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Selenium Uptake and Transfer in an Aquatic Food Chain and its Effects on Fathead Minnow Larvae[1]

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Abstract. The transfer of Se (Na_2SeO_4) was followed through a laboratory food chain (water-algae-rotifer-larval fish) and its effect on larval fathead minnows (*Pimephales promelas*). Selenium transfer between algae (*Chlorella pyrenoidosa*) and rotifers (*Brachionus calyciflorus*) was a function of time and food availability. Selenium concentrations in the rotifers ranged from 46 to 91 μg Se · g^{-1} dry weight after 5 hr of feeding. Selenium concentrations (±SD) in larval fish reached 61.1 ± 1.1 μg Se · g^{-1} dry weight for 9 day-old larvae and 51.7 ± 1.6 μg Se · g^{-1} for 17 day-old larvae after 7 and 9 days of feeding with Se-contaminated rotifers, respectively. Final dry weights of larvae fed Se-contaminated rotifers were significantly lower than those of controls, although acute toxicity (mortality) was not demonstrated. The biological half-life of food-derived Se in the larvae was 28 days.

Selenium in the aquatic environment comes from the weathering of selenite-containing shale deposits and soils (Bertine and Goldberg 1971; National Research Council 1976), combustion of fossil fuels (Andren and Klein 1975; Gutenmann *et al.* 1976), metal smelting and refining (Demayo *et al.* 1979) and leaching from agricultural areas (Gissel-Nielsen and Gissel-Nielsen 1978). Selenium has both beneficial and toxic effects depending on conditions. In trace amounts, Se serves as an essential component of the glutathione peroxidase enzyme system (Rotruck *et al.* 1973). At higher concentrations, inorganic Se follows sulfur biochemical pathways in bacteria and plants becoming incorporated into selenomethionine and selenocysteine and, ultimately, into proteins (Wrench 1978; Bottino *et al.* 1984). Selenium toxicity resulting from high Se exposures is well documented; however, the mechanisms of toxicity have not been elucidated.

Selenium passes through the food web primarily in an organic form (National Research Council 1976), whereas waterborne Se is primarily inorganic (Cutter 1982). While the relative toxicity of organic vs inorganic Se is poorly defined, Se bioaccumulation is most pronounced when administered through the food (Sandholm *et al.* 1973; Turner and Swick 1983) or in organic form (Kleinow and Brooks 1986). In fish, the early life history stages are the most sensitive to toxic agents (Benoit *et al.* 1982). The objectives of this study were to 1) construct a reliable system to produce algae and rotifers with varying Se concentrations for use as larval fish food, 2) study the transfer of waterborne Se to larval fish via a laboratory food chain and 3) observe the biological effects of Se on larval fish.

Materials and Methods

The alga *Chlorella pyrenoidosa*, the rotifer *Brachionus calyciflorus* and larvae of the fathead minnow *Pimephales promelas* were used. Time courses of Se uptake in rotifers given high and low algal biomass concentrations, of Se uptake and depuration in fish larvae, and Se food chain transfer and Se effects on 2 and 8 day-old fish larvae were measured. All measurements of biomass and Se concentrations were expressed in terms of dry mass.

Algae were supplied from batch cultures grown at room temperature, mixed by filtered air and exposed to continuous cool-white fluorescent light at 100 μE · m^{-2} · sec^{-1}. Culture medium

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starting from distilled deionized water contained 2×10^{-3}M $NaNO_3$; 4×10^{-5}M Mg SO_4; 2×10^{-4}M $CaCl_2$; 5.5×10^{-5}M K_2HPO_4; 5 μg · L^{-1} vitamin B_{12}; and 0.1 mL · L^{-1} Hutner's trace element solution (Hutner *et al.* 1950). When algal concentrations in experimental cultures reached ~400 μg dry mass · mL^{-1}, Se concentrations were increased to 2.5 μg Se · mL^{-1}. In order to achieve maximal Se transfer from medium to algae without producing acute toxicity, the S:Se ratio in all cultures was maintained at 0.44 (Shrift 1954). The cultures were allowed 3 days to reach Se equilibrium with the medium. Selenium-75 (as $H_2{}^{75}SeO_4$, New England Nuclear) was used as a radioactive tracer.

Algal selenium concentrations (μg Se · g^{-1} dry mass algae) were calculated from ^{75}Se activity and algal dry mass obtained through the use of a dry mass-biovolume conversion factor of 0.57 g dry algal mass · cm^{-3} fresh algal cell volume (Boraas 1983). Biovolumes were measured by an electronic particle counter (Particle Data Celloscope).

Rotifers used in each experiment were supplied by a 3-stage chemostat system (Boraas 1983; Bennett 1984), which was inoculated with adult females obtained from existing cultures. The first stage consisted of three 14 L algal culture vessels, which fed a second stage that consisted of one 14 L rotifer culture vessel. Overflow from the rotifer vessel (stage 2) was collected in a third stage. Rotifers were harvested, washed and resuspended in 50 mL of control medium before use in a given experiment. Rotifer biomass was calculated by multiplying numbers per mL by 0.17 μg · $rotifer^{-1}$, which assumes rotifer guts to be half full of algae (Boraas 1983).

Selenium uptake by rotifers was measured with two ratios of algae to rotifers: 25:50 and 57:240 μg algae · mL^{-1} to μg rotifer · mL^{-1}. 500 mL suspensions were continuously agitated in the dark for 5 hr. The rotifers were separated from the algae by filtering 40 mL samples through a 57 μm mesh Nitex screen. These rotifers were washed and immediately heat killed at 50°C to reduce metabolic Se losses. Replicates of this suspension were filtered by suction through pre-weighed 10 μm Nuclepore polycarbonate filters and washed. Filtered rotifers were counted for ^{75}Se activity, dried for 24 hr at 60°C, and weighed to allow determination of μg Se · g $rotifer^{-1}$ (including mictic and amictic eggs).

Selenium uptake by larval fish was measured in three experiments. Se-contaminated and control rotifers for feeding to larval fish were prepared as described above at the low algae:rotifer ratio. Rotifers were allowed to feed for 5 hr before being harvested. Daily, equal volumes of rotifers were divided among five 800 mL polypropylene larval chambers. Three chambers received Se-contaminated rotifers and two received controls. Each chamber contained 500 mL of Lake Michigan water (as Milwaukee, WI dechlorinated tap water with <0.001 μg Se · mL^{-1}). At the initiation of an experiment either 47, 14 or 13 fish larvae were placed in the chambers for experiments 1, 2, and 3, respectively. Selenium losses from heat-killed rotifers were measured in a separate chamber without fish larvae.

Fish larvae were obtained from breeding pairs maintained in the laboratory. After hatching, active larvae to be used in the Se uptake and depuration experiment (experiment 1), were divided equally among the larval test chambers. Larvae were initially fed rotifers raised on control algae. At 4 day post-hatch, test larvae were fed Se-contaminated rotifers for 7 days and then returned to a control diet for an additional 19 days. Control larvae received control rotifers throughout. Daily, larvae of a given replicate were removed from their chamber, washed, placed in a 20 mL vial, and counted alive for 20 min to determine ^{75}Se activity.

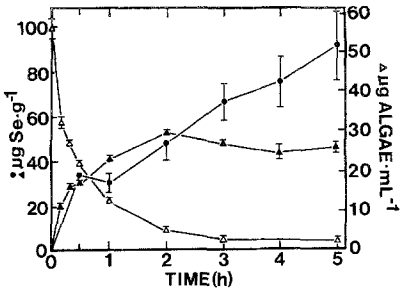

Fig. 1. Time course of the uptake of Se by rotifers from algae at high (●) and low (▲) initial algal:rotifer biomass ratios. Selenium concentrations expressed on dry mass basis. (△) Residual algal concentrations over the low algae:rotifer ratio time course. Data expressed as means ± SD

All larvae were then placed in chambers with fresh food rations. At termination of the experiment, dry mass was determined for each larva. The dry mass of larvae at time intervals prior to termination was extrapolated from an age-log dry mass plot constructed from measured initial and final dry mass data (Bennett 1984). Larvae in experiment 2 were fed uncontaminated rotifers until 8 day post-hatch. Test groups then received Se-contaminated rotifers for 9 days. On day 19 the larvae were removed from their chambers, washed, counted for ^{75}Se activity, dried and weighed. Experiment 3 larvae were treated in a similar manner, except administration of Se-contaminated rotifers began 2 days post-hatch and continued for 7 days before termination.

Results

The selenium in rotifers in the low algae to rotifer ratio experiment reached a concentration of 45 μg Se · g $rotifer^{-1}$ after 2 hr of feeding and remained steady at ~40 μg Se · g $rotifer^{-1}$ (Figure 1). Algal biomass decreased exponentially during the course of this experiment from 57 ± 3 to 5.1 ± 0.6 μg · mL^{-1} after 2 hr (Figure 1). Selenium uptake in the high algae:rotifer experiment continued until termination of the experiment. Initial Se concentrations of the algae were 3,100 μg Se · g^{-1} and 2,600 μg Se · g^{-1} for high and low algae:rotifer ratio experiments, respectively. All error terms noted here and below are ±1 standard deviation.

Selenium uptake by larval fish feeding on Se-contaminated rotifers was 1.46 ng Se · $larva^{-1}$ · day^{-1} (including gut contents) during the first 4 days and remained steady during the next 3 days (Figure 2). When control rotifers were provided as food, day 7 through day 28, the Se depuration constant was -0.025 · day^{-1}.

Mean selenium concentrations in the larvae reached a peak of 51.5 ± 2.8 μg Se · g^{-1} on day 4 followed by a gradual decrease in Se concentration, even though the Se content per larva was still in-

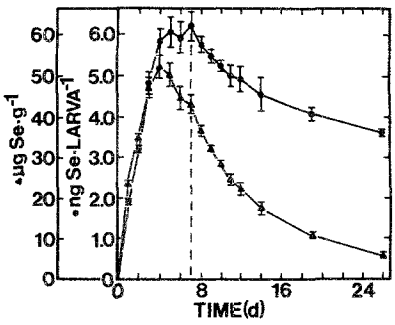

Fig. 2. Uptake and depuration of Se by test larvae. (▲) Dry mass Se concentration of the larvae. (●) Mean selenium content per larva. Dashed line indicates time when administration of Se-contaminated food was ended and control food begun (day 7). Data are expressed as means ± SD

creasing (Figure 2). Selenium content and concentration of Se in the larvae at the end of Se-contaminated food exposure are given in Table 1. Rotifer selenium concentration in this experiment, estimated from algae:rotifer ratios of feeding suspensions, was at least 70 µg Se · g^{-1}. Mean dry masses of test larvae at experiment termination were shown by 1-way ANOVA (Sokal & Rohlf 1981) to be significantly different from controls (Table 1).

During experiment 2, where larvae received Se-contaminated rotifers 8 days after hatch, the mean algal Se concentration was 3,170 ± 120 µg Se · g^{-1}. The mean Se concentration of the rotifers over the 9-day feeding period, along with Se content and concentration in test larvae at experiment termination, are given in Table 1. The mean dry mass of test larvae at experiment termination were significantly different from controls (Table 1). During the 9-day period, the mean values for waterborne Se leaching from dead rotifers and arising from larval depuration were 0.84 ± 1.6 × 10^{-3} µg Se · mL^{-1} for chambers containing only rotifers and 1.1 ± 0.3 × 10^{-3} µg Se · mL^{-1} for chambers containing both rotifers and larvae.

The results of experiments in which larvae received Se-contaminated rotifers 2 days after hatch (experiment 3) are summarized in Table 1. Mean selenium concentration in the larvae at experiment termination was shown by Chi-square analysis (Sokal and Rohlf 1981) to be significantly different than Se concentrations in experiment 2 larvae. Mean dry mass of test and control larvae were significantly different at the 90% confidence level but not at the 95% level.

Discussion

Mean Se concentrations for algae in all experiments confirmed values predicted from Shrift's data using *Chlorella vulgaris* and a medium with a similar S:Se ratio. This ratio is important because S and Se are competitive antagonists and their relative proportions can greatly influence S-Se uptake kinetics (Shrift 1954).

Transfer of Se between algae and rotifers was dependent on the algae:rotifer ratio (Figure 3). Rotifers in the high algae:rotifer experiment showed a steady increase in Se concentration over the 5 hr feeding period. The Se concentration in rotifers grown for experimental food chain studies can, therefore, be controlled by either feeding time (Figure 1) or algae:rotifer ratio (Figure 3). Initial Se uptake during the first 20 min can be attributed to the replacement of control algae by Se-contaminated algae in rotifer guts. This time period is equal to gut passage times observed for *B. calyciflorus* by Starkweather and Gilbert (1977). The increase of Se concentration after 20 min must, therefore, be due to bioaccumulation within the rotifers. Rotifers actively feeding on Se-contaminated algae were lethargic after a few hours compared to controls at all algae:rotifer ratios and there was observational evidence of decreased feeding activity.

Selenium uptake by the fish larvae (Figure 2) may have reached equilibrium with Se levels supplied through the food. The food supply was sufficient to sustain growth of the larvae, as shown by the increase in larval mass and the concomitant decrease in Se tissue concentration. However, the rate of Se uptake was not sufficient to facilitate increased Se accumulation. A similar plateau in Se concentration was observed by Bertram and Brooks (1986), using adult *P. promelas*. The plateau they observed was caused by a decrease in Se-contaminated food availability, an observation similar to that found for rotifer experiments in the present study.

The Se depuration rate constant for the larvae ($-0.025 \cdot d^{-1}$) was faster than that observed for adult *P. promelas* ($-0.014 \cdot d^{-1}$) given Se-contaminated food ranging in concentration from 1.33 to 7.32 µg Se · g^{-1} (Bertram and Brooks 1986). This difference was possibly due to differences in food Se concentrations, as shown by Hilton *et al.* (1982) that depuration rates increase with increasing dietary Se levels. Differences between larval and adult physiology and metabolic rate may also be contributing factors. The faster decline in Se concentration per unit biomass over total Se content per individual can be explained by Se dilution by larval growth.

In larval experiments 2 and 3, test larvae received food of nearly the same Se concentration, but administration was initiated at different post hatch ages, 8 days and 2 days respectively. Faster

Table 1. Summary of data pertaining to larval fish experiments 1, 2 and 3. Where applicable, data are expressed as means ± SD

Expt.	Mean rotifer Se conc. ($\mu g \cdot g^{-1}$)	Approximate daily larval food ration (μg rotifer $\cdot$ larva^{-1})	Mean initial[a] larval dry wt (μg)	Mean final larval dry wt (μg) control	test	Mean final larval Se content (ng Se $\cdot$ larva^{-1})	Mean final larval Se concentration ($\mu g \cdot g^{-1}$)
1	>70	50	90[b]	1470 ± 160	800 ± 70[d]	6.2 ± 0.4[c]	43.0 ± 2.9[c]
2	68 ± 9	1330	400[b]	1888 ± 897	1354 ± 545[d]	70.0 ± 4.9	51.7 ± 1.6
3	55 ± 10	1190	100 ± 6	475 ± 154	416 ± 118	24.8 ± 2.9	61.1 ± 1.1[e]

[a] Time when feeding on Se-contaminated rotifers was initiated
[b] Extrapolated from age-log dry mass regression
[c] Values when feeding of Se-contaminated food was ended (day 7)
[d] Significantly different ($P < 0.05$) from control
[e] Significantly different ($P < 0.01$) from experiment 2 value

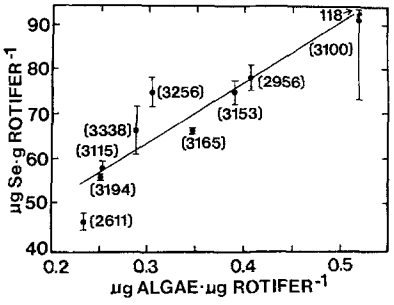

Fig. 3. Compiled data showing the relationship between the ratio of initial algal:rotifer biomass and the Se concentration in rotifers after 5 hr of feeding. The Se concentrations per algal dry mass provided to the rotifers are indicated in parentheses. Data are expressed as means ± SD

Se uptake and higher Se content in experiment 2 larvae was due to their larger size and ability to consume more rotifers per unit time. Although the larger larvae used in experiment 2 received Se-contaminated food for a longer period of time, final mean Se concentration was significantly lower ($p < 0.01$) than the smaller larvae which were offered Se-contaminated food 2 days post-hatch (experiment 3). Several other studies involving trace element bioaccumulation have also found this concentration-weight relationship (Boyden 1974; Fowler and Benayoun 1976). These results could be due to larger larvae containing proportionately more muscle tissue (a poor Se accumulator), proportionately less liver and kidney tissue (Se accumulators) (Turner and Swick 1983; Hodson *et al.* 1980; Kleinow and Brooks 1986), or development of more efficient Se elimination systems. In terms of food availability, the food ration on a μg rotifer $\cdot$ μg larva^{-1} basis was lower for the larger larvae and may have contributed to the lower Se concentrations.

Although no mortalities were attributed to Se toxicity, Se-exposed larvae were significantly smaller ($p < 0.05$) in mass than controls for two of three experiments. This has also been observed in adult fish (Hilton *et al.* 1980, 1982). Lower mass could be attributed to malnourishment, *i.e.*, the larvae did not consume adequate amounts of Se-contaminated rotifers due to unpalatability (Underwood 1971), or to Se toxicity. Hilton *et al.* (1980), using selenite-contaminated food and rainbow trout, reported a chronic dietary Se toxicity level between 3 and 13 μg Se $\cdot$ g^{-1}. However, the values pertain to an inorganic Se moiety and may not be comparable to food web studies.

Comparisons of Se toxicity between waterborne, inorganic forms and organic, food-derived forms, results in different conclusions (Niimi and LaHam 1976). For example, acute toxicity was not demonstrated in the present study with larvae consuming food with 55-68 μg Se $\cdot$ g^{-1}. However, with the same fish species, mortalities have been demonstrated to occur within a week at waterborne Se levels less than 10 μg Se $\cdot$ mL^{-1} (Cardwell *et al.* 1976; Halter *et al.* 1980). Boyum (1984), using *Chlamydomonas* and *Daphnia*, demonstrated that Se administered via the food decreased the toxicity of waterborne Se. Organically bound Se apparently is less toxic than inorganic Se on a concentration basis.

A major problem confronting larval fish toxicity studies with food vectors is the inability to prepare naturally derived contaminated food sources which contain toxicants in a form palatable to the fish. Through procedures employed in this study, it has been demonstrated that these problems can be overcome. The use of a water-algae-rotifer culture system is satisfactory for the preparation of Se-contaminated larval fish food. These procedures may also be useful for studies of other toxic substances.

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