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Decreased Survival and Teratogenesis During Laboratory Selenium Exposures to Bluegill, *Lepomis macrochirus*

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Selenium from coal ash disposal has been associated with declines of fish populations in a power plant cooling reservoir (Cumbie and Van Horn 1978). Field studies suggested these declines were caused by the effects of excessive selenium on survival and reproduction. Bluegill (*Lepomis macrochirus*) embryo-larval studies using eggs of feral fish from a selenium-contaminated reservoir found low larval survival and teratogenesis when ovarian selenium concentrations were high (Carolina Power & Light 1985; Gillespie and Baumann 1986). In these cases selenium bioaccumulated in the food web, resulting in exposure of the adult fish to both dietary and waterborne sources of selenium. Since dietary sources supply most of the selenium that accumulates in fish (Sandholm et al. 1973), we hypothesized that reproductive impairment could occur if the dietary concentrations were sufficiently elevated and the chemical form of selenium was equivalent to the form experienced by the fish in the environment. This hypothesis was studied in a partial life cycle test by comparing the effects of chronic dietary exposure of selenomethionine (SeMet) and selenite (Se^{4+}) to bluegill sunfish. We also tested one combination of dietary SeMet and waterborne Se^{4+}. SeMet was selected as the organic form because in a preliminary study its accumulation was most similar to that of naturally occurring selenium in bluegill (Woock et al. 1984). Se^{4+} was selected as the inorganic form because it is the predominant oxidation state in some contaminated cooling reservoirs (Cutter 1986).

We demonstrate that for larval bluegill (1) elevated dietary selenium exposure to parent fish causes teratogenesis and decreases larval survival, (2) parental dietary organoselenium is more toxic than dietary inorganic selenium, and (3) the combination of parental dietary, plus waterborne exposure, is more toxic than dietary exposure alone.

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MATERIALS AND METHODS

Parent bluegill (P1) were obtained from Windmill Hatchery (Kernersville, North Carolina) as juveniles (1 to 16 g, average 5.1 g wet wt.) and were stocked approximately 100 per 450-L glass tank. Five replicate tanks were stocked for each treatment. The bluegill were acclimated 7 weeks while being fed a control diet.

Water temperature and photoperiod were controlled to simulate North Carolina seasonal changes. Average weekly temperatures ranged from 7.4°C (winter) to 28.8°C (summer). Corresponding light periods were from 9 to 16 h. The first summer period was extended 3 months to maximize growth so that larger fish would be available for the spawning experiments. During spawning experiments, water temperature was held at 28±2°C and light period at 14.25 h/d.

The water for the study was from Harris Lake (Wake County, North Carolina), which is an instream impoundment supplying makeup water for a power plant cooling tower. To assure consistent quality, the water was passed through 2 sand-and-gravel filters followed by 2 activated carbon columns. P1 tanks and embryo-larval (F1) cups (320 mL, polyethylene) received the filtered water continuously at rates exceeding 4.8 tank volumes per 24 h. Air was provided to the P1 tanks. Chemical quality of the water was routinely monitored and averaged: hardness, 16 mg/L $CaCO_3$; alkalinity, 14 mg/L $CaCO_3$; total organic carbon, 6.7 mg/L; dissolved solids, 46 mg/L; sulfate, 5.2 mg/L; pH, 6.5; and conductivity, 57 µmhos/cm. Dissolved oxygen ranged 3.4-12.5 and 6.3-7.6 mg/L in P1 tanks and embryo-larval cups, respectively.

Each P1 tank with its associated embryo-larval cups was serviced by a single mixing vessel. Se4+ stock solution (Na_2SeO_3 in deionized water) was delivered to the appropriate mixing vessels to provide a nominal selenium concentration of 10 µg/L. Measured total waterborne selenium averaged 10.2 µg/L (n = 116, SD = 2.2) in the five tanks receiving Se4+ and < 1 µg/L (n = 24, maximum = 1µg/L) in the others. Two speciation analyses showed that Se4+ was the predominant (> 99%) oxidation state of selenium in the P1 tanks.

The diets of the P1 were a trout chow (Zeigler Brothers, Gardners, Pennsylvania) supplemented during manufacture with either dl-SeMet or Na_2SeO_3. Purity of SeMet was 90%-99% (Sigma Chemical Company, St. Louis, Missouri). The fish chow contained 3728 kcal/kg and consisted of (w/w) 8.0% moisture, 40.0% protein, 2.3% crude fiber, 8.7% fat, 9.6% ash, and 33.7% carbohydrates.

Food samples were digested for selenium analysis in perchloric, sulfuric, and nitric acids. Selenium from water samples and diet digests was measured by hydride generation atomic absorption spectroscopy following the analytical procedures of Cutter (1986).

Recovery of selenium in analytical tissue standards (Kodak® TEG-50-B gel; NBS bovine liver SRM 1577-A) ranged from 84.5% to 120% (n = 111) and in water standards ranged from 100% to 105% (n = 251). Precision of these measurements on individual tissue and water standards ranged from 0% to 13.1% relative standard deviation.

Table 1 shows the selenium treatments. The concentrations were in the range reported for food web components in contaminated aquatic systems (Cumbie and Van Horn 1978; Woock and Summers 1984). Exposures, which continued for 323 d, were initiated by switching from the control diet to the treatment diets and starting delivery of Se4+ solution to the appropriate mixing vessels. The daily feeding rate was the same for all treatments but was varied from 5% to 0.25% of mean body weight, being adjusted as necessary when water temperature changes influenced consumption. The mean weight of fish in each treatment was estimated initially and on days 15, 34, 62, 89, and 182 by weighing 5% to 9% of the fish in each treatment. Fish were not fed at least 16 h prior to sampling and were weighed fresh to nearest 0.1 g. On day 259 all remaining fish except those selected for spawning experiments were weighed. Mortalities and behavioral observations were recorded daily.

Table 1. Treatments and selenium concentrations in test diets.

Treatment	Nominal conc. µgSe/g	Measured conc. µgSe/g‡
Control	< 1	0.79 (0.77-0.87)
Dietary SeMet	3	3.6 (3.1-4.0)
Dietary SeMet	13	13 (12-15)
Dietary SeMet	30	30 (27-32)
Dietary SeMet plus aqueous Se4+ @ 10 µg/L	13	13 (12-15)
Dietary Se4+	13	14 (13-15)
Dietary Se4+	30	31 (28-35)

‡Means (range) for 3 batches of diets prepared during the study.

Spawning experiments were initiated following 260 d of P1 exposure. Groups of 7 fish containing approximately 5 females and 2 males were selected and returned to their original tanks (excess fish were removed). The spawning and embryo-larval procedures were similar to Eaton (1970). Clay saucers (25.4-cm diameter) were substituted for Eaton's concrete artificial spawning nets, and embryo-larval cups were placed in separate F1 chambers instead of in the P1 exposure tanks. The clay saucers were readily utilized by the bluegill as spawning beds. Saucers were examined at least twice daily for 38 d for presence of fertilized eggs. Nine to twenty sets of fertilized eggs per treatment were tested. For

percent hatch determinations, 150 fertilized eggs (50 per embryo-larval cup) were selected from each set of fertilized eggs that was tested. After hatching, larvae were counted, examined for deformities, and returned to the embryo-larval chamber. Larval survival and malformations were counted at the swim-up stage, i.e., after yolk absorption when the larvae entered the water column in search of food. No attempt was made to feed the larvae. Larval survival in this report is defined as the percentage of the larvae that swam up since other larvae that survived for a time lying on the substrate, but did not swim up, were usually severely malformed and eventually died. All live larvae were examined for malformations; dead larvae were usually too decomposed to reliably examine.

P1 cumulative mortality data were normalized for periodic removals of live fish for other studies. The data were normalized to "fish days" by multiplying the number of fish left in each tank after a sampling period by the number of days in each interval between removals. The number of mortalities was divided by the sum of fish days obtained for each interval, giving mortalities in total fish days of exposure. We also transformed adult fish lengths and weights ($\log_e$) and hatch and larval survival ratios (arcsin square root) for one-way ANOVA and Duncan's multiple range test to determine differences among treatments. Differences were considered significant at alpha = 0.05.

RESULTS AND DISCUSSION

Effects of chronic selenium exposure on the parent bluegill are summarized in Table 2. Final, cumulative mortalities were not significantly different (Table 2) in the 30 µg/g SeMet and Se4+ treatments but significantly exceeded those seen in the lower exposure concentrations. Mortalities began first in the 30 µg/g Se4+ group, then increased rapidly in both 30 µg/g treatments after 120 d and diminished after 200 d. Dying fish often, but not always, exhibited clinical symptoms including food aversion, edema, lethargy, melanism, tetany, and erratic, spiral, or circular swimming. These signs were also noted occasionally in fish receiving the 13 µgSe/g diets.

Opaqueness of both eye lens (true lens cataracts) was noted after 15 d in P1 bluegill being fed the highest SeMet diet but was not noted in other treatments. By 260 d, cataracts were noted in 37% of the affected group (Table 2). Mean final body weights and lengths of the P1 bluegill (prior to spawning) were significantly lower in the 30 µg/g Se4+ treatment (Table 2; only weight data given). The P1 bluegill which were exposed simultaneously to selenium in their water and food had no significant differences in weight, cataracts, or mortalities compared with dietary selenium exposure alone (Table 2).

Parental selenium exposure significantly affected mean larval survival, whereas percent hatch was not significantly affected (Table 3). At comparable levels in the parents' diet, SeMet was more toxic to the larvae than Se4+. None of the larvae from

Table 2. Final body weight (g), percent occurrence of lens cataracts, and cumulative mortalities per 10,000 fish days in the parent bluegill after 260 d dietary selenium exposure. Data are the mean and range of all fish in a treatment for weight and are mean and range of replicate tank values for cataracts and mortalities. Treatment means followed by different letters differ significantly, $p < 0.05$.

Nominal conc., µgSe/g	Weight, g			Cataracts, %			Mortalities		
	N	Mean	Range	N	Mean	Range	N	Mean	Range
< 1	261	19.1a	(2.9-117)	5	0	(0)	5	0.5a	(0-0.9)
SeMet									
3	263	17.1a	(2.9-139)	5	0	(0)	5	0.4a	(0-1.0)
13	268	17.3a	(2.9-138)	5	0	(0)	5	0.7a	(0-1.4)
13¶	223	17.6a	(2.2-125)	5	0	(0)	5	0.9a	(0-1.9)
30	211	16.4a	(3.1-125)	5	37	(24-46)	5	5.1b	(0.9-9.5)
Se4+									
13	266	17.1a	(2.7-129)	5	0	(0)	5	1.1a	(0-2.4)
30	188	14.0b	(2.2-145)	5	0	(0)	5	4.6b	(2.4-7.4)

¶Also exposed to waterborne Se4+, 10 µgSe/L.

parents that were fed the SeMet diet at 30 µgSe/g swam up, while, on average, 75% of larvae from parents fed the 30 µg/g Se4+ diet survived (Table 3). A much greater decrease in mean larval survival occurred between 13 and 30 µg/g of the SeMet diet compared with the decrease between 13 and 30 µg/g of the Se4+ diet (Table 3). Also, except for the 30 µg/g SeMet treatment, variability of larval survival was great among individual spawns (Table 3).

The variability in response within exposure concentrations may be partly related to the amount of selenium accumulated by the female parent and transferred to the egg. A similar relationship was suggested in studies with feral fish containing elevated selenium (Carolina Power & Light 1985; Gillespie and Baumann 1986).

Additional exposure of the P1 and the F1 to waterborne Se4+ significantly lowered survival of the larvae compared with those larvae from parents fed the comparable diet without waterborne exposure (Table 3).

The occurrence of terata in larvae was also related to treatment, affecting 100% of larvae of parents in the highest SeMet treatment (Table 3). These larvae always exhibited edema and usually lordosis and lower jaw gape (Figure 1). A few also exhibited scoliosis or kyphosis. Essentially all larvae that did not swim up in the other treatments, but were alive at the time swim-up counts were made, exhibited one or more terata. The number of multiple terata in individual larva generally decreased with decreased selenium concentration in the parents' diet. Usually less than 5% of the larvae that swam up had deformities, with no apparent differences among treatments.

Table 3. Reproductive effects following dietary selenium exposure of parent bluegill for 287-324 d. Data are the mean and range of all spawns tested within each treatment. Treatment means followed by different letters differ significantly, $p < 0.05$.

Nominal conc., µgSe/g	% Hatch			% Larval survival			% Larvae with terata		
	N	Mean	Range	N	Mean	Range	N	Mean	Range
< 1	12	87a	(68-95)	12	95a	(89-99)	12	2	(0-6)
SeMet									
3	20	87a	(63-98)	20	93a	(60-100)	20	3	(0-28)
13	18	85a	(49-95)	18	81ab	(33-97)	18	10	(0-56)
13¶	9	82a	(68-95)	10	41c	(0-94)	10	50	(3-100)
30	12	87a	(70-99)	13	0d	(0)	13	100	(100)
Se4+									
13	12	82a	(65-95)	13	93a	(77-98)	13	2	(0-5)
30	10	82a	(58-91)	11	75b	(2-99)	11	15	(1-97)

¶P1 and F1 also exposed to waterborne Se, 10 µgSe/L.

Some of the effects we noted in the P1 bluegill have been reported in other studies. Accounts exist of "cloudy lens" in fish from selenium-contaminated reservoirs, and Se4+ injection is known to induce cataracts in rats (Ostadalova et al. 1978). However, this is the first report documenting cataracts in laboratory fish exposed to selenium.

Chronic mortality, decreased body weight, and clinical symptoms similar to those we noted in the P1 bluegill were reported for trout fed Se4+ in dry pellet diets at 10-13 µgSe/g (Goettl and Davies 1978; Hilton et al. 1980). Finley (1985) reported mortality in 3 of 4 bluegills in 44 d of feeding mayfly nymphs containing 13.6 µgSe/g from a contaminated reservoir. In our study, significant mortalities occurred only at dietary concentrations greater than 13 µgSe/g. Comparison of results between studies suggests that trout may be more sensitive than bluegill to high dietary selenium because one chemical species and the type of diet were similar in our study and those of Goettl and Davies (1978) and Hilton et al. (1980), yet concentrations causing significant mortality were higher for the bluegill in our study.

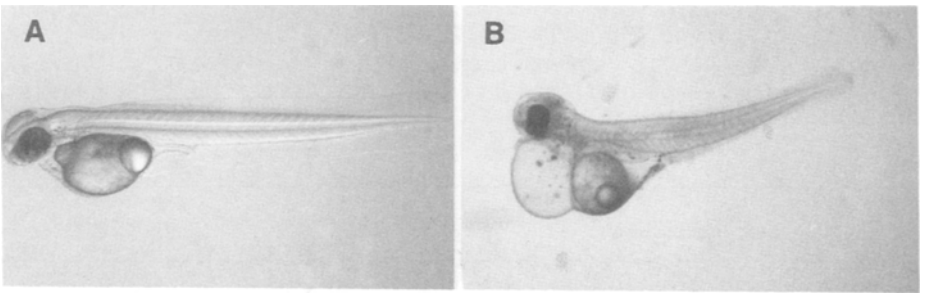

Figure 1. Bluegill larvae 4 days post hatch. A: Normal larva from control parents. B: Malformed larva from parents fed selenomethionine at 30 μgSe/g diet.

In comparing Finley's (1985) data to ours, differences in dietary concentrations causing mortality may be partly attributable to the amount of protein ingested by the fish. Several studies have shown that increased protein quantity and quality decreased selenium toxicity (see review in National Research Council 1976). Though mortalities were lower in our study, we calculate that Finley's bluegill received slightly lower selenium doses (≈ 0.57-0.81 μgSe/g • fish/d) than our bluegill in the highest SeMet and Se4+ diets (≈ 1.7 μgSe/g • fish/d during the first few weeks of the study). However, the ratio of ingestible selenium per ingestible protein of our highest SeMet and Se4+ diets was only 75 μgSe/g protein compared to 194 μgSe/g protein of the mayfly nymphs. Thus, the relatively greater quantity of protein per unit of selenium in our diets may have ameliorated selenium toxicity compared with toxic effects which may occur with selenium-contaminated natural foods of bluegill.

These are the first reproductive effects reported in which larval fish survival and teratogenesis have been related to known dietary selenium exposure of the parent fish. Gillespie and Baumann (1986) reported similar results in field studies; however, parental selenium exposure was unknown. Niimi and LaHam (1975) found no embryo mortality but increased larval mortality of zebrafish when the larvae were directly exposed to waterborne selenium ≥ 3 mg/L. Conversely, Hodson et al. (1980) reported no effect on swim-up larvae but a significant small mortality of eyed trout eggs exposed to waterborne selenium ≥ 28 μg/L. Our data indicate that dietary sources of selenium and the combination of dietary and waterborne selenium at elevated environmental levels may severely affect survival of young spawned by exposed adult fish.

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