Bull. Environ. Contam. Toxicol. (1988) 40:771–778

Bulletin of Environmental Contamination and Toxicology

Dietary Exposure of Bluegills (*Lepomis macrochirus*) to (75) Se: Uptake and Distribution in Organs and Tissues

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Under natural conditions fish concentrate selenium (Se) in visceral tissues, attaining concentration factors (tissue Se/water Se) of 590-35,000 (Lemly 1985). Food chain bioaccumulation is an important route of Se exposure for fishes (Sandholm et al. 1973; Nassos et al. 1980; Rudd et al. 1980; Carolina Power and Light Company 1984), and organic forms may be more readily concentrated than inorganic forms (Sandholm et al. 1973; Sharma and Davis 1980). Fish concentrate Se in liver, kidney, and heart under laboratory conditions (Adams 1976; Hodson et al. 1980; Hilton et al. 1982; Lemly 1982).

High Se concentrations in bluegills (Lepomis macrochirus) are associated with decreased reproduction in Se-enriched reservoirs, and Se appears to concentrate in the ovaries of these fish (Cumbie and VanHorn 1978; Baumann and Gillespie 1986). Furthermore, Se may be a teratogenic agent causing abnormalities in bluegill larvae (Gillespie and Baumann 1986), and high Se concentrations in the ovaries of female bluegills may be responsible for these abnormalities. Therefore, we proposed to compare the accumulation of (75) Se in gonads with its accumulation in other tissues and organs to assess the deposition of Se in bluegills.

MATERIALS AND METHODS

Adult bluegills were purchased from Fender's Fish Hatchery, Baltic, Ohio, and acclimated in aerated municipal drinking water for at least 2 wk before experiments began. Bluegills were isolated individually in 20-L aquaria and conditioned to feed on mealworms (Tenebrionidae) for at least 1 wk before treatment. About half the water in each tank was replaced weekly; all solid waste materials were siphoned off as required.

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A microliter syringe was used to inject aqueous L-(75) Se-selenomethionine (purchased from Amersham International, Arlington Heights, Illinois) into mealworms immediately before they were fed to the bluegills. We also fed these same bluegills uninjected mealworms to achieve a daily diet of 1-2% of body weight (Carlander 1977).

Bluegills (N= 16; 57-138 g, wet wt) were fed three mealworms per week that were injected with 1 uL of (75) Se-selenomethionine (specific activity = 7.4 mCi/mg). This was done to ensure adequate gamma activity for detection without causing toxicity. After 6, 8, 10, and 12 wk, four fish were randomly selected, anesthetized with quinaldine, and dissected. One to three tissue samples each of skeletal muscle, heart, gonad, liver, and blood from the pericardium (8 wk exposure only) were collected from each fish and extracted overnight in 70% ethanol. The ethanol was removed, tissues were dissolved in 1% sodium lauryl sulfate, and 20 uL of each sample were assayed for total protein and 1 mL for (75) Se activity. Activity was also determined for one sample each of muscle, heart, ovary, and liver in 70% ethanol. To estimate the amount of isotope lost to unincorporated products such as carbohydrates and amino acids, we measured activity in the ethanol remaining after extraction at 4 wk of exposure. Additionally, samples of muscle, heart, liver and gonads were dissected from bluegills not dosed with (75) Se, extracted in 70% ethanol overnight, weighed, and dissolved in 1% sodium lauryl sulfate; 20 uL were then assayed for total protein.

Total protein determinations were made by using a modified Lowry's method (Markwell et al. 1978) with a known standard of bovine albumin (100 ug/mL). Activity of (75) Se was measured with a Princeton Gamma-Tech, lithium drifted, germanium detector equipped with a Canberra Model 8180 multichannel analyzer. Spectra were analyzed with a PDP-11/05 minicomputer interfaced with the multichannel analyzer and programmed for direct determination of isotope activity, with corrections for decay. The activity of (75) Se was expressed as uCi/g protein. One-way analysis of variance (ANOVA) and a multiple comparison procedure (Duncan's multiple range) were used to determine statistical differences of (75) Se activity for each tissue type at different sampling times over the duration of exposure.

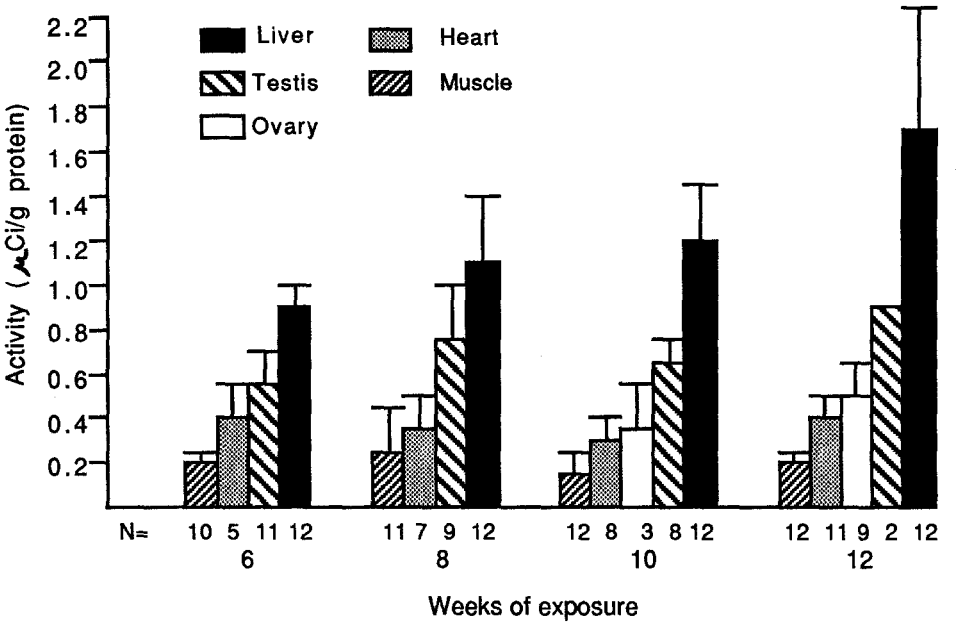

Figure 1. Mean activity of (75) Se for tissues of bluegills fed (75) Se-selenomethionine for 6 to 12 wk. Standard deviations are represented by brackets; NC= not calculated; N= number of tissue samples analyzed from four bluegills.

RESULTS AND DISCUSSION

Uptake of (75) Se varied among tissues and through time (Fig. 1). More isotope was accumulated by liver than by any other tissue (range of mean activity 0.89 to 1.66 uCi/g protein). Testes accumulated more isotope (0.55 to 0.90 uCi/g protein) than any other tissue except liver, whereas ovary, heart, blood, and skeletal muscle accumulated the least (0.19 to 0.51 uCi/g protein). Mean isotope activity in blood samples at 8 weeks of exposure was relatively low (0.30 uCi/g protein). Activity increased in the liver (ANOVA, $p<0.05$) and testis[1] over the 12-week exposure period, whereas that in skeletal muscle and heart remained nearly constant (ANOVA, $P>0.05$).

The activity of (75) Se for gamma assays was normalized to total protein because of the tendency of protein to

[1] Gamma activity in testis could not be analyzed statistically at 12 weeks because only two males were collected.

bind Se (Schultz et al. 1980; Reilly et al. 1984). If this activity had been normalized by using the weight of tissue, the relative distribution of (75) Se activity would have differed. Protein per unit weight was lowest (9-10%) in liver and ovary and highest (30%) in testis (Table 1). Muscle and heart were similar in percent protein.

Ethanol-soluble materials accounted for 43-65% of total activity in tissue samples (Table 1). The association of Se with unincorporated (ethanol-soluble) molecules, such as free amino acids and simple carbohydrates, was the major component of total Se in algal cells (Bottino et al. 1984). Selenium associated with unincorporated products in the tissues of bluegills used for this study (43-65%) also appeared to be a major component of Se residues.

Because the concentrations of Se are consistently lower in skeletal muscle than in other tissues and are less variable with respect to exposure concentrations (Adams 1976), they provide an appropriate background concentration of Se with which all other tissues can be compared.

Table 1. Mean percent protein (by wt) in bluegill tissues and percent of isotope extracted by ethanol in tissues of bluegills after 4 wk of feeding. ND= no data.

Tissue	Mean Percent Protein	Activity (uCi x 10^3)			Percent Extracted
		Total	Ethanol	Tissue	
Muscle	17	4.41	2.83	1.58	64
Heart	17	18.75	12.28	6.47	65
Ovary	10	0.52	0.29	0.23	56
Liver	9	5.10	2.21	2.89	43
Testis	30	ND	ND	ND	ND

Therefore, we normalized the activity of (75) Se to the levels in skeletal muscle to compare the uptake and distribution of (75) Se-selenomethionine from this study with results from other studies (Table 2). The

concentration of (75) Se-selenomethionine in liver and ovary generally agreed with those found in other studies. The relative concentration of (75) Se in testis, however, was high compared with the concentration of Se in testes of feral fish. The concentration of (75) Se in heart and blood was less than that found in laboratory studies with waterborne selenite.

Table 2. Concentration factors of (75) Se-selenomethionine (12 wk) in bluegills and ranges of concentration factors for Se in tissues of fishes from selenium-enriched reservoirs and laboratory studies. Mean Se values for each organ were divided by Se values in skeletal muscle or whole body Se. ND= no data; Water= waterborne Se exposure; Diet= dietary Se exposure.

Tissue	(75) Se Activity	Reservoir Studies	Laboratory Studies (selenite)	
			Water	Diet
Liver	7.5	1.6-4.4[e-j]	4-45[a-c]	2-38[d]
Testis	4.1	0.5-1.7[e-j]	ND	ND
Ovary	2.3	0.9-6.0[e-j]	ND	ND
Gonads	ND	ND	0.8-1.7[c]	ND
Heart	1.9	ND	5-8[bc]	ND
Blood	1.2	ND	3.5-10[a-c]	ND

a Adams (1976)
b Hodson et al. (1980)
c Lemly (1982)
d Hilton et al. (1982)
e CP&L (1981, 1983)
f Cumbie and VanHorn (1978)
g CP&L (1980)
h Sager and Cofield (1984)
i CP&L (1979)
j Baumann and Gillespie (1986)

The relative uptake of (75) Se in heart and blood measured during our study was lower than that reported in other studies (Adams 1976; Lemly 1982). However, fish in these studies were exposed to waterborne selenite, which should result in higher concentrations of Se in gills, blood, and heart than occur after exposure to dietary Se (Hilton et al. 1982).

Our results support this observation; isotope activity was only slightly greater in heart and blood than in skeletal muscle of bluegills after dietary exposure to (75) Se. The difference between (75) Se activity in heart and skeletal muscle was slight; the greater (75) Se in heart may have been due to the greater tendency of selenoproteins to be formed in this organ (Lemly 1982). The average daily diet of (75) Se fed to bluegills was about 0.04 mg/kg wet weight of food or 0.27 mg/kg dry weight of food assuming an 85% moisture content for meal worms. This dietary concentration of selenomethionine is near the range of dietary selenite (0.35 mg/kg - 1.3 mg/kg dry wt of food) at which the accumulation of Se was highest in liver of rainbow trout, Salmo gairdneri (Hilton et al. 1982). At low dietary concentrations, bluegills also accumulate selenomethione at greater concentrations in liver than other organs after 12 wk of exposure.

In field studies of Se in fish taken from Se-enriched reservoirs during the spawning season, testicular Se was always less than ovarian Se. However, this difference was slight at low concentrations of Se and increased (Se in ovary became greater) as the body concentration of Se increased (Baumann and Gillespie 1986). The concentrations of Se in the diet of bluegills in the present study (0.04 mg/kg) were low in comparison with concentrations of Se in available food (0.1-23 mg/kg) for fish from selenium-enriched reservoirs (CP&L 1979,1983,1984). Therefore, the greater activity of (75) Se in testis than in ovary may have been caused, in part, by the low concentration of Se in the diet or the relatively short exposure period for our study.

The lack of mature ova in bluegills from this study also may have contributed to low levels of (75) Se activity in the ovary. In bluegills from Se-enriched reservoirs, Se content is significantly greater in ovaries than in carcass during the spawning season (Baumann and Gillespie 1986), but only slightly greater than in muscle when averaged for the entire year (Sager and Cofield 1984). If the eggs in the bluegills used in our study had matured during exposure, ovaries might have had greater (75) Se activity levels in comparison with other tissues. However, even at low concentrations of dietary Se and at reproductively immature stages, the levels of (75) Se activity in gonads were 2 to 4 times that of skeletal muscle. These results suggest that Se concentrates in ovaries and testes of fishes.

Acknowledgments. We thank the Environmental Services Section of the Carolina Power and Light Comapany, New Hill, North Carolina, for funding this research. We also thank Merlin Curtis, Renee Watters (Deartment of Zoology, Ohio State University), Dr. Rick Myser, and Joe Talnagi (Nuclear Research Laboratory, Ohio State University) for their technical assistance.

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Received November 20, 1987; accepted December 30, 1987.