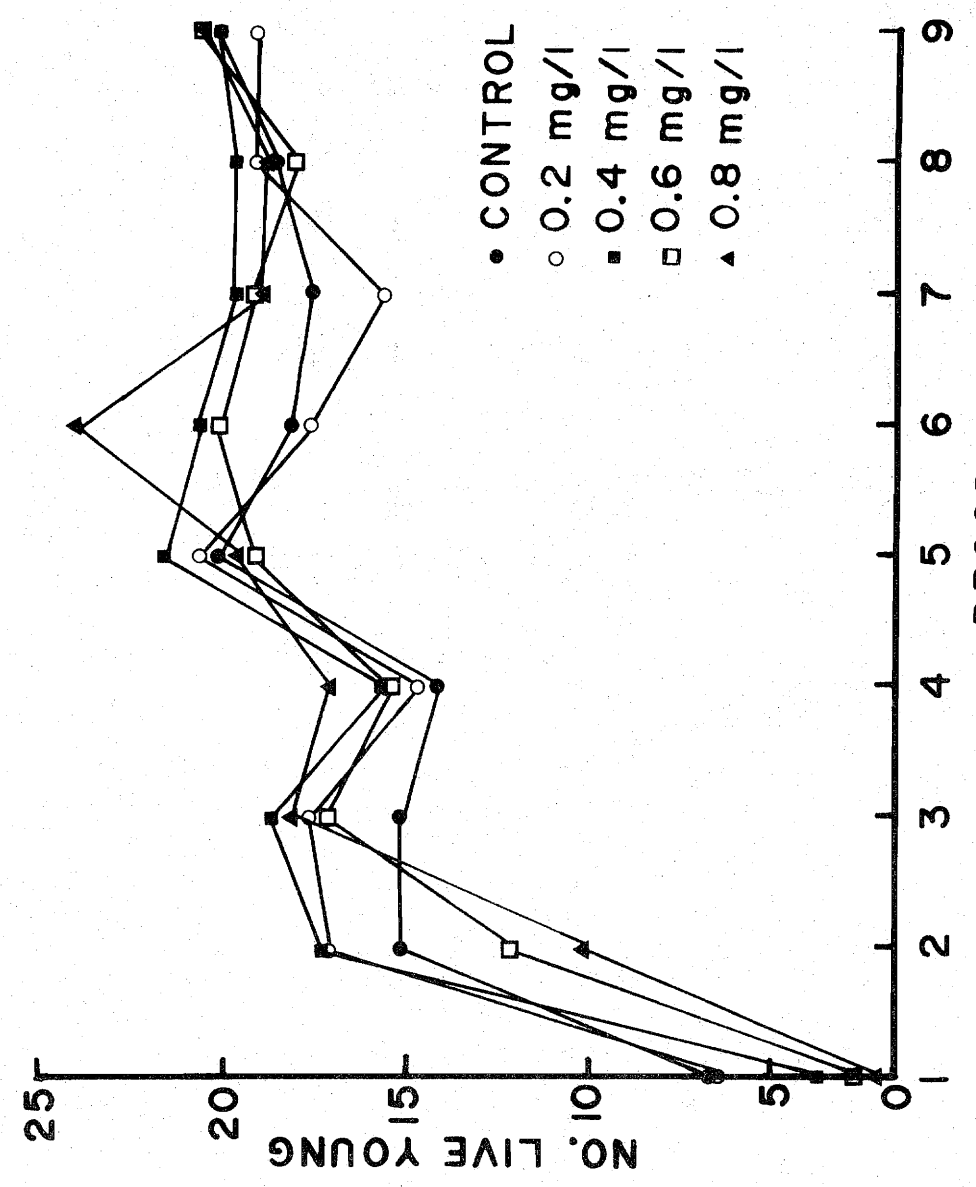

Figure 5. Mean number of live young by brood of Daphnia pulex during chronic exposure to selenite-selenium.

Table 6. Summary descriptive statistics for number of deteriorated eggs and number of dead young with and without caudal spines in Daphnia pulex due to chronic exposure to selenite-selenium.

PARAMETER Statistic	Selenium Concentration, mg/l Control	0.2	0.4	0.6	0.8
DETERIORATED EGGS					
No. of animals	17	20	19	20	19
Mean per animal (SD)	1.18 (2.77)	2.10 (2.51)	2.95 (3.36)	4.80 (3.43)	4.53 (7.43)
Maximum no. per animal in a brood	10	9	10	11	23
DEAD YOUNG WITH SPINES					
No. of animals	17	20	19	20	19
Mean per animal (SD)	1.00 (4.12)	0.45 (1.79)	0.42 (0.77)	2.15 (4.40)	0.63 (1.34)
Maximum no. per animal in a brood	17	8	3	20	5
DEAD YOUNG WITHOUT SPINES					
No. of animals	17	20	19	20	19
Mean per animal (SD)	0.00 (0.00)	0.25 (0.64)	0.58 (1.02)	0.90 (1.48)	1.21 (1.96)
Maximum no. per animal in a brood	0	2	4	5	7

Table 7. Full and partial abortions observed in Daphnia pulex during chronic exposure to selenite-selenium.[a]

Brood	Parameter	Selenium Concentration, mg/l				
		Control	0.2	0.4	0.6	0.8
1	No. of animals	17	19	18	20	9
	Full abortions	0	0	0	4	4
	Partial abortions	0	0	3	7	2
2	No. of animals	15	17	17	20	9
	Full abortions	0	0	0	0	0
	Partial abortions	0	0	0	1	5
3	No. of animals	15	17	17	20	9
	Full abortions	0	0	0	0	0
	Partial abortions	0	0	0	0	1
4	No. of animals	15	17	17	19	9
	Full abortions	0	1	0	0	0
	Partial abortions	0	0	0	0	0

[a]No abortions occurred after brood 4.

Table 8. Summary descriptive statistics for reproduction in Daphnia pulex after 28 days exposure to selenite-selenium.

Parameter	Selenium Concentration, mg/l				
	Control	0.2	0.4	0.6	0.8
No. of animals	17	20	19	20	19
Mean total no. of eggs per animal ± SD	126 ± 52	118 ± 59	138 ± 61	140 ± 46	76 ± 83
Mean no. of live young per animal ± SD	124 ± 53	115 ± 59	135 ± 59	133 ± 47	70 ± 76
Mean brood size	13.78	12.79	14.95	14.72	7.78
Mean percent dead per animal	1.72	2.37	2.87	5.59	8.34
Mean no. of full abortions per animal ± SD	0.0	0.05 ± 0.22	0.0	0.20 ± 0.41	0.21 ± 0.42
Mean no. of partial abortions per animal ± SD	0.0	0.0	0.16 ± 0.37	0.40 ± 0.50	0.42 ± 0.69
Total no. of abortions	0	1	3	12	12
Mean no. of young trapped per animal ± SD	0.0	0.10 ± 0.31	0.26 ± 0.56	1.00 ± 1.08	1.00 ± 1.56

Figure 4 and Table A4 (Appendix) depict the results for percent dead young (the percentage of eggs that do not result in live young). Reproductive dysfunction increased with increasing selenium concentration, and decreased with succeeding broods, statistically significant differences occurred during broods 1, 2 and 8. Some reproductive dysfunction is probably normal in the early broods as indicated by the 7.5 percent value for the control group in brood 1, but it was much less than the 48, 65 and 73 percent values for the 0.4, 0.6 and 0.8 mg/l selenite-Se groups respectively.

Summary descriptive statistics for dead young with and without caudal spines and deteriorated eggs are presented in Table 6. Generally the frequency of all three parameters increased with increasing selenium concentration. The values for the group exposed to 0.8 mg/l selenite-Se are somewhat biased on the low side due to large numbers of mortalities occurring in the preadult instars. The high mean number of dead young with caudal spines in the control group is due to one individual who had 17 dead young with spines in one brood. With the exception of broods 1 and 2, these parameters make up a biologically insignificant portion of the total eggs produced.

The other two reproductive dysfunction parameters, full and partial abortions, are summarized in Table 7. Like the

other reproductive dysfunction parameters there was an increase in frequency as selenium concentration increased and the frequency decreased with subsequent broods. No abortions occurred in the control group. The last partial abortion occurred in brood 3 and the last full abortion occurred in brood 4. As with percent dead young, except at the higher concentrations in the first two broods, partial and full abortions were of little biological significance.

The results for number of live young are presented in Figure 5 and Table A5 (Appendix). The groups at the lower selenium concentrations and the control group had higher numbers of live young in the early instars, and the reverse was true for later broods. As with length, selenium seems to have a stimulatory effect on live young in the later broods. Statistically significant differences relative to the control group occurred during broods 1, 2, and 6, with marginal differences in brood 3. During broods 1 and 2 the control group and the groups at the lower concentration(s) produced a very significantly greater number of live young. In brood 6 the group exposed to 0.8 mg/l selenite-Se had significantly more live young than those exposed to 0.2 mg/l selenite-Se and the control group. The number of live young per brood for all groups including those exposed to selenium were within the range normally observed in D. pulex (Anderson et al., 1937; Green, 1956; Buikema, 1973).

Summary descriptive statistics over the entire test for reproductive parameters are presented in Table 8. For total eggs per animal, live young per animal, and mean brood size, there were no real effects throughout the test except at 0.8 mg/l selenite-Se. All the reproductive dysfunction parameters show a pattern of increasing with increasing selenium concentration. The results for all parameters at 0.8 mg/l selenite-Se are biased on the low side due to the mortalities at that concentration.

There are few studies in the literature on the effects of selenium on reproduction of fish or aquatic invertebrates. Most are studies on fish egg incubation time and hatching (Table 3) (Adams, 1976; Huckabee and Griffith, 1974; Niimi and LaHam, 1975). Other studies included fish embryo-larva toxicity tests (Birge, 1978), a study on abnormal development in oyster embryos (Glickstein, 1978), and a study on rate of albinism in fry hatched from exposed eggs (Westerman and Birge, 1978). However, Halter et al., (in press) reported that reproduction in Daphnia magna was unaffected at all concentrations tested (0.03 to 0.28 mg/l selenite-Se) during chronic exposure.

Summarizing the results of the chronic study, there was a significant depression in length at the highest concentrations during two preadult molts. No differences in adult length occurred until brood 9 when the group at the

highest concentration was significantly larger. These differences disappeared at brood 10. There appeared to be a general pattern of stimulation in length at the higher concentrations in the later broods.

For total number of eggs no clearcut pattern was evident, although there appeared to be a slight stimulation due to selenium in the later instars. Percent dead young significantly increased at the higher concentrations in the early broods, and these differences disappeared later. A similar pattern was observed for live young; however, stimulatory effects were observed in the later instars, particularly in brood six.

Length appeared to be a sensitive parameter during the preadult instars, but may not be quite as sensitive during adult instars; immature stages of a life cycle are generally more sensitive to toxicant exposure than adult stages (Buikema and Benfield, 1979). The reproductive parameters, with the exception of total number of eggs, appeared to be the best indicators of selenium stress. In general, it appeared that selenium had inhibitory effects during the early broods. These effects disappeared in later broods when selenium may even produce slightly stimulatory effects. These results indicate that the Daphnia may be acclimating to the selenium stress.

Based on a brood by brood analysis there were no

significant effects on length or any of the reproductive parameters at 0.2 mg/l selenite-Se. There was a slightly elevated mortality rate, but is probably not of biological significance. Only minimal effects were observed at 0.4 mg/l selenite-Se. An estimate of the maximum allowable toxicant concentration (MATC) from this study would then be between 0.2 and 0.4 mg/l (geometric mean = 0.28 mg/l). This agrees fairly well with the results of other invertebrate chronic studies, USEPA (1978a) reported a similar range for the marine Mysidopsis of 0.127-0.143 mg/l Se (geometric mean = 0.135 mg/l). Halter et al. (in press) reported an MATC for D. magna of 0.28 mg/l selenite-Se, however that was the highest concentration they tested, and no effects were observed. Kimball (unpublished) using selenious acid, reported the results of a life cycle test for D. magna as 0.19 to 0.30 mg/l Se (geometric mean = 0.24 mg/l).

The criterion USEPA proposes to use for establishing an MATC is total live young produced per animal over the course of the test. If that method were used in this study, the no effects concentration estimate would be 0.6 rather then 0.2 mg/l (Table 8), due to the slight stimulatory effects on reproduction which occurred in the later broods. However, on the basis of the brood by brood analysis and the results of the reproductive dysfunction parameters (which USEPA ignors), 0.2 mg/l is a much better estimate of the no

effects level and gives a MATC value close to the others available from the literature.

Effects of Selenium on the Release of Young

Young Daphnia apparently trapped in the shed exoskeleton of a parent were observed during the chronic study. Apparently the effect was due to selenium because it never was observed in the controls and it generally increased in frequency with increasing selenium concentration (Tables 8 and 9). Like the other reproduction dysfunction parameters, it was much more common in the early broods, with up to 45 percent of the females at a concentration being affected. The trapped young never amounted to more then 5 percent of the total eggs. Thus, although the trapping of young is of biological interest, it may not be toxicologically significant.

Forty-seven young were observed to be trapped during the course of the study: twenty-eight with caudal spines were alive when observed, nineteen, all but one without a caudal spine, were dead. Whether alive or dead at the time they were observed, they were included in one of the dead young catagories because in a natural situation they would be functionally dead because they were trapped.

The lack of a caudal spine in all but one of the dead trapped young indicates that they had died in an early stage of embryonic development and suggests that they re-

Table 9. The percentage of female Daphnia pulex with trapped young and the percentage of eggs resulting in trapped young, during chronic exposure to selenite-selenium.

Brood	Parameter	Control	Selenium Concentration, mg/l 0.2	0.4	0.6	0.8
1	Percentage of females	-	10.5	16.7	5.0	11.1
	Percentage of eggs	-	1.3	2.3	0.7	3.2
2	Percentage of females	-	-	-	45.0	44.4
	Percentage of eggs	-	-	-	4.3	5.1
3	Percentage of females	-	-	-	5.0	22.2
	Percentage of eggs	-	-	-	0.3	1.8
4	Percentage of females	-	-	-	-	-
	Percentage of eggs	-	-	-	-	-
5	Percentage of females	-	-	6.2	16.7	-
	Percentage of eggs	-	-	0.6	1.7	-
6	Percentage of females	-	-	-	-	-
	Percentage of eggs	-	-	-	-	-
7	Percentage of females	-	-	-	-	-
	Percentage of eggs	-	-	-	-	-
8	Percentage of females	-	-	-	-	22.2
	Percentage of eggs	-	-	-	-	3.3
9	Percentage of females	-	-	-	5.9	11.1
	Percentage of eggs	-	-	-	0.3	0.5

present "normal" dead young which had died before the release of any young occurred. For the alive trapped young with caudal spines, the spines generally were curved around the posterior margin of the animal. The curved spines indicate that these animals were in the final stage of embryonic development (Obreshkove and Fraser, 1940). The postabdominal movement of the parental Daphnia normally is enough to eject even dead young; the trapping of these young suggests an interuption of the brood release-molting sequence of the parental Daphnia. Another possible explanation is that the live trapped young due to their earlier stage of development, or the selenium, or both, did not possess the morphological features or behavioral response of trying to swim free of the brood chamber, upon vigorous movement of the parents postabdomen. In one instance, however, the shed exoskeleton was ruptured with a probe and the young released. During several minutes of observation, these released young appeared to behave normally.

Effects of Selenium on Oxygen Consumption

Changes in the respiratory activity of fish exposed to pollutants has been used as a indicator of pollutant stress (Sparks et al., 1972; Rice et al., 1977; Hughes, 1976). Few studies, however, have used oxygen consumption of invertebrates as an indicator of pollutant stress. These studies include research on tubificid worms (Whitley and

Sikora, 1970), freshwater snails (Sheanon and Trama, 1972), bivalves (Capuzzo and Sasner, 1977) and Daphnia (Sherr and Armitage, 1973; Buikema et al., 1978; Gieger, 1979). This study represents the first attempt to measure selenium stress on an invertebrate by using oxygen consumption.

The major reason for looking at oxygen consumption is related to the biological function of selenium. In mammals selenium is involved in preventing oxidative damage to cell and organelle membranes, 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 (Frosh and Lish, 1975). This suggested that oxygen consumption might be a good parameter to monitor selenium stress.

The results of the oxygen consumption studies are presented in Table 10. There appeared to be a slight non-significiant increase in oxygen consumption due to the selenium. Each table value represents the pooling of replicates from two separate tests, and looking at the standard deviations, there was quite a bit of variation among the replicates. In one test, due to a shortage of young animals, none were exposed to 0.2 mg/l selenite-Se; for some reason in that test, the results were higher than in the

Table 10. Effects of acute exposure to selenite-selenium on the oxygen consumption 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.

Selenium Concentration	No. of Replicates	Oxygen Consumption ul/mg dry wt/hr (SD)
0.2 mg/l	6	6.85 (1.56)[a]
Control	3	6.64 (1.92)
0.4 mg/l	7	9.70 (1.76)[b]
0.8 mg/l	7	9.31 (2.37)
0.6 mg/l	7	9.15 (2.34)
Control	4	9.05 (3.59)

[a]ANOVA P value = 0.8648

[b]ANOVA P value = 0.9676

other tests for the controls and all concentrations, though the trend among the different concentrations remained the same. In order to prevent the 0.2 mg/l selenite-Se value from being biased, a second control value was included in Table 10. Although the results were somewhat variable, there was no significant impact of selenium on the oxygen consumption of the Daphnia. On the basis of this study oxygen consumption does not appear to be a good indicator of pollutant stress.

Effects of Selenium on Filtering Rate and Gut pH

The filtering rate of zooplankton has been used to study secondary production (Rigler, 1971). Recently this technique has been applied as a measure of pollutant stress in Daphnia (Cooley, 1977; Buikema et al., 1978; Geiger, 1979). The reason for conducting filtering rate and gut pH studies was an observation made in the chronic study, most of the animals exposed to 0.8 mg/l selenite-Se that died did not have a green gut characteristic of feeding on Chlamydomonas. Two possible explanations for this observation are a decreased level in feeding, and/or alteration in digestive enzyme function due to a shift in pH.

The results for the filtering rate study are presented in Table 11. The values are the result of the pooling of two separate tests. The only statistically significant differences occurred between the group exposed to 0.2 mg/l

Table 11. 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.

Selenium Concentration	No. of Replicates	Filtering Rate ml/animal/~~hr~~ day. (SD)
0.2 mg/l	6	3.15 (1.03)[a]
Control	6	2.78 (1.91)
0.4 mg/l	6	1.77 (1.28)
0.8 mg/l	6	1.41 (0.33)
0.6 mg/l	5	1.19 (0.69)

[a]ANOVA P value = 0.0378

selenite-Se and those at 0.6 and 0.8 mg/l selenite-Se. The 0.2 mg/l group had a slightly elevated filtering rate relative to the controls. For the other three concentrations there was an increasing depression in filtering rate with increasing selenium concentration. The elevated filtering rate at 0.2 mg/l selenite-Se may be a compensatory response to low level selenium stress. At the higher concentrations there was a definite pattern of depression which may account for the effects observed during the chronic study.

Filtering rate appears to be a useful indicator of pollutant stress. The trends observed during the 24 hr filtering rate test were similar to those observed in reproduction during the chronic study.

The dye study indicated no differences in gut pH among the control and selenium-exposed groups. All animals observed displayed a pattern of red/rose dye color in the anterior portion of the gut grading into orange/yellow at the posterior end. This indicates a normal pH gradient for Daphnia (Hasler, 1935). If there is an effect of selenium on the digestive enzyme function it was not due to pH.

EVALUATION OF THE SELENIUM WATER QUALITY CRITERION FOR FRESH WATER

Evaluation of the Criteria to Date

Recently, two sets of water quality criteria have been published: the first by the National Academy of Sciences (NAS, 1972), and the second by the United States Environmental Agency (USEPA, 1976). Under provisions of the Federal Water Pollution Control Act, and the Clean Water Act of 1977, the USEPA is required to publish lists of toxic pollutants and to develop and periodically update water quality criteria for them. Currently the USEPA is producing criterion documents for the first list of 65 toxic pollutants (USEPA, 1978c) and this list includes selenium and its compounds.

In attempting to set a water quality criterion to protect freshwater aquatic life for selenium and its compounds, one must contend with a compound that is both toxic and a required trace element, at least for species of mammals, birds, fish and dinoflagellates (NAS, 1976; Poston et al., 1976; Lindstrom and Rodhe, 1978). A difficulty arises because of the very narrow margin between the level at which it is required and the level at which it becomes toxic. Copeland (1971) suggested that the required and toxic dietary doses of selenium for humans are 0.2 and 5.0 mg/day, respectively. The resulting safety factor of 25 fold is narrower than that for any other required trace

element.

Setting a freshwater criterion for selenium is also complicated by synergistic and antagonistic interrelationships with many substances such as sulfate, arsenic, cadmium, and mercury (NAS, 1976). In fish selenium antagonizes mercury toxicity (Kim et al., 1977; Heisinger et al., 1979). Depending on the relative concentrations, selenium may behave synergistically or antagonistically with mercury (Huckabee and Griffith, 1974; Glickstein, 1978).

Geochemical interactions are also important when setting a criterion, because of their impact on environmental concentrations. Because of geochemical interactions, selenate, the least toxic of the selenium compounds (Table 3), potentially is the most dangerous form of selenium (NAS, 1976). Under alkaline, oxidizing conditions, selenate is the favored chemical form. However, under acidic, reducing conditions the equilibrium shifts to selenite, the most toxic form in laboratory studies (Table 3).

Because of interactions with iron, selenite and biselenite concentrations in aerobic aquatic systems are much lower then would be expected. Selenite and biselenite ions combine with iron to form insoluble ferric selenites and biselenites, and in aerated waters over a pH range of 2 to 8, both selenite and biselenite ions are strongly absorbed by hydrous ferric oxides (Howard, 1977). At pH 7 to 8,

ferric hydroxide can absorb 90 to 99 percent of the selenite ions in natural waters (Howard, 1971). Under acidic conditions absorbed selenite is rapidly reduced to very insoluble elemental selenium (NAS, 1976). Because of the above geochemical interactions, the potential pollution hazard of the selenites appears minimal (NAS, 1976).

Unfortunately, factors such as synergism and geochemistry have not received much attention when water quality criteria have been developed. The criteria have been derived by using application, correction, and sensitivity factors. Many times the resulting criteria are ultraconservative and often are not scientifically sound. The freshwater selenium criterion of the USEPA (1976), as well as the criterion in Interim Draft No. 1 of the Selenium Criterion Document (USEPA, 1977), are the product of an application factor of 0.01 times a 96 hour LC50 value (Table 12). A freshwater criterion for selenium was not considered by the NAS (1972). The selection of the 0.01 application factor in the Interim Draft No. 1 was based on data which indicated that selenium was teratogenic, but there is nothing in the literature which has established that a 0.01 application factor is appropriate for teratogenic compounds (Hartung, pers. comm.).

NAS (1972) states that 0.01 and 0.1 are "universal" application factors which are used when safe levels of a

Table 12. A comparison of suggested water quality criteria for selenium in fresh water and empirically derived maximum acceptable toxicant concentration (MATC) values for freshwater organisms.

WATER QUALITY CRITERIA			MATC		
Reference	Application Factor	Criterion	Organism	MATC ug/l Se	Reference
NAS (1972)	-	none	Daphnia magna[c]	280	Adams (1976)
USEPA (1976)	0.01	0.01 X 96 hr LC50	Daphnia magna[d]	240 (190-300)[a]	Kimball, unpbl.
USEPA (1977)	0.01	20 ug/l	Pimephales promelas[d]	110 (153-303)[b]	Kimball, unpbl.
USEPA (1978d)	Guidelines (USEPA, 1978e)	9.7 ug/l as 24 hr average and not greater then 22 ug/l at anytime	Daphnia pulex[c]	283 (200-400)[a]	This study.

[a]MATC derived by taking the geometric mean of the life cycle test limits included in parentheses.

[b]MATC derived by correcting embryo-larva tests limits included in parentheses to life cycle test limits by following the guidelines (USEPA, 1978e) and taking the geometric mean of those limits.

[c]Compound used was sodium selenite.

[d]Compound used was selenious acid.

chemical have not been experimentally determined. Although not stated explicitly by USEPA (1976) when developing its criterion, an application factor of 0.01 instead of 0.1 was used for materials which are known, or suggested to be bioaccumulative or persistent, as suggested by NAS (1972).

To evaluate the USEPA (1976) freshwater criterion for selenium two questions must be answered. First, does the available data suggest that selenium is bioaccumulative or persistent, thereby supporting the use of the 0.01 application factor? Second, irrespective of the NAS (1972) guidelines for the use of "universal" application factors, is their use appropriate in the case of selenium?

The limited data available suggests that selenium does moderately bioaccumulate and is persistent. Adams (1976) in a single species laboratory study, reported bioaccumulation factors for fathead minnows on a wet weight basis of 29.2 for the whole fish, 18 in muscle, and 149.9 in the visera, after 96 days exposure to 0.01 mg/l selenite-Se. Nicholson (1977) using a freshwater model ecosystem dosed with 0.05 mg/l selenate-Se, reported 50-day bioaccumulation factors as high as 496 on a dry weight basis. Mosquitofish, bluegills, Elodea, dragonfly nymphs, snail flesh, and algae exhibited bioaccumulation factors of 238, 206, 184, 277, 466, and 496, respectively. Calculating bioaccumulation factors for selenium from the data of Guthrie and Cherry

(1979) for a coal-ash basin drainage system; bacteria, mosquitofish, crayfish, and tadpoles accumulated the greatest amounts of selenium, with bioaccumulation factors on a wet weight basis of 109, 85, 65, and 59, respectively.

Even less information is available on the persistence of selenium in aquatic organisms. Adams (1976) reported the half-life of selenium in fathead minnows to be in excess of 50 days, while Gissel-Nielsen and Gissel-Nielsen (1973) reported a half-life of 27 days for the eel, Anguilla anguilla, and 13 days for the guppy, Lebistes reticulatis. A half-life of 29 days was reported for rainbow trout by Gissel-Nielsen and Gissel-Nielsen (1978).

The use of a "universal" application factor does not seem to be prudent in the case of selenium. These factors were designed to be conservative in order to protect even the most sensitive of species. However, in the case of selenium with such a narrow range between required and toxic levels, by using these conservative factors we run the risk of setting a criterion at or below a required level for an organism.

The USEPA (1978e) recently produced a set of guidelines for setting water quality criteria; these guidelines require the application of a series of sensitivity and correction factors to empirical data. The scientific basis for these guidelines appears shaky. In their evaluation of the

biological data used in deriving the EPA guidelines, Cairns et al. (1978) reported among other things that actual MATC values were ignored in favor of calculated ones, water quality interactions were ignored, the data used to develop the guidelines were biased toward pesticides and soft waters, units of measurement were interchanged, LC50 and EC50 values were treated as equivalents, and differences in species sensitivity and methodologies ignored.

The revised selenium water quality criterion (USEPA, 1978d) was based on alternative procedures which consisted of using these guidelines along with unpublished data. The revised freshwater criterion for selenium is 9.7 ug/l as a 24 hour average, and not greater than 22 ug/l at any time.

The 22 ug/l value, known in the guidelines as the final acute value, is the lowest of the two values calculated from acute toxicity data for fish and invertebrates. In this case it was based on three LC50 values for freshwater invertebrates, including a questionable 48 hour LC50 value for D. magna (USEPA, 1978a).

The 24 hour average value of 9.7 ug/l was based on chronic toxicity data. In deriving this value, estimates of chronic toxicity were calculated from fish and invertebrate chronic data, and by estimation from acute toxicity data (0.44 times the final acute value). In the case of selenium the last method was selected, as a result this chronic

criterion is ultraconservative. The fish and invertebrate empirical chronic estimates were rejected for a lower calculated estimate.

Looking at Table 12 which summarizes all of the water quality criteria for selenium in fresh water, as well as all of the empirically derived MATC values for selenium, several trends can be observed. First, the current revised criterion (USEPA, 1978d) is more conservative than any of the previous selenium criteria. Second, all of the MATC values, including the one from this study, agree fairly well with one another. Finally, on the basis of these MATC values, all of the criteria to date are too conservative.

The results of this study, which is more extensive than any published in the literature on selenium sublethal effects, indicate that 200 ug/l is a no effects level. At that concentration no significant effects relative to the controls were found on growth, reproduction, reproductive dysfunction, oxygen consumption or filtering rate. There was a slightly elevated chronic mortality at that concentration, but it is probably not of any biological significance, unfortunately no statistical analysis could be carried out.

Reevaluation of a Criterion-Belews Lake

Selenium was reported to be the cause of an absence in 1976 of young-of-year fish in Belews Lake, North Carolina

(Cumbie, 1978). The lake is an impoundment which receives a heated discharge as well as an ash basin effluent from a coal-fired electric power plant. The mean selenium concentration for 1976-1977 was 10 ug/l, with a maximum of 20 ug/l. Cumbie (1978) attributed the absence of young-of-year fish to reproductive failure, due to the elevated selenium concentrations in the ovaries of the few reproductively active females that he was able to obtain. He assumed the elevated selenium concentrations were due to biomagnification via crustacean zooplankton.

Cumbie did no laboratory studies to support his conclusions. The conclusions he draws are questionable, and he ignores several alternative explanations. Transitions are normal occurrences in new impoundments; a transition in important food organism populations or the fish populations themselves could explain what happened. Weiss and Anderson (1978) reported a sharp decline in the rotifer populations in the lake that same year, rotifers are an important food for larval fish (Siefert, 1972) and this may explain the lack of young-of-year fish. Synergistic interactions between the many elements in the ash basin effluent may also be an explanation.

Although there are serious questions about Cumbies (1978) conclusions, the fact that the mean selenium concentration in the lake was at the revised selenium criterion

(USEPA, 1978d), requires that further investigations be made. What happened at Belews Lake presents a dilemma, it indicates that a criterion for selenium below 10 ug/l is appropriate, however all of the laboratory data indicates that a criterion much higher is appropriate. If the cause of the lack of young-of-year fish was selenium, with the pH regime and redox potentials of Belews Lake, it was due to the selenate form (Cumbie, 1978). This supports the NAS (1976) conclusion that selenate is potentially the most dangerous form of selenium in the environment. Under these circumstances, and because of what is known about selenite interactions with iron, a dual standard might be appropriate, a strict one for selenate-selenium for waters with alkaline and oxidizing conditions, and a more lenient one for selenite- and biselenite-selenium for waters with acidic and reducing conditions. However, due to the uncertainty about what actually occurred in Belews Lake, more research on selenate-selenium is needed before a criterion for it can be developed.

CONCLUSIONS

Based on acute toxicity tests, the effects of acute sublethal exposure on oxygen consumption and filtering rate, and from studies on sublethal effects due to chronic exposure, selenite-selenium appears to be only moderately toxic to Daphnia pulex.

Specific conclusions are listed below.

(1) Daphnia pulex was acutely the most sensitive of the three species tested, the 48 hr LC50 was 3.87 mg/l selenite-Se. The 96 hr LC50 and EC50 values for Gambusia affinis and Physa sp. respectively were 12.56 and 24.08 mg/l selenite-Se.

(2) Chronic mortality to Daphnia was greatest at 0.8 mg/l selenite-Se. Fifty-three percent mortality occurred at the end of 28 days compared to 18 percent in the control group. At 0.6 and 0.4 mg/l selenite-Se mortality was less than or equal to that of the control group. A slightly elevated mortality of 12 percent above the control group was noted at 0.2 mg/l selenite-Se; it probably was not of any biological significance.

(3) During 28 days of exposure Daphnia growth, as measured by length, was depressed during the preadult instars and slightly stimulated during the later adult instars. Number of live young per brood was depressed at 0.4, 0.6 and 0.8 mg/l selenite-Se during the early broods, and appeared slightly stimulated in the later broods. Reproductive dysfunction

as evident by dead young, deteriorated eggs, and abortions was significant at the higher concentrations in the early broods, but almost never occurred in the later broods. It appeared that the Daphnia were acclimating to the selenite-selenium stress.

(4) Based on summary statistics for the entire 28 days of chronic exposure, there was no effect on mean number of total eggs per animal, mean number of live young per animal, or mean brood size, except at the highest concentration 0.8 mg/l selenite-Se where they were depressed. All of the reproductive dysfunction parameters increased as selenite-selenium concentrations increased.

(5) The effect of selenite-selenium on Daphnia oxygen consumption was variable. After twenty-four hours exposure there were no significant differences between any of the test groups.

(6) The filtering rates of Daphnia were significantly higher at 0.2 mg/l selenite-Se, than at 0.6 and 0.8 mg/l selenite-Se after 24 hours exposure. There was a non-significant increase at 0.2 mg/l selenite-Se relative to the controls, and marked non-significant depressions at 0.4, 0.6 and 0.8 mg/l selenite-Se.

(7) Based on these results, 0.2 mg/l appears to be the no effects level of toxicity for selenite-selenium (MATC= 0.28 mg/l). However based solely on the production of live

young over the entire 28 days of the test, as recommended by USEPA, the no effects concentration would be 0.6 mg/l.

(8) The results of this study as well as those in the literature, suggest that all of the water quality criteria for selenium in fresh water proposed by USEPA are inadequate. Based on the toxicological, bioaccumulation and geochemical data available, a dual criterion seems to be appropriate, a strict criterion for selenate-selenium and a lenient criterion for selenite- and biselenite-selenium.

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APPENDIX 1

Table A1. Mean lengths of preadult Daphnia pulex during chronic exposure to selenite-selenium and the results of ANOVA and Duncan's new multiple range tests. Lengths not underscored by a continuous line were found to be significantly different by Duncan's test at the 0.05 level.

Molt	ANOVA P value	Selenium Concentration (No. of animals)				
0[a]	0.2915	0.2 mg/l(20) 0.79 mm	0.6 mg/l(20) 0.80 mm	0.8 mg/l(19) 0.82 mm	0.4 mg/l(19) 0.83 mm	Control (17) 0.83 mm
1[b]	0.1765	0.8 mg/l(18) 0.96 mm	0.2 mg/l(20) 0.99 mm	0.6 mg/l(20) 1.00 mm	Control(17) 1.01 mm	0.4 mg/l(18) 1.02 mm
2	0.0548	0.8 mg/l(13) 1.20 mm	0.6 mg/l(20) 1.26 mm	0.2 mg/l(20) 1.27 mm	0.4 mg/l(18) 1.31 mm	Control (17) 1.31 mm
3	0.0125	0.8 mg/l(11) 1.44 mm	0.6 mg/l(20) 1.52 mm	0.2 mg/l(18) 1.58 mm	Control(15) 1.60 mm	0.4 mg/l(17) 1.61 mm
4	0.9788	0.4 mg/l(1) 1.58 mm	0.6 mg/l(3) 1.60 mm	0.8 mg/l(3) 1.61 mm	0.2 mg/l(2) 1.64 mm	

[a]Molt 0 was the instar the animal was in when the chronic study began.

[b]Molt 1 was the instar after the first molt to occur during the chronic study.

Table A2. Mean lengths of adult Daphnia pulex during chronic exposure to selenite-selenium, and the results of ANOVA and Duncan's new multiple range tests. Lengths not underscored by a continuous line were found to be significantly different by Duncan's test at the 0.05 level.

Brood	ANOVA P value	Selenium Concentration (No. of animals)				
1	0.2330	0.6 mg/l(20) 1.81 mm	Control(17) 1.84 mm	0.8 mg/l(9) 1.86 mm	0.2 mg/l(19) 1.88 mm	0.4 mg/l(18) 1.88 mm
2	0.5940	0.6 mg/l(20) 2.04 mm	Control(15) 2.05 mm	0.2 mg/l(17) 2.09 mm	0.8 mg/l(9) 2.09 mm	0.4 mg/l(17) 2.10 mm
3	0.3994	Control(15) 2.17 mm	0.6 mg/l(20) 2.18 mm	0.2 mg/l(17) 2.22 mm	0.4 mg/l(17) 2.23 mm	0.8 mg/l(9) 2.25 mm
4	0.5174	Control(15) 2.26 mm	0.6 mg/l(20) 2.26 mm	0.2 mg/l(17) 2.28 mm	0.4 mg/l(17) 2.31 mm	0.8 mg/l(9) 2.33 mm
5	0.3590	Control(15) 2.33 mm	0.2 mg/l(16) 2.37 mm	0.6 mg/l(19) 2.37 mm	0.8 mg/l(9) 2.40 mm	0.4 mg/l(16) 2.41 mm
6	0.2780	Control(14) 2.39 mm	0.2 mg/l(16) 2.42 mm	0.6 mg/l(19) 2.44 mm	0.4 mg/l(16) 2.47 mm	0.8 mg/l(9) 2.48 mm
7	0.1511	Control(14) 2.42 mm	0.2 mg/l(14) 2.46 mm	0.6 mg/l(18) 2.48 mm	0.4 mg/l(16) 2.51 mm	0.8 mg/l(9) 2.52 mm
8	0.1288	Control(14) 2.46 mm	0.2 mg/l(14) 2.49 mm	0.6 mg/l(18) 2.51 mm	0.4 mg/l(16) 2.55 mm	0.8 mg/l(9) 2.56 mm
9	0.0381	Control(14) 2.49 mm	0.2 mg/l(14) 2.51 mm	0.6 mg/l(17) 2.57 mm	0.4 mg/l(16) 2.59 mm	0.8 mg/l(9) 2.59 mm
10	0.0749	Control(14) 2.54 mm	0.2 mg/l(13) 2.54 mm	0.4 mg/l(14) 2.61 mm	0.6 mg/l(15) 2.61 mm	0.8 mg/l(9) 2.64 mm

Table A3. Mean total number of eggs of Daphnia pulex during chronic exposure to selenite-selenium, and the results of ANOVA and Duncan's new multiple range tests. Values not underscored by a continuous line were found to be significantly different by Duncan's test at the 0.05 level.

Brood	ANOVA P value	Selenium Concentration (No. of animals)				
1	0.9676	0.8 mg/l(9) 7.00	Control(17) 7.29	0.4 mg/l(18) 7.33	0.6 mg/l(20) 7.60	0.2 mg/l(19) 7.79
2	0.0038	0.6 mg/l(20) 12.85	Control(15) 14.93	0.8 mg/l(9) 15.33	0.2 mg/l(17) 16.82	0.4 mg/l(17) 17.47
3	0.1498	Control(15) 15.33	0.6 mg/l(20) 17.25	0.2 mg/l(17) 17.47	0.4 mg/l(17) 18.71	0.8 mg/l(9) 18.89
4	0.8424	0.2 mg/l(17) 14.94	0.4 mg/l(17) 15.53	0.6 mg/l(19) 15.63	Control(15) 15.67	0.8 mg/l(9) 17.00
5	0.6029	0.6 mg/l(18) 19.22	0.8 mg/l(9) 19.33	Control(15) 20.07	0.2 mg/l(16) 20.25	0.4 mg/l(16) 21.75
6	0.0015	Control(14) 17.86	0.2 mg/l(16) 18.50	0.4 mg/l(16) 20.31	0.6 mg/l(18) 21.22	0.8 mg/l(9) 23.89
7	0.0714	0.2 mg/l(14) 15.79	Control(14) 17.43	0.8 mg/l(9) 18.89	0.6 mg/l(18) 19.39	0.4 mg/l(16) 19.63
8	0.5823	0.6 mg/l(18) 18.17	Control(14) 18.57	0.2 mg/l(14) 19.14	0.4 mg/l(16) 19.75	0.8 mg/l(9) 20.33
9	0.8118	0.2 mg/l(14) 18.93	Control(14) 19.79	0.4 mg/l(16) 19.81	0.8 mg/l(9) 20.56	0.6 mg/l(17) 20.71

Table A4. Mean percent dead young of Daphnia pulex during chronic exposure to selenite-selenium, and the results of ANOVA and Duncan's new multiple range tests. Values not underscored by a continuous line were found to be significantly different by Duncan's test at the 0.05 level.

Brood	ANOVA P value	Selenium Concentration (No. of animals)				
1	0.0001	Control(16) 7.50%	0.2 mg/l(17) 16.29%	0.4 mg/l(18) 47.71%	0.6 mg/l(20) 64.86%	0.8 mg/l(9) 72.99%
2	0.0001	Control(15) 0.00%	0.2 mg/l(17) 0.00%	0.4 mg/l(17) 1.28%	0.6 mg/l(20) 7.90%	0.8 mg/l(9) 38.92%
3	0.3040	0.4 mg/l(17) 0.23%	0.2 mg/l(17) 0.74%	0.6 mg/l(17) 1.00%	Control(15) 2.00%	0.8 mg/l(9) 5.34%
4	0.4882	0.4 mg/l(16) 0.00%	0.8 mg/l(9) 0.00%	0.6 mg/l(18) 1.63%	0.2 mg/l(17) 6.73%	Control(15) 8.89%
5	0.2311	Control(14) 0.00%	0.2 mg/l(16) 0.00%	0.8 mg/l(9) 0.00%	0.4 mg/l(16) 0.90%	0.6 mg/l(18) 1.61%
6	0.4724	Control(14) 0.00%	0.4 mg/l(16) 0.00%	0.8 mg/l(9) 0.00%	0.6 mg/l(18) 4.76%	0.2 mg/l(16) 7.86%
7	0.4372	Control(14) 0.00%	0.4 mg/l(16) 0.00%	0.8 mg/l(9) 0.00%	0.6 mg/l(18) 0.80%	0.2 mg/l(13) 1.92%
8	0.0114	Control(14) 0.00%	0.2 mg/l(14) 0.00%	0.4 mg/l(16) 0.63%	0.6 mg/l(17) 0.69%	0.8 mg/l(9) 7.01%
9	0.2807	Control(14) 0.00%	0.2 mg/l(14) 0.00%	0.4 mg/l(15) 0.00%	0.6 mg/l(17) 0.57%	0.8 mg/l(9) 0.65%

Table A5. Mean number of live young of Daphnia pulex during chronic exposure to selenite-selenium , and the results of ANOVA and Duncan's new multiple range tests. Values not underscored by a continuous line were found to be significiantly different by Duncan's test at the 0.05 level.

Brood	ANOVA P value	Selenium Concentration (No. of animals)				
1	0.0001	0.8 mg/l(9) 1.67	0.6 mg/l(20) 2.60	0.4 mg/l(18) 3.72	0.2 mg/l(19) 6.47	Control(17) 6.65
2	0.0001	0.8 mg/l(9) 9.78	0.6 mg/l(20) 12.00	Control(15) 14.93	0.2 mg/l(17) 16.82	0.4 mg/l(17) 17.24
3	0.2082	Control(15) 15.13	0.6 mg/l(20) 17.05	0.2 mg/l(17) 17.35	0.8 mg/l(9) 18.00	0.4 mg/l(17) 18.65
4	0.6219	Control(15) 14.13	0.2 mg/l(17) 14.29	0.6 mg/l(19) 15.37	0.4 mg/l(17) 15.53	0.8 mg/l(9) 17.00
5	0.5779	0.6 mg/l(18) 18.89	0.8 mg/l(9) 19.33	Control(15) 20.07	0.2 mg/l(16) 20.25	0.4 mg/l(16) 21.56
6	0.0134	0.2 mg/l(16) 17.63	Control(14) 17.86	0.6 mg/l(18) 20.22	0.4 mg/l(16) 20.31	0.8 mg/l(9) 23.89
7	0.0531	0.2 mg/l(14) 15.50	Control(14) 17.43	0.8 mg/l(9) 18.89	0.6 mg/l(18) 19.22	0.4 mg/l(16) 19.63
8	0.8035	0.6 mg/l(18) 18.06	Control(14) 18.57	0.8 mg/l(9) 18.78	0.2 mg/l(14) 19.14	0.4 mg/l(16) 19.63
9	0.8535	0.2 mg/l(14) 18.93	Control(14) 19.79	0.4 mg/l(16) 19.81	0.8 mg/l(9) 20.44	0.6 mg/l(17) 20.59

APPENDIX 2

Observations on the Effects of Selenium on Chlamydonomas

The toxicity of selenium to Chlamydomonas reinhardti was observed twice during this study. During some preliminary tests, an attempt was made to evalute the toxicity of selenite-selenium using the basic procedure in the Algal Bottle Test (USEPA, 1971). The alga was grown in Bolds Basal Medium (See Materials and Methods) modified by the substitution of chloride salts for sulfate salts, because sulfate is a selenium antagonist (Kumar and Prakash, 1971). The test was run in 250 ml flasks with 60 ml of test solution at an initial cell density of approximately 50,000 cells/ml. The range finding concentrations tested were between 5 and 150 mg/l selenite-Se. The test was conducted at the same temperature and light regimes as used for the algae that are fed to the Daphnia (see Materials and Methods).

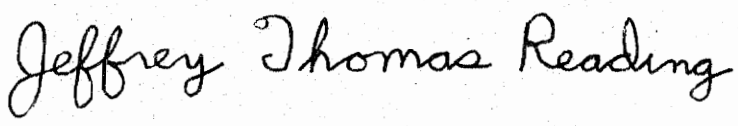
Due to a problem with the electronic particle counter the experiment was terminated. However, the algae were allowed to grow in the selenite-selenium spiked media for two weeks. Each flask was then uniformly mixed, and their optical density read at 680 nm on a spectrophotometer. Based on that data, selenite-selenium has a toxic effect on algal growth below 5 mg/l. One of the replicates at 25 mg/l was examined microscopically after 96 hr and the algal cells were observed to be encysted.

During the filtering rate studies, the effects of selenite-selenium on Chlamydomonas were also observed. An algal control, all at the same initial cell density, was run at each selenite-selenium concentration (0.2, 0.4, 0.6 and 0.8 mg/l). In one of the tests, even though the cell densities of all the algal controls had increased after 24 hours, a pattern of decreasing cell density with increasing selenium concentration was observed. A decrease of 3 to 15 percent was found when comparing the cell density of an algal control at one concentration to the algal control of the next lower concentration. This pattern was not observed in the second test. However it appears that the algae had divided just prior to the beginning of that test, and no appreciable increase in cell density was observed in any of the algal controls after 24 hours.

VITA

Jeffrey Thomas Reading was born on May 26, 1954 in Trenton, New Jersey where he was graduated from Ewing High School in 1972. In 1976 he received a Bachelor of Science degree with high honors in environmental science from Rutgers University, New Brunswick, New Jersey.

He began studies toward a Master of Science degree in Zoology at Virginia Polytechnic Institute and State University, Blacksburg, Virginia in 1976. From 1976 to 1977 he was a Graduate Research Assistant in Biology, and was a Graduate Teaching Assistant in Biology from 1977 to 1979. He is a member of Alpha Zeta, the Phi Sigma Society, the Association of Southeastern Biologists and the Water Pollution Control Federation.



ACUTE AND CHRONIC EFFECTS OF SELENIUM ON *Daphnia pulex*

by

Jeffrey Thomas Reading

(ABSTRACT)

Acute toxicity tests with selenium were conducted with three freshwater species. All data are expressed as selenite-selenium. *Daphnia pulex* had a 48 hr LC50 of 3.87 mg/l selenium. The 96 hr LC50 and EC50 values for *Gambusia affinis* and *Physa* sp. respectively, were 12.56 and 27.08 mg/l selenium.

The sublethal effects of 0.2, 0.4, 0.6 and 0.8 mg/l selenium on survival, growth and reproduction of *Daphnia pulex* were monitored for twenty-eight days. These results were analyzed statistically by brood. Appreciable mortality only occurred at 0.8 mg/l selenium. Growth, as measured by body length, was depressed at the highest concentration during the early instars and was slightly stimulated during the later instars. Number of live young per brood was depressed at 0.4, 0.6 and 0.8 mg/l selenium during the early broods and may have been stimulated in later broods. Reproductive dysfunction (i.e., dead young, deteriorated eggs, and abortions) only was significant at the higher concentrations in the early broods. It appeared that the *Daphnia* were acclimating to the selenium stress. Based on these

studies, the MATC for selenite-selenium was 280 ug/l.

The effects of selenium on oxygen consumption and filtering rate during 24 hr exposure were also tested at the above concentrations. There were no significant effects of selenium on oxygen consumption. Selenium slightly stimulated filtering rate at 0.2 mg/l and depressed it at the higher concentrations.

An evaluation of the water quality criteria for selenium in fresh water indicates that all of the methods for deriving these proposed criteria are inadequate. Based on my evaluation a dual criterion seems appropriate: a strict criterion for selenate-selenium and lenient criterion for selenite- and biselenite-selenium.