Bull. Environ. Contam. Toxicol. (1990) 45:775–782

Environmental Contamination and Toxicology

Tissue Distribution of Mercury and Selenium in Minnows, *Phoxinus phoxinus*

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The protective effect of selenium against mercury toxicity has been extensively demonstrated in a number of studies (Burke *et al.* 1977; Kasuya 1976). Since mercury uptake is not always diminished by the presence of selenium (Kim *et al.* 1977) and neither does selenium enhance the elimination of mercury (Lucu and Skreblin 1981; Cuvin and Furness 1988), these findings indicate that the mechanism for the observed protective action of selenium against mercury toxicity lie along different lines. It is believed that the rechanelling of mercury from one organ or tissue to another is one of the general mechanisms involved in the protective action of selenium against mercury toxicity. This is supported by the fact that one of the observed effects of selenium treatment on mercury-intoxicated animals is the apparent modification of the distribution pattern of mercury in the different organs and tissues. Decreased mercury levels in the kidney after selenium treatment has been demonstrated in rats by Chen *et al.* (1974) and Potter and Matrone (1974).

The following study aims to determine the effect of selenium on the distribution pattern of mercury in a common freshwater fish, the minnow *Phoxinus phoxinus* (Order Cypriniformes; Family Cyprinidae). Conversely, the effect of mercury on the tissue distribution of selenium will also be studied.

MATERIALS AND METHODS

Adult minnows were collected from Drumptian Ford, Killearn in the Strathclyde region of Scotland. The site was relatively free from agricultural and industrial wastes. The fish used ranged from 60-80mm. After at least 48 hours of acclimatization to laboratory conditions, the fish were divided into 2 groups for the following experiments.

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For the study on tissue distribution of mercury one group of 14 fish was placed in an aquarium containing 0.1 ug/l mercury as mercuric chloride ($HgCl_2$) and the other in an aquarium with 0.1 ug/l selenium as sodium selenate (Na_2SeO_4). The fish were allowed to take-up mercury for 7 days. The water was changed on the third day to maintain mercury and selenium levels. After the uptake period, the fish were washed in tapwater and then transferred to aquaria containing clean aged tap water. The fish were kept in these aquaria for 6 hours to remove the mercury which may have adhered externally to the skin and gill epithelia. The fish were then sacrificed and the fish for each treatment were divided into two groups of 7 fish each. Dissection was carried out and similar tissues for the 7 fish were pooled for mercury anlysis. The tissues analyzed were the following: gut, liver, kidney, muscle, brain, gonads, gills and visceral remains. The rest of the tissues (mainly skeleton and skin) were lumped together and mercury determinations were carried out on these as well. Mercury analysis was done using a mercury vapour detector after acid digestion of freeze-dried fish samples based on the procedure of Armstrong and Uthe (1971) and Muirhead (1986).

For the study on tissue distribution of selenium, eight adult minnows were placed in beakers containing 10uCi (370 kBq) of ^{75}Se as sodium selenite ($Na_2{}^{75}SeO_3$). Another group was simultaneously exposed to 0.01 ug/l mercury and 10 uCi ^{75}Se. Both groups were allowed to take-up radioactive selenium for 4 days. After the uptake period, the fish were washed in tap water to remove superficial radioactivity. The fish were transferred to beakers containing uncontaminated tapwater and were kept there for 6 hours. This was to wash away any radioactive selenium adhering to the external surfaces, particularly in the gill epithelia. The fish were pooled and radioactivity was determined using a Packard NaI gamma scintillation counter. Selenium activity was expressed as counts per minute (cpm) and concentration as cpm/g. The tissues analyzed were the same as that of the mercury distribution study.

RESULTS AND DISCUSSION

The modification of tissue distribution pattern of mercury has been reported as one of the observed effects of selenium treatment. Table 1 shows the tissue distribution of mercury in minnows with and without exposure to selenium. Although the kidney makes up less than 0.5% of the total body weight, it contained

Table 1. Tissue distribution of mercury in minnows. Each value is the mean of two replicate samples of tissues pooled from 7 fish. Values in parentheses are standard deviations.

Tissue	Treatment*	Mean dry weight, g	Mean Hg concentration, ug/g
Gut	Hg	0.171 (0.045)	0.244 (0.037)
	Hg + Se	0.129 (0.016)	0.302 (0.003)
Liver	Hg	0.093 (0.051)	0.223 (0.039)
	Hg + Se	0.088 (0.044)	0.302 (0.124)
Kidney	Hg	0.030 (0.018)	1.032 (0.381)
	Hg + Se	0.046 (0.002)	0.617 (0.089)
Gonad	Hg	1.250 (0.392)	0.031 (0.002)
	Hg + Se	0.796 (0.250)	0.038 (0.014)
Vis. rem.	Hg	0.106 (0.033)	0.135 (0.013)
	Hg + Se	0.093 (0.015)	0.167 (0.043)
Gill	Hg	0.151 (0.616)	0.270 (0.086)
	Hg + Se	0.144 (0.006)	0.303 (0.102)
Muscle	Hg	1.808 (0.328)	0.085 (0.001)
	Hg + Se	1.562 (0.019)	0.081 (0.014)
Brain	Hg	0.043 (0.022)	0.273 (0.019)
	Hg + Se	0.035 (0.010)	0.295 (0.104)
Others	Hg	2.054 (0.418)	0.092 (0.026)
	Hg + Se	1.800 (0.059)	0.079 (0.007)

* Hg: the fish were exposed to 0.1 ug/l mercury; Hg + Se: the fish were exposed to 0.1 ug/l mercury and 0.1 ug/l selenium. Exposure period was 7 days prior to analyses.

the highest concentration of mercury, with 1.032 ug/g. The liver, gut, gills and brain contained comparable levels, ranging from 0.223 to 0.273 ug/g. These were followed by the visceral remains and the muscle. The gonads had the lowest mercury concentration despite the fact that they contributed 21.65% of the total body weight since the fish used were collected during their breeding season and the females were gravid.

In the fish exposed simultaneously to 0.1 ug/l mercury and selenium, the kidney also showed the highest mercury content among the tissues analysed. This was followed by the brain, gut, liver and gills all of which had similar mercury concentrations (0.295 to 0.303 ug/g). The visceral remains, muscle and gonads had the lowest mercury levels.

T-test showed there was no significant difference ($p>0.05$) between the 2 treatments. Except for

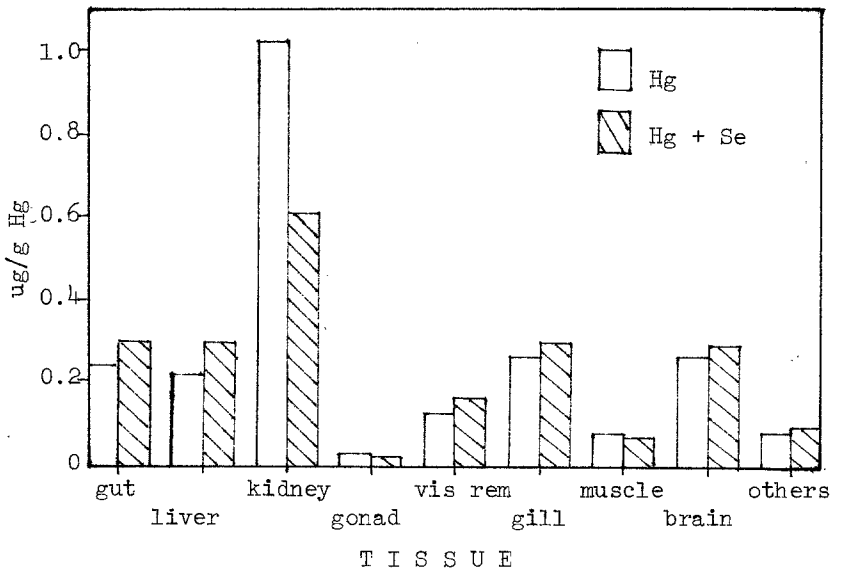

Figure 1. Mercury levels in different tissues of *P. phoxinus* with and without exposure to selenium.

the kidney, mercury concentrations in the other tissues were, generally, slightly higher in the selenium-treated groups. Figure 1 shows that mercury in the kidney was decreased by approximately one-third with simultaneous administration of selenium.

A number of studies have already demonstrated that the kidney is a primary target organ of inorganic mercury (Parizek and Ostadalova 1967; Gritzka and Trump 1968). The reduction of kidney mercury in the selenium treated group, even if not statistically significant in minnows seems to agree with several studies with rats wherein mercury uptake by the kidney was decreased with selenium treatment (Chen *et al.* 1974; Potter and Matrone 1974). Tissue distribution of mercury is dependent on the dose of selenium employed. Maximum reduction of renal mercury levels is produced by approximately equimolar doses of mercury and selenium (Fang 1977). Since the mercury and selenium doses used for the studies with minnows were not equimolar, this could explain why the difference in mercury levels in the kidney between the 2 treatments were not so great as to be statistically significant. It has also been reported that selenium also causes lower retention of mercury in the same organ in killifish (Sheline and Schmidt-Nielsen 1977).

Since it has been previously shown that the whole-body uptake of mercury by minnows is not diminished by the presence of selenium (Cuvin and Furness 1988), this means that mercury from the kidney is diverted to other tissues. Results here showed that with the exception of the kidney, mercury levels in other tissues were generally higher in selenium treated groups.

The distribution of radioactive selenium in minnows with and without simultaneous administration of mercury is shown in Table 2. The gut and liver had the highest concentrations of selenium with 132475 and 116940 cpm/g, respectively. These were closely followed by the kidney with a slightly lower selenium content. The visceral remains and gills also had comparable levels of selenium. The brain showed 29604 cpm/g and the gonads 21107 cpm/g, with the lowest concentration in the muscle.

Table 2. Tissue distribution of selenium. Each value is the mean of 2 replicate samples pooled from 4 fish each. Values in parentheses are standard deviations.

Tissue	Treatment*	Mean dry weight, g	Mean Se concentration, cpm/g
Gut	Hg	0.047 (0.004)	132475 (68422)
	Hg + Se	0.040 (0.010)	72296 (20154)
Liver	Hg	0.017 (0.000)	116940 (5606)**
	Hg + Se	0.018 (0.001)	82205 (6760)
Kidney	Hg	0.010 (0.001)	103926 (56720)
	Hg +Se	0.010 (0.000)	86380 (7251)
Gonad	Hg	0.115 (0.053)	21107 (2924)**
	Hg +Se	0.059 (0.019)	31048 (907)
Vis. rem.	Hg	0.023 (0.006)	61426 (30565)
	Hg + Se	0.034 (0.003)	34573 (10904)
Gill	Hg	0.041 (0.001)	51729 (8073)
	Hg + Se	0.039 (0.004)	31909 (703)
Muscle	Hg	0.494 (0.041)	6947 (571)
	Hg + Se	0.445 (0.010)	7600 (1090)
Brain	Hg	0.017 (0.004)	29604 (6610)
	Hg + Se	0.014 (0.002)	22952 (989)
Others	Hg	0.558 (0.063)	8964 (229)**
	Hg + Se	0.533 (0.300)	10405 (184)

* Hg: the fish were exposed to 10 uCi ^{75}Se; Hg + Se: the fish were exposed to 0.1 ug/l mercury and 10 uCi ^{75}Se. Exposure period was 4 days prior to analyses.
** Se concentration between the 2 treatments statistically significant at $p<0.05$.

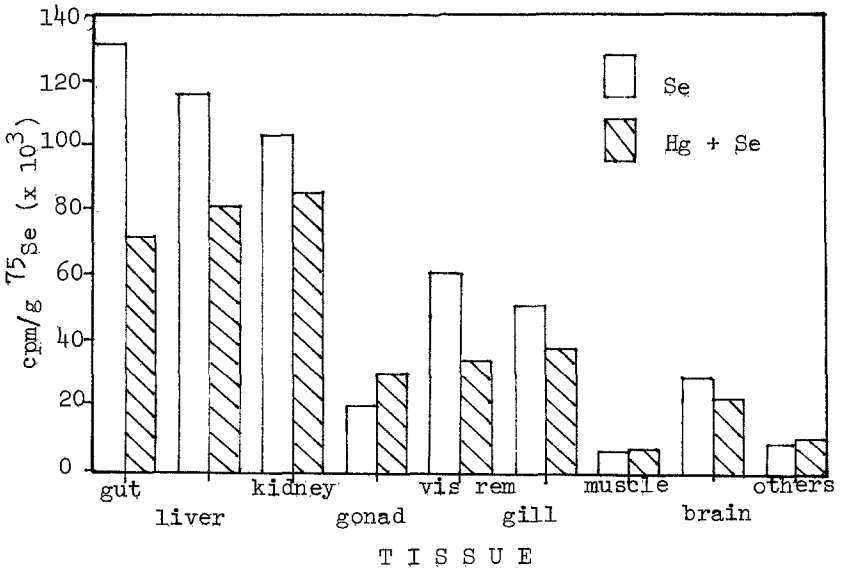

Figure 2. Selenium levels different tissues of *P. phoxinus* with or without exposure to mercury.

The distribution of radioactive selenium in the tissues of fish exposed simultaneously to selenium and mercury showed that the kidney, liver and gut had similar levels of selenium with 86380, 82205 and 72296 cpm/g, respectively. The visceral remains, gills and gonads contained comparable concentrations of selenium. The brain had 22952 cpm/g, while the muscles had the lowest concentration of selenium.

Comparison of the percentage distribution of radiaoctive selenium between the group treated with mercury and those which were not is shown in Figure 2. Selenium concentration in the liver was significantly lower ($p<0.05$) in the mercury-treated group. Although not statistically significant, selenium concentrations in the gut of mercury-treated fish was only half of those exposed only to selenium. Selenium levels in kidney, visceral remains, gills and brain were also lower for the mercury-treated group. The remaining tissues had slightly higher selenium concentrations with mercury treatment. However, only the gonad and "others" had significantly higher selenium concentrations.

It appears that selenium accumulates specifically in the gut and liver, unlike mercury which is particularly accumulated in the kidney.

The comparatively high levels of mercury in the gut and gills in both the selenium-treated and non-selenium treated groups suggest that these two organs play important roles as the major routes of uptake of mercury in minnows. Pentreath (1976) also reported relatively high mercury concentrations in the gut of plaice, although the mercury levels he found in the gill filaments were lower than those found in minnows.

In the case of selenium, the gut showed much higher levels than the gills in both treatments. This suggests that the route of uptake of selenium is primarily through the gut. Studies with pike have shown that 20 times more selenium is accumulated from food than water (Turner and Swick 1983), which further supports observations.

Acknowledgments. The authors gratefully acknowledge the support of Dr. S.J. Muirhead for the help in mercury analysis and Mr. K. Ensor for the collection of the fish used in this study. MLACA was supported by a Postgraduate Studentship from the British Council.

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Received April 18, 1990; Accepted May 3, 1990.