THE TOXICITY AND RESIDUE DYNAMICS OF SELENIUM IN FISH AND AQUATIC INVERTEBRATES

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ABSTRACT

THE TOXICITY AND RESIDUE DYNAMICS OF SELENIUM IN FISH AND AQUATIC INVERTEBRATES

By

William James Adams

Selenium concentrations in water, sediment, zooplankton and fish from the western basin of Lake Erie were determined by spectrophotometric procedures. The average concentration of selenium (dry weight) in ten fish species ranged from 8.12 ± 1.02 ppm for sheepshead to 1.80 ± 0.12 ppm for common shiners. The selenium concentration in yellow perch increased in proportion to the length and weight of the fish. Higher concentrations in unfiltered water (0.023 ± 0.004 ppm) than in filtered water (0.003 ± 0.001 ppm) were attributed to selenium adsorbed on suspended solids and contained in the plankton. The average concentration of selenium in sediment and zooplankton samples (dry weight) was 0.36 ± 0.07 ppm and 2.54 ± 0.14 ppm, respectively.

The concentrations of selenium in the fish from western Lake Erie are higher than reported for other areas of the Great Lakes and this may be attributed to the fact that western Lake Erie is heavily industrialized and a recipient of multiple waste discharges.

The acute toxicity of sodium selenate and sodium selenite to several species of fish and invertebrates was determined by static and continuous flow tests. Sodium selenite was found to be more toxic than sodium selenate and both compounds were more toxic in the continuous flow tests than in the static tests. A slow accumulative

mortality occurred in all continuous flow tests and it was found that an exposure period of at least 96 days is needed to determine the asmyptotic LC50 for inorganic selenium compounds.

The 48 day LC50 values for sodium selenate and sodium selenite were 2.0 mg/l and 1.1 mg/l, respectively, with fathead minnows. The comparative 48 day LC50 values for sodium selenite with bluegills and rainbow trout were 0.40 mg/l and 0.50 mg/l. The 96 day LC50 value for rainbow trout exposed to sodium selenite was 0.28 mg/l. Coho salmon larvae appeared to be more sensitive to sodium selenite than other species of fish as indicated by the 48 day LC50 value of 0.16 mg/l. The 96 hour LC50 for sodium selenate with *Hyallela azeteca* was 0.76 mg/l. The hatchability of fathead minnow eggs exposed to sodium selenite at concentrations of 1-40 mg/l was unaffected, however, the eggs hatched prematurely at concentrations greater than 15 mg/l and the median survival time of the resulting fry was reduced at all concentrations.

The uptake of selenium by fathead minnows exposed to 10, 25 and 50 µg/l Se occurred in a curvilinear manner with a rapid period of accumulation during the first 8 days followed by a slower rate of accumulation over the next 88 days. The average concentration of selenium (wet weight), after 96 days exposure to 50 µg/l Se, in the viscera, gill, head-tail, and muscle was 2.44, 0.58, 0.54 and 0.44 mg/Kg. The data suggests that the accumulation of selenium in the various tissues is directly related to the exposure concentration.

The elimination of selenium from fathead minnows occurred in a curvilinear manner and was asmyptotic with the time axis after 96 days. Elimination from the viscera was most rapid (half-life 5.1 days); the half-life of selenium in other tissues exceeded 50 days.

Rainbow trout exposed to sodium selenite (0.22 mg/l) for 48 days had average concentrations of 81.7, 61.9, 29.4, 8.5, 7.0, 4.6, 4.5, 3.5, 1.9 and 0.45 mg/Kg in the spleen, liver, heart, pyloric caeca, kidney, intestine, blood, brain, gill and muscle, respectively. Selenium concentration in the muscle was consistently lower than in other tissues with only 20 percent of the total body residue in the muscle of rainbow trout and 27 percent in the muscle of fathead minnows. Trout which died after exposure to sodium selenite contained 1.61 ± 0.18 mg/Kg as compared to 0.90 ± 0.11 mg/Kg in the muscle of the trout which survived a 96 day period of exposure.

Bioconcentration factors for both fathead minnows and rainbow trout were inversely related to the exposure concentration.

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By

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INTRODUCTION

The element selenium can be traced in an orderly sequence from its origin in the earth's crust to specific geologic formations, to distribution of specific genera and groups of plants which require the element for growth, to the accumulation in vegetation and to its subsequent toxicity to birds or mammals that consume the seleniferous feeds (Allaway et al., 1966; Hoffman et al., 1973).

The disease syndrome produced by selenium is a disease of antiquity and has been described in widely separated areas of the world. It has been reported as early as 1295 by Marco Polo (1926) when he wrote of his travels in China and by Stein (1912) when his horses became poisoned in 1906 while traveling in Turkestan and Western China. The earliest account of the form of selenium poisoning known as alkali disease was reported in the United States in 1860 by T. C. Madison (1860). Since this time numerous other incidences of selenium poisoning have been reported (Anderson et al., 1961; Franke and Moxon, 1936; Franke and Painter, 1938; Moxon and Rhian, 1943; Muth and Binns, 1964; and Rosenfeld and Beath, 1964). More recently, Hosseinian et al. (1972) have reported selenium poisoning in a mixed flock of sheep and goats in Iran. The symptoms of selenium poisoning are typical of heavy metal poisoning, including nervous disorders, loss of hair and nails and malformation and abortion of embryos.

Until 1957 the main concern about selenium has been due to its toxicity. In the last 19 years several compelling reasons for the

reexamination of the role of selenium in biology have arisen. Selenium was discovered by Schwarz and Foltz (1957) to be an essential component of a factor, called factor three, that prevented liver degeneration in rats maintained in diets low in vitamin E (tocopherol). This was immediately followed by the finding that selenium is effective in the prevention of a number of economically important diseases of farm animals (Erwin et al., 1961; Nesheim and Scott, 1958; Patterson et al., 1957; Rahman et al., 1960; Schwarz et al., 1957; and Scott et al., 1967). Selenium has recently been shown by Thompson and Scott (1969) to be an essential nutrient in its own right, independent of a complimentary effect of vitamin E. The amount of selenium required by livestock varies greatly depending on the amount of vitamin E present although levels of 0.01-0.1 mg/kg in the diet are known to protect cattle and poultry from white muscle disease and exudative diathesis, respectively (Schubert et al., 1961; Thompson and Scott, 1969). The role of selenium as an essential nutrient became more clear when Rotruck et al. (1972) discovered that selenium is an integral part of glutathione peroxidase, an enzyme responsible for destroying lipid peroxides, preventing erythrocyte hemolysis and oxidative destruction of cell membrane lipids. Selenium is also being reexamined in view of the fact that it may be of some benefit in treating certain forms of cancer (Mickelsen, 1970; Shamberger and Frost, 1969) and because it has been shown to be of benefit in detoxifying other metals including silver, mercury, cadmium, lead and some forms of arsenic (Burch et al., 1973; Ganther et al., 1972a; Ganther et al., 1974; Groth et al., 1972; Hill, 1972, 1974; Levander and Argrett, 1969; and Parizek and Ostadalova, 1967).

At present, nearly all cases of selenium poisoning have been in domestic livestock and not in humans, nevertheless, environmental contamination of selenium is of special importance because of the possibility of human injury resulting from the consumption of meat, vegetables, dairy products and fish from affected areas. Extensive investigations of the poisoning of animals have already been made (Cerwenka and Cooper, 1961; Cousins and Cairney, 1961; Daize and Beath, 1935; Kubota et al., 1967; Moxon and Rhian, 1943; Rosenfeld and Beath, 1964; and Schroeder, 1967) and limited studies on consumption by humans have been reported (Hamilton and Hardy, 1949; Smith et al., 1936; Smith and Lillie, 1940; Schroeder et al., 1970; and Thompson et al., 1975), but research on the concentration of selenium in aquatic organisms and the toxicity of selenium compounds to fish and other aquatic organisms is lacking.

Several sources of selenium pollution do exist. Selenium is mainly produced as a by-product of copper refining and is used extensively as a decolorizer for glass and ceramics. It is also used for photo cells, xerography, rectifiers, solar batteries, television cameras, traffic lights, enamels, brighteners for copper plating, vulcanizing, antioxidant in oils, insecticides and fungicides, paint, varnish, glue remover and various other uses. Environmental contamination from the misuse of these materials could result in a possible threat to aquatic life.

A major source of selenium in the aquatic environment has been attributed to fallout from stack emissions of fossil fuel power plants and industries (Pakkala et al., 1972). Kesseler et al. (1971) and Shah et al. (1970) have reported the concentration of selenium in coal

and oil to be as high as 5.0 ppm and 1.4 ppm, respectively. Other potential sources of selenium pollution have been demonstrated by Hashimoto et al. (1970), Johnson (1970) and Pillay et al. (1974) who have found concentrations of selenium in excess of 1.0 ppm in coal, petroleum, and rubber products, up to 8.0 ppm in solid wastes and as high as 14.0 ppm in particulate stack emissions. The proximity of fossil fuel power plants, steel mills and refining and metal plating factories along the shores of the Great Lakes increases the chance of contaminating the aquatic biota with selenium. Agricultural runoff may also cause some contamination because selenium is used in certain pesticides (Moxon and Rhain, 1943) and is reported to be present as an impurity in fertilizers (Wells, 1966).

A survey conducted by Copeland (1971) in Lake Michigan showed an average of 0.5 ppm selenium in bottom sediments and as high as 7.0 ppm in zooplankton. Ayers (1970) has reported levels of selenium in Lake Michigan for phytoplankton, zooplankton and benthos to be as high as 1.05 ppm, 3.90 ppm and 3.10 ppm, respectively. Copeland (1970) originally suggested that selenium may accumulate in the aquatic ecosystem through the food chain with successive trophic levels accumulating greater amounts of selenium. However, more recent data (Copeland et al., 1973) have not substantiated this theory. A recent survey of fish from New York waters, Lake Erie and Lake Ontario indicated that the level of selenium in fish from these areas is usually between 0.1 and 1.0 ppm (wet weight). Similar values have been reported for Lake Michigan by Copeland et al. (1973). Sandholm et al. (1973) have found similar levels of selenium in fish taken from Finland, Baltic Sea and the Atlantic Ocean with the concentrations

ranging between 0.9 ppm and 2.3 ppm (dry weight). Schroeder et al. (1970) have reported somewhat higher concentrations ranging between 1.0 ppm to 2.2 ppm (wet weight) in certain sea foods including smelt, lobster, shrimp, herring, and fish flour. Similar findings have been reported by Kifer et al. (1969), Lindberg (1968), Lunde (1968, 1970), and Soares and Miller (1970).

While the above types of data provide information concerning the availability of selenium in aquatic environments they do not clearly indicate whether selenium is accumulated through the food chain, nor do they suggest what influence selenium may have on fish populations. Only a minimal amount of research has been undertaken to interpret these environmental levels in terms of the effects on fish populations and studies have not been conducted to determine the distribution and rates of uptake and elimination of selenium in fish. In addition, the toxicity of various selenium compounds to fish has been just recently investigated (Huckabee and Griffith, 1974; Niimi and LaHam, 1975; and Weir and Hine, 1970). Although a paucity of data exists on the effects of selenium on aquatic organisms, the death of stocked game fish in a Colorado reservoir has been associated with high levels of selenium in the bottom sediments (Barnhart, 1958).

In view of the scarcity of information on the presence of selenium in the aquatic environment and its affect on aquatic organisms the following three areas of investigation were chosen: (1) to determine the selenium content in selected components of the aquatic biota of western Lake Erie; (2) to measure the acute toxicity of selenium to several species of fish; and (3) to determine the distribution and rates of uptake and elimination of selenium in selected species of fish.

SECTION I: A SURVEY OF THE SELENIUM CONTENT IN THE AQUATIC BIOTA OF WESTERN LAKE ERIE

METHODS AND MATERIALS

The method selected for selenium analysis was a spectrophotometric procedure described by Cummins et al. (1965). This procedure is based on the method of complexing selenium with diaminobenzidine as described by Hoste and Gillis (1955) and refined by Cheng (1956). Some modifications of the method were made including the use of a Beckman DK-2A spectrophotometer instead of a spectronic 20, a pH meter was used for all pH adjustments and the digestion solution was changed to a 1:1 mixture of sulfuric and perchloric acids. Twenty grams of sodium molybdate dissolved in 100 milliliters of water were added to each liter of digestion solution. A certified standard stock solution of selenium dioxide (Fisher Scientific Co.) was used to establish standard curves. The digestion procedure was tested by analyzing fish which had been exposed to radioactive Selenite-75. The average loss of selenium during the digestion was 2.78 percent (Table A1). The minimum detectable concentration by this method is 0.1 mg/kg. The percent recovery of selenium added to fish muscle was found to be 103.0 ± 1.0 percent (Table A2). In order to ascertain the accuracy of this method fish samples were independently analyzed for selenium by fluorometric analysis and compared with analysis of the same tissues using the described spectrophotometric procedure (Table 1).

Table 1. Comparison of selenium concentrations in fish samples analyzed by both spectrophotometric and fluorometric methods of analysis.

Sample No.	Species	Concentration of Selenium (ppm)	
		Spectrophotometric[1]	Fluorometric[2]
183	Fathead minnow	0.36±0.02	0.40
153	Yellow perch	0.42±0.03	0.41
157	Yellow perch	0.47±0.06	0.46

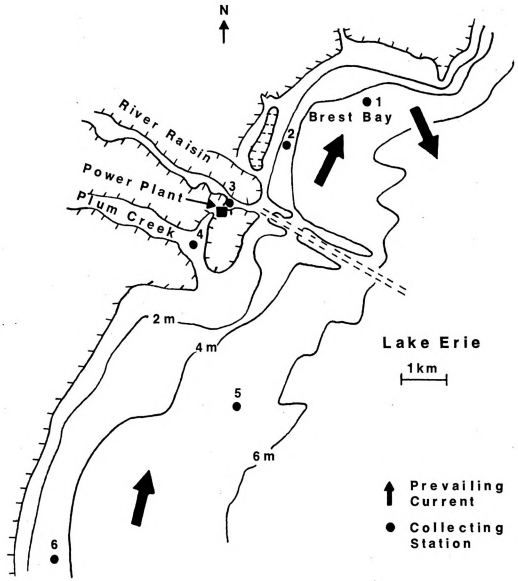
[1]The spectrophotometric values are given with their respective standard errors. Each sample was analyzed four times.

[2]The fluorometric analysis was conducted independently in the Dept. of Animal Science, Mich. State Univ., E. Lansing, Mich. Each sample was analyzed twice.

Fish, zooplankton, sediment and water samples were collected during the summers of 1973 and 1974 from six different stations in western Lake Erie (Figure 1). The fish were collected with an otter trawl and gill nets, placed in plastic bags and frozen until each fish could be fileted, skinned, and dry homogenized with a Polytron Blender. The homogenate was placed in glass vials and kept frozen until analyzed. Water samples were collected in glass bottles and analyzed the same day to minimize the loss of selenium due to adsorption. Plastic bottles were found to adsorb greater amounts of selenium than glass bottles and therefore they were not used. Samples of filtered (0.45 μ Millipore filter) and non-filtered water were analyzed using 100 milliliters of water and following the same procedure as before except that the samples were not heated. The method of analyzing for selenium in water was checked by employing Selenite-75 and comparing stable and radioactive determinations on the same samples. The average deviation of the stable analysis from the radioactive analysis was 1.85 ± 0.99 percent (Table 3A).

Zooplankton samples were collected with a 500 micron plankton tow net and kept frozen until analyzed. An Ekman dredge was used to collect sediment samples. A subsample of about 100 grams was taken for analysis from the Ekman hauls at each station. Both the zooplankton and sediment samples were first air dried in a drying oven at 60°C for 24 hours. Experiments with radioactive Selenite-75 indicated that no significant loss due to volatilization of selenium from the samples should occur at this temperature for up to 30 hours of heating (Tables 4A and 5A). One gram of fish and sediment, and 0.5 grams of zooplankton were normally used for analysis. Sediment and zooplankton values were

Figure 1. A map of the study area located along the near shore area of western Lake Erie at Monroe, Michigan.


N
River Raisin
Power Plant
Plum Creek
Brest Bay
1
2
3
4
5
6
2 m
4 m
6 m
Lake Erie
1km
Prevailing Current
Collecting Station

calculated on a dry weight basis whereas the fish values were calculated on a wet weight basis and converted to dry weight using the average percent moisture for each species.

Standard statistical procedures (variance, correlation, t-tests, and Duncan's multiple range test) were used to analyze the data (Steel and Torrie, 1955). All values were tested at the 0.05 probability level and the term significance is used to indicate this throughout this section. Variation about the mean is denoted by the standard error. Logarithims to the base 10 were used for all data transformations.

The fish collected and analyzed for selenium content were yellow perch (*Perca flavescens*), common shiners (*Notropis cornutus*), spottail shiners (*Notropis hudsonius*), sheepshead (*Aplodinotus grunniens*), carp (*Cyprinus carpio*), white bass (*Roccus chrysops*), goldfish (*Carassius auratus*), gizzard shad (*Dorosoma cepedianum*), walleye (*Stizostedion vitreum*), and white sucker (*Catastomus commersoni*).

RESULTS AND DISCUSSION

The average concentration of the unfiltered water samples for each collection date was found to be significantly higher than in the filtered samples (Table 2). This difference was attributed to adsorption of selenium on plankton and suspended solids in the unfiltered samples. No relationship was found between the concentration of selenium in the water and the site of sample collection. The concentration of selenium in the filtered samples is in agreement with the values reported by Bowen (1966) (0.02 ppm or less) as commonly occurring in freshwater lakes, however, these values are significantly higher

Table 2. Concentration of selenium in four sets of water samples collected at six locations in western Lake Erie.

Station	Date	Concentration of Selenium (ppm)	
		Filtered	Unfiltered
1	5/11/73	0.005	0.012
2	"	0.006	0.026
3	"	0.006	0.015
4	"	0.005	0.012
5	"	0.005	0.019
6	"	0.005	0.020
Mean±S.E.		0.005±0.001	0.017±0.001
1	5/30/73	0.001	0.036
2	"	0.001	0.036
3	"	0.002	0.017
4	"	0.001	0.025
5	"	0.005	0.032
6	"	0.003	0.071
Mean±S.E.		0.002±0.001	0.036±0.008
1	7/18/73	0.001	0.028
2	"	0.002	0.031
3	"	0.005	0.043
4	"	0.005	0.024
5	"	0.003	0.020
6	"	0.003	0.024
Mean±S.E.		0.003±0.001	0.028±0.003
1	8/28/74	0.001	0.011
2	"	0.001	0.012
3	"	0.002	0.010
4	"	0.002	0.009
5	"	0.001	0.010
6	"	0.001	0.015
Mean±S.E.		0.001±0.001	0.011±0.001

than those reported by Copeland and Ayers (1972) (0.083 ppb) for Lake Michigan.

Two sets of sediment samples collected on 6/24/73 and 8/28/74 had average concentrations of 0.56 ± 0.06 and 0.16 ± 0.04 ppm dry weight, respectively (Table 3). In the first set of samples, stations five and six were found to be significantly higher than station two and station six was also significantly higher than station three. There were no significant differences between stations in the second set of samples. The selenium concentration was not correlated with the organic content of the sediments at any of the stations. Sediment selenium values obtained in the present study are lower than the values reported by Copeland and Ayers (1972) for Lake Michigan. They found the average concentration of selenium in bulk sediments to be 0.60 ppm wet weight. Wiersma and Lee (1971) surveyed the selenium content in the sediment from several lakes in Wisconsin and found the concentrations to range from 1.0-3.0 ppm dry weight. The data obtained in this study indicates that the selenium content in the sediments of western Lake Erie appears to be less than in other areas of the Great Lakes region, however, the data does fall within the range of values (0.05-0.60 ppm wet weight) reported by Bowen (1966) for sediments on a world wide basis.

Three sets of zooplankton samples were collected with the average concentration of all the samples being 2.54 ± 0.14 ppm dry weight (Table 4). There were no significant differences between the sets of samples nor did any of the stations tend to show consistently higher concentrations of selenium in the zooplankton. The data obtained in this study agrees with the concentrations of selenium found in zooplankton in Lake Michigan by Ayers (1970) and Copeland and Ayers (1972).

Table 3. Selenium concentration in two sets of sediment samples collected at six locations in western Lake Erie. Sample one was collected on 6/24/73 and sample two was collected on 8/28/74.

	Selenium (ppm dry wt.) ± 1 Std. Error	
Station	Sample #1	Sample #2
1	0.52±0.06	0.10±0.01
2	0.35±0.07	0.17±0.03
3	0.47±0.12	0.37±0.07
4	0.60±0.04	0.10±0.01
5	0.65±0.12	0.10±0.01
6	0.75±0.05	0.10±0.01
Mean	0.56±0.06	0.16±0.04

Table 4. Selenium concentration of three sets in zooplankton samples collected at six locations in western Lake Erie. Samples one, two and three were collected on 6/24/73, 7/26/73 and 8/24/74, respectively.

	Selenium (ppm dry wt.) ± 1 Std. Error		
Station	Sample #1	Sample #2	Sample #3
1	2.71±0.23	2.27±0.12	2.17±0.22
2	2.21±0.16	2.07±0.07	---
3	---	1.70[a]	0.78[a]
4	2.12±0.14	3.51[a]	---
5	2.74±0.27	3.13±0.12	3.88±0.19
6	2.11±0.17	---	2.70±0.03
Mean	2.38±0.14	2.54±0.34	2.38±0.64

[a]Inadequate amount of zooplankton was collected to allow for more than one analysis.

The average dry weight concentration for all of the fish samples combined was 3.59 ± 0.17 ppm (0.74 ± 0.03 ppm wet weight). The average dry weight concentrations ranged from 1.80 ± 0.12 ppm for common shiners to 8.12 ± 1.02 ppm for sheepshead (Table 5). Both dry and wet weight values for all the fish analyzed are presented in Table 6A. Yellow perch were examined most intensively because they are one of the most abundant species in western Lake Erie and are often taken by sports and commercial fishermen. The average concentration of selenium in 79 yellow perch was found to be 3.32 ± 0.22 ppm dry weight. Although no definite relationships between selenium content in fish samples and collection site were established, yellow perch were found to have significantly higher concentrations at station one than at any other station.

Pakkala et al. (1972) surveyed the selenium content in yellow perch from eastern Lake Erie and reported the average concentration to be 0.32 ± 0.01 ppm (wet weight) as compared to 0.74 ± 0.05 ppm (wet weight) in the present study. The mean concentration of selenium in yellow perch taken from Lake Michigan was 0.57 ± 0.03 ppm wet weight (Copeland et al., 1973). The average concentration (wet weight) of selenium in sheepshead, white bass, and walleye from eastern Lake Erie was found to be 0.43 ± 0.02 ppm, 0.42 ± 0.02 ppm, and 0.29 ± 0.02 ppm, respectively (Pakkala et al., 1972). These values are significantly lower than the values obtained for the same species in the present study (Table 5). The range of values reported by Copeland et al. (1973) for Lake Michigan fish, are only slightly less than the values obtained in this study. They reported the average concentration of

Table 5. Concentration of selenium in fish collected at six locations in western Lake Erie.

Species	Station	Number of Fish	Average Concentration (ppm) ± 1 S.E. Wet Weight	Dry Weight
Yellow perch	1	20	0.89 ± 0.07	4.00 ± 0.31
"	2	25	0.74 ± 0.11	3.33 ± 0.51
"	5	15	0.57 ± 0.09	2.54 ± 0.46
"	6	19	0.65 ± 0.09	2.90 ± 0.39
Average		79	0.74 ± 0.05	3.32 ± 0.22
Common shiner	2	11	0.44 ± 0.05	1.80 ± 0.19
"	5	10	0.43 ± 0.04	1.76 ± 0.17
Average		21	0.44 ± 0.03	1.80 ± 0.12
Spottail shiner	1	10	0.52 ± 0.05	2.12 ± 0.21
"	6	6	0.96 ± 0.18	3.94 ± 0.73
Average		16	0.69 ± 0.09	2.82 ± 0.37
Sheepshead	1	3	0.97 ± 0.17	5.23 ± 0.92
"	3	1	1.48	7.96
"	4	2	1.13	6.08
"	6	7	1.85 ± 0.23	9.95 ± 1.25
Average		13	1.51 ± 0.19	8.12 ± 1.02
Carp	5	3	1.02 ± 0.19	4.48 ± 0.83
"	6	3	0.61 ± 0.01	2.67 ± 0.38
Average		6	0.82 ± 0.13	3.57 ± 0.55
White bass	2	1	0.80	4.17
"	5	3	0.82 ± 0.19	4.29 ± 0.98
Average		4	0.82 ± 0.13	4.26 ± 0.69
Goldfish	4	1	1.60	6.96
"	5	1	1.11	4.83
Average		2	1.36	5.89

Table 5 (cont'd)

Species	Station	Number of Fish	Average Concentration (ppm) ± 1 S.E. Wet Weight	Dry Weight
Gizzard shad	5	4	0.73 ± 0.07	3.69 ± 0.34
Walleye	4	3	0.32 ± 0.07	1.52 ± 0.35
"	5	4	0.67 ± 0.14	3.13 ± 0.65
Average		7	0.52 ± 0.11	2.44 ± 0.52
White sucker	2	2	0.59	3.01

selenium in all species of fish combined to be 0.54 ± 0.01 ppm (wet weight) as compared to 0.74 ± 0.04 ppm (wet weight) in this study.

Selenium content was not significantly different between the sexes for any of the species collected. A significant correlation between selenium concentration and both length and weight was found for yellow perch at station one (Figure 2). Collections at other stations did not include sufficient size range to allow this type of comparison for yellow perch or for other species.

In order to compare the concentrations of selenium in the fish from western Lake Erie with fish from other areas which are subject to less municipal and industrial wastes, seven yellow perch from the northern tip of Lake Huron were analyzed and found to contain significantly less selenium than the yellow perch from western Lake Erie. The average values for the Lake Huron and Lake Erie yellow perch were 0.60 ± 0.04 ppm (wet weight) and 0.74 ± 0.05 ppm, respectively. Although the difference between these two means is statistically significant, there is insufficient data to determine whether or not man's activities have influenced the concentration of selenium in western Lake Erie. Beal (1974), however, has indicated that selenium levels in Canadian fish generally declined from areas of high population to areas of low population density. He reported that fish from the Great Lakes had an average concentration of 0.5 ppm (wet weight) whereas fish from Northwestern Ontario, Manitoba, Saskatchewan, Alberta and Northwest Territories had a combined average concentration of 0.2 ppm.

A comparison of the concentration of selenium in the various trophic levels indicated that selenium progressively increased from water to sediment to zooplankton and to fish (Figure 3). Similar results

reported by Copeland et al. (1973) showed the concentration of selenium to increase from water to sediment and from water to phytoplankton to zooplankton, but the levels in the fish did not exceed those found in the zooplankton. Insufficient data has been collected to determine whether this increase in selenium concentration through the trophic levels is the result of biological magnification or simply a reflection of the relative rates of accumulation of the organisms at each trophic level.

Figure 2. A correlation of the concentration of selenium in fish muscle with the length and weight of the fish.

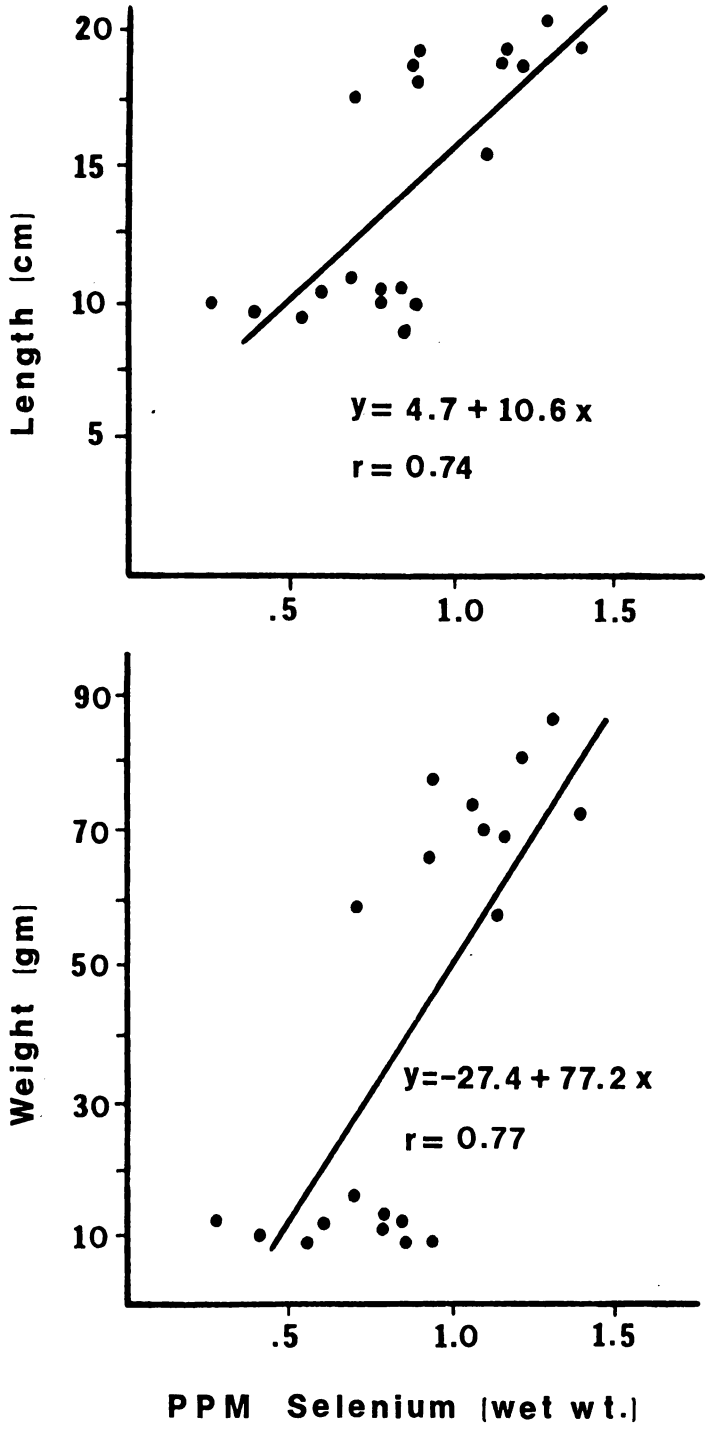
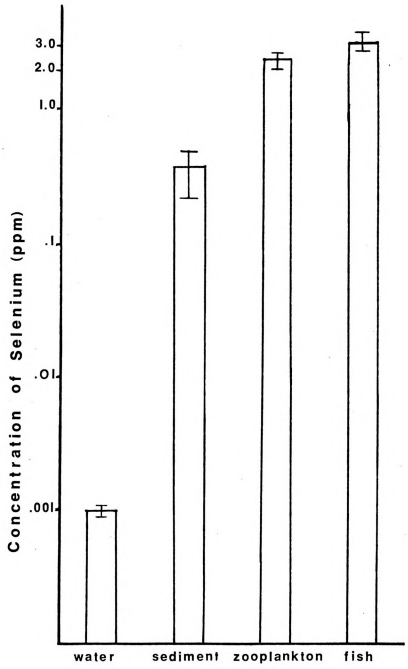

Length (cm)
20
15
10
5
y= 4.7 + 10.6 x
r= 0.74
.5
1.0
1.5
Weight (gm)
90
70
50
30
10
y=-27.4 + 77.2 x
r= 0.77
.5
1.0
1.5
PPM Selenium (wet wt.)

Figure 3. A comparison of the concentration of selenium in water, sediment, zooplankton and fish samples collected from western Lake Erie. All values are reported as ppm dry weight except water.


Concentration of Selenium (ppm)
3.0
2.0
1.0
.1
.01
.001
water
sediment
zooplankton
fish

SECTION II: TOXICITY AND SELENIUM RESIDUE DYNAMICS IN FISH AND AQUATIC INVERTEBRATES

METHODS AND MATERIALS

Acute Toxicity Tests

Static and continuous flow toxicity tests were used to measure the toxicity of sodium selenate and sodium selenite to fish and aquatic invertebrates. The acute toxicity of sodium selenite to fathead minnows (*Pimephales promelas*) (at several temperatures), fathead minnow eggs and rainbow trout (*Salmo gairdneri*) was determined by static toxicity tests. Static tests were also conducted with sodium selenate and fathead minnows.

Continuous flow toxicity tests were used to measure the toxicity of sodium selenate to fathead minnows and amphipods (*Hyallela azeteca*). The toxicity of sodium selenite to fathead minnows, bluegills (*Lepomis macrochirus*), rainbow trout fingerlings and alevin, and coho salmon (*Oncorhynchus kisutch*) alevins was also determined by continuous flow toxicity tests.

All tests were conducted according to the general methods outlined in Standard Methods for the Examination of Water and Wastewater (American Public Health Association, 1971) and the recommendations of the Committee on Methods for Toxicity Tests with Aquatic Organisms (Stephan, 1975). The term "asmyptotic LC 50", as described by Brown (1973), is used in this paper to refer to the concentration of selenium at which 50 percent of the test organisms can survive for an indefinite period of time.

Uptake, Distribution and Elimination Experiments

As an initial attempt to measure the uptake and elimination of selenium in fish, juvenile fathead minnows were exposed, under static conditions, to radioactive selenite-75 (as H_2SeO_3). Uptake was measured over a 28 day period after which the remaining fish were placed in a continuous supply of water without selenium for 96 days and the elimination of selenium was measured.

Adult fathead minnows were exposed for 96 days to a mixture of radioactive selenite-75 and stable sodium selenite by means of a continuous flow delivery system. The uptake and distribution of selenium in the fish was measured during this time. At the termination of the exposure period the fish were placed in a continuous supply of water without selenium for 96 days and the elimination of selenium from the fish was measured.

Fingerling rainbow trout were also exposed to a mixture of radioactive selenite-75 and stable sodium selenite by means of a continuous flow delivery system. The exposure concentrations were selected so that fish would be expected to die at the higher concentrations but not at the lower concentrations. Ten larger fish (12.0 ± 0.5 cm) were placed in the tank with the lowest exposure concentration (0.22 mg/l) so that tissues not easily dissected from the smaller fish (6.5 ± 0.1 cm) could be analyzed for selenium content. The percentage of dead fish at each concentration was used to calculate a 96 day LC 50 value for the smaller trout. The experiment was terminated after 48 and 96 days for the larger and smaller trout, respectively, and all fish remaining alive were sacrificed and analyzed for selenium content.

Fish Source and Maintenance

Fathead minnows and bluegills were obtained from populations maintained in ponds at the Michigan State University, Department of Fisheries and Wildlife Research Facility. Fish of a known age were obtained by collecting young of the year and maintaining them in the laboratory in 70 gallon fiberglass tanks supplied with a continuous flow of well water. All fish were maintained indoors for at least one month prior to being used. The fish were acclimated to test temperatures for one week before initiating the toxicity tests.

Rainbow trout and coho salmon were reared in the laboratory from eggs obtained from the Michigan Department of Natural Resources' Platte River Fish Hatchery. Alevin trout and salmon were initially fed Ewos salmon starter diet (Aktiebolaget Ewos Co., Sodertalje, Sweden) several times each day and were later fed twice each day with a 1:1 mixture of #4 Ewos pellets and Oregon Moist diet. The fish were not fed 24 hours prior to or during the static toxicity tests. In all other experiments the fish were fed #4 Ewos pellets once each day with the amount of food corresponding to 2 percent of their body weight. Debris was siphoned from the tanks every other day, all tanks were checked in the morning and evening, and dead fish were removed and recorded.

Water Characteristics and Source

Well water, which was passed through an aeration tank and sand filter, was the water source for all experiments. The chemical characteristics of the well water are summarized in Table 6. Water chemistry, including dissolved oxygen, pH, temperature, total

Table 6. Chemical and physical characteristics of the test water.

Characteristic	Filtered Well Water
Alkalinity (mg/l $CaCO_3$)	331.9
Ammonia (mg/l-N)	0.42
Carbon, total (mg/l-C)	79
Chloride (mg/l-Cl)	0.6
Specific conductance (umoho/cm^3 at 25 C)	610
Copper (mg/l-Cu)	<0.05
Dissolved oxygen (mg/l)	8.2
Hardness (mg/l-$CaCO_3$)	329.1
Iron (mg/l-Fe)	1.0
Lead (mg/l-Pb)	<0.3
Nitrate (mg/l-N)	0.03
Nitrite (µg/l-N)	<5
Nitrogen, total kjeldahl (mg/l-N)	1.11
Phosphorus, total (mg/l-P)	0.01
Selenium (µg/l-Se)	<1
Solids, total (mg/l)	317
Sulfate (mg/l-SO_4)	5.0
Temperature (C)	12

alkalinity and hardness, was measured at the beginning and end of each static toxicity test (Table 7).

Total alkalinity, hardness and pH were measured weekly and dissolved oxygen three times a week for all continuous flow toxicity tests and uptake, distribution and elimination experiments (Tables 8 and 9). All measurements were made in the control tanks except for temperature which was measured in a different exposure tank each day. A Beckman Chem-mate pH meter was used to measure pH and all other measurements were done according to Standard Methods for the Examination of Water and Wastewater (American Public Health Association, 1971).

Exposure Systems

Static Toxicity Tests with Fathead Minnows and Rainbow Trout

The test containers consisted of 15 liter glass aquaria placed in a temperature controlled water bath. The aquaria were filled with water one day prior to the beginning of a test and aerated for 12 hours. The test tanks were not aerated during the tests. The toxicant was added to the aquaria by thoroughly mixing an appropriate amount of concentrated stock solution with the water in the test tanks. Duplicate test tanks were used for each test concentration and 10 fish were placed in each tank according to a random sorting scheme. At the termination of each test the 20 control fish were weighed and measured (Table 7).

Fathead Minnow Egg Exposure

Fathead minnow eggs were collected from breeding populations maintained in the laboratory. Eggs 1 to 2 days old were placed in

Table 7. Description of the fish and chemical and physical characteristics (mg/l) of the test water used for static toxicity tests.

Compound and Species	Fish Size: Length (cm)	Fish Size: Weight (gm)	Temperature	Dissolved Oxygen	pH	Hardness as ($CaCO_3$)	Partial Alkalinity as ($CaCO_3$)	Total Alkalinity as ($CaCO_3$)
Sodium selenate (fathead minnows)								
Initial	----	----	14.0	10.0	8.40	334.0	40.0	336.0
Mean ± 1 S.E.	2.45±0.06	0.08±0.01	14.6±0.09	----	----	----	----	----
Final	----	----	15.0	8.3	8.30	312.0	0	300.0
Sodium selenite (fathead minnows)								
Initial	----	----	13.5	9.2	7.35	324.0	0	340.0
Mean ± 1 S.E.	4.31±0.13	0.58±0.06	12.9±0.08	----	----	----	----	----
Final	----	----	13.8	7.1	7.35	300.0	0	305.0
Sodium selenite (fathead minnows)								
Initial	----	----	20.0	8.6	8.30	310.0	10.0	316.0
Mean ± 1 S.E.	4.60±0.11	0.70±0.70	20.0±0.01	----	----	----	----	----
Final	----	----	19.9	6.2	8.00	296.0	0	280.0
Sodium selenite (fathead minnows)								
Initial	----	----	25.0	8.6	8.30	304.0	10.0	306.0
Mean ± 1 S.E.	4.97±0.13	0.92±0.08	25.1±0.05	----	----	----	----	----
Final	----	----	24.8	4.7	8.20	280.0	0	271.0

Table 7 (cont'd)

Compound and Species	Fish Size: Length (cm)	Fish Size: Weight (gm)	Temperature	Dissolved Oxygen	pH	Hardness as ($CaCO_3$)	Partial Alkalinity as ($CaCO_3$)	Total Alkalinity as ($CaCO_3$)
Sodium selenite (rainbow trout)								
Initial	----	----	13.5	9.8	8.40	330.0	18.0	328.0
Mean ± 1 S.E.	4.40±0.09	0.69±0.05	14.9±0.25	----	----	----	----	----
Final	----	----	15.0	5.6	8.30	330.0	12.0	328.0

Table 8. Description of the fish and chemical and physical characteristics (mg/l) of the test water used for continuous flow toxicity tests.

Compound	Species	Fish Size: Length (cm)	Fish Size: Weight (gm)	Temperature	Dissolved Oxygen	pH	Hardness as ($CaCO_3$)	Total Alkalinity as ($CaCO_3$)
Sodium selenate	Fathead minnow	5.08[a] 0.13[b] (4.2-6.0)[c]	0.87 0.66 (0.48-1.70)	16.5 0.2 (14.8-18.8)	7.83 0.10 (7.3-8.6)	7.40[e] ---- (7.3-8.1)	337.9 1.5 (328-346)	313.2 1.1 (304-320)
Sodium selenite	Fathead minnow	4.77 0.08 (4.2-6.1)	0.77 0.06 (0.5-2.0)	15.6 0.1 (14.7-16.9)	8.20 0.17 (8.1-8.7)	7.80 ---- (7.3-8.1)	337.5 2.7 (320-352)	321.7 3.2 (310-341)
"	Bluegill sunfish	5.22 0.14 (4.4-6.4)	1.72 0.14 (0.78-2.48)	15.7 0.2 (13.0-20.0)	7.78 0.18 (6.2-9.2)	7.75 ---- (7.7-8.0)	318.0 1.4 (312-328)	330.2 3.7 (290-336)
"	Rainbow trout fingerling	6.57 0.10 (5.1-10.1)	2.73 0.13 (1.27-12.08)	14.6 0.1 (13.5-16.5)	6.33 0.12 (5.5-7.4)	7.40 ---- (7.2-7.5)	324.8 0.6 (320-328)	343.2 0.6 (340-348)
"	Rainbow trout fry	2.78 0.04 (2.4-3.0)	0.15 0.01 (0.07-0.23)	17.4 0.4 (14.5-20.3)	7.53 0.31 (5.7-9.0)	7.30 ---- (7.2-7.4)	334.0 1.4 (330-340)	312.5 2.6 (298-318)
"	Coho salmon fry	3.10 0.05 (2.4-3.7)	0.31 0.06 (0.18-0.40)	14.9 0.03 (14.3-15.0)	8.00 0.16 (7.5-8.5)	7.75 ---- (7.4-7.8)	325.2 1.5 (324-328)	346.0 2.2 (338-350)

Table 8 (cont'd)

Compound	Species	Fish Size: Length (cm)	Fish Size: Weight (gm)	Temperature	Dissolved Oxygen	pH	Hardness as ($CaCO_3$)	Total Alkalinity as ($CaCO_3$)
Sodium selenate	*Hyallela azeteca*	----	----	15.9[d]	7.83	7.40	336.8	312.0

[a,b]Mean value plus or minus one standard error.
[c]Range of values.
[d]The mean of the samples taken at the beginning and end of the four day test.
[e]Median pH value.

Table 9. Description of the fish and chemical and physical characteristics (mg/l) of the test water used for measuring uptake and elimination of selenium in fathead minnows.

Type of Test	Fish Size		Temperature	Dissolved Oxygen	pH	Hardness as ($CaCO_3$)	Total Alkalinity as ($CaCO_3$)
	Length (cm)	Weight (gm)					
Juvenile Fish							
Static (uptake)	4.19[a]	0.53	25.1	4.95	7.60[d]	363.7	339.9
	0.07[b]	0.04	0.5	0.66	----	8.8	2.0
	(3.8-5.3)[c]	(0.35-1.27)	(21.1-27.6)	(3.2-7.0)	(7.2-7.8)	(352-372)	(330-352)
Continuous Flow (elimination)	4.18	0.45	19.9	7.51	7.30	342.8	330.4
	0.07	0.03	0.3	0.39	----	6.2	2.4
	(3.7-5.3)	(0.25-0.92)	(18.4-20.8)	(6.2-8.3)	(7.1-7.5)	(335-350)	(325-336)
Adult Fish							
Continuous Flow (uptake)	7.03	2.81	15.6	6.14	7.60	323.8	341.6
	0.06	0.09	0.1	0.13	----	0.7	1.7
	(6.0-8.0)	(1.6-4.3)	(15.0-16.0)	(4.9-7.7)	(7.4-7.8)	(318-328)	(322-344)
Continuous Flow (elimination)	6.89	2.98	13.9	8.04	7.30	324.4	343.8
	0.10	0.18	0.1	0.05	----	0.8	0.8
	(6.2-8.2)	(1.9-5.9)	(13.0-15.0)	(7.8-8.4)	(7.2-7.4)	(320-328)	(340-348)

[a,b]Mean value plus or minus one standard error.

[c]Range of values.

[d]Median pH value.

egg cups made of nitex screening and 1 inch polyvinyl chloride pipe. The egg cups were suspended in 1 liter beakers which contained eight concentrations of sodium selenite ranging from 1 to 40 ppm. Each test concentration was duplicated once and 25 eggs were used in each test container. The water and toxicant were changed every other day and the temperature was maintained at 25 C by means of a water bath. An air stone was placed under each egg cup and allowed to bubble slowly to provide some agitation of the water around the eggs. The air was turned off after hatching was complete. The larval fish were kept in the same beakers after hatching to compare percent mortality with the controls. No attempt was made to feed the larval fish.

Exposure System for Continuous Flow Toxicity Tests

A proportional diluter (Mount and Brungs, 1967) was used to deliver 500 ml of test water per cycle to each exposure tank. Water for the diluter system was fed by gravity flow from a 70 gallon fiberglass head tank where it was heated by 2 100-watt aquaria heaters to maintain a temperature near 15 C in all test tanks (Table 8).

Test water flow rates averaged 125 ml/min for all toxicity tests except for rainbow trout fingerlings which averaged 150 ml/min. The replacement times (90%) were 7 and 9 hours, respectively (Sprague, 1969). These values agree with the replacement times suggested by Alabaster and Abram (1965) and Sprague (1969).

The test tanks were screen-covered glass aquaria with water volumes of 30 liters. All aquaria were covered with black plastic and the entire diluter system was surrounded by plastic curtains to

protect the fish from any laboratory disturbances. Overhead fluorescent lamps provided light on a 13 hour/day photoperiod.

Five concentrations of sodium selenate or sodium selenite and a control were used for each test, except when testing fingerling rainbow trout in which case only four concentrations and a control were used. Thirty fish were randomly assigned to each tank. At the beginning of each test an additional 25 fish of the same size and species as being tested were removed from the holding tanks and weighed and measured (Table 8).

Egg-Alevin Exposure

The effects of sodium selenite on rainbow trout eggs-alevin and coho alevin were tested for 31 days and 43 days, respectively. One hundred rainbow trout eggs, in the eyed stage, and 75 2-day old coho alevin were placed in plastic frame boxes (19 cm x 9 cm x 9 cm) with nitex bottoms and sides. The boxes were placed so that the water delivered to the exposure tanks flowed through the boxes and over the eggs and alevin. The rainbow trout eggs were in the boxes 8-10 days before hatching was complete. On the 11th day the number of rainbow trout alevin was reduced to 50 per box. The rainbow trout and coho alevin were held in these boxes for 20 days and then released into the test tanks. The eggs and alevin were protected from direct light by covering the tops of the test tanks with black plastic sheets.

At the conclusion of the experiment, a subsample of 25 surviving rainbow trout fry from each test tank were weighed and measured. Mean weights and lengths were compared by Duncan's multiple range test (Steel and Torrie, 1960). This was not possible with the coho fry because of the high mortality rate during the experiment.

Amphipod Exposure

Immature *Hyallela azeteca* were exposed to sodium selenite for 96 hours by means of a continuous flow delivery system. Thirty amphipods were placed in each of six plastic-frame, nitex-screen boxes of the same type as previously described. The amphipods were originally collected from the Red Cedar River at Michigan State University and cultured indoors for several months prior to this experiment.

Fathead Minnows - Static Exposure to Selenite-75

Twenty juvenile fathead minnows were randomly assigned to each of eight 30 liter glass aquariums. The fish in four tanks were used to measure uptake of selenite from water (Table 9) and those in the remaining four tanks were used to measure elimination rates. One tank in each set of four contained the control fish.

The exposure concentration in all tanks was 0.083 ng/l (13.53 nCi/l). During the 28 day period of exposure one half of the water in each aquarium was replaced every other day. A sufficient amount of radioactive selenite was added to each exposure tank with the replacement water to maintain a concentration of 0.083 mg/l. The radioactive water which was removed from the exposure tanks during this experiment and was filtered on succeeding experiments through several layers of charcoal and polyurethane foam before it was discharged into the sanitary sewer.

At the end of the exposure period the fish saved for measurement of elimination were placed in clean aquaria containing 30 liters of water and no selenite. Water was supplied at a rate of 125 ml/min and the replacement time (90%) was 9 hours.

Fathead Minnow and Rainbow Trout Exposure - Uptake, Distribution and Elimination Experiments

Proportional diluters were used to deliver a mixture of radioactive selenite-75 and stable selenite to adult fathead minnows and fingerling rainbow trout. Flow rates, during the period of uptake and elimination for the fathead minnows, were maintained at 100 ml/min and 150 ml/min, respectively, and the replacement times (90%) were 12 and 9 hours, respectively. The flow rate and replacement time (90%) for the trout during the period of uptake was 150 ml/min and 9 hours, respectively. The diluter system was modified to deliver four duplicate concentrations, 0, 10, 25, and 50 µg/l for the fathead minnows. Five concentrations ranging from 0.22 mg/l to 0.95 mg/l, were tested with rainbow trout (Table 10).

Sixty fathead minnows were placed in each of eight 30 liter tanks and 30 rainbow trout were placed in each of four 15 liter tanks. Ten trout were also placed in a 15 liter tank and used to measure the distribution of selenium in various fish tissues.

Preparation of Stock Solutions

Stock solutions were made with demineralized water and reagent grade sodium selenate (Na_2SeO_4) and sodium selenite (Na_2SeO_3) (A. P. Mackay Inc. and Alfa Products, respectively). Stock solutions for all experiments using a continuous flow delivery system were placed in a 7 liter mariotte bottle and connected to the diluter system as described by Mount and Warner (1965).

Two mCi of radioactive selenite-75 (H_2SeO_3) (New England Nuclear) were used to prepare the stock solutions for all uptake, distribution and elimination experiments. The half-life was 120.4 days and the

Table 10. The mean concentration of selenium (± 1 S.E.) in the water used for continuous flow toxicity tests. Nominal concentrations are in parentheses. A single water sample was analyzed from each test chamber once a week.

Compound	Species	Selenium Concentration (mg/l) in Six Test Chambers					
		Control	1	2	3	4	5
Sodium selenate	Fathead minnow	< 0.001	0.52 ± 0.04 (0.62)	1.23 ± 0.04 (1.25)	2.70 ± 0.17 (2.50)	5.32 ± 0.19 (5.0)	10.95 ± 0.39 (10.0)
Sodium selenite	"	"	0.51 ± 0.04 (0.62)	1.03 ± 0.08 (1.25)	1.99 ± 0.23 (2.50)	4.39 ± 0.38 (5.0)	9.58 ± 0.97 (10.0)
"	Bluegill sunfish	"	0.18 ± 0.01 (0.20)	0.23 ± 0.02 (0.30)	0.34 ± 0.04 (0.50)	0.54 ± 0.07 (0.80)	0.90 ± 0.08 (1.25)
"	Rainbow trout (fingerling)	"	0.22 ± 0.01 (0.20)	0.31 ± 0.01 (0.30)	0.41 ± 0.01 (0.50)	0.57 ± 0.01 (0.80)	0.95 ± 0.01 (1.25)
"	Rainbow trout (fry)	"	0.05 ± 0.01 (0.06)	0.13 ± 0.01 (0.125)	0.20 ± 0.01 (0.25)	0.41 ± 0.01 (0.50)	0.90 ± 0.10 (1.00)
"	Coho salmon (fry)	"	0.18 ± 0.01 (0.20)	0.27 ± 0.01 (0.30)	0.36 ± 0.03 (0.50)	0.52 ± 0.02 (0.80)	1.00 ± 0.03 (1.25)
Sodium selenate	*Hyallela azeteca*	"	0.56 (0.62)	1.25 (1.25)	2.81 (2.50)	5.70 (5.0)	11.28 (10.0)

specific activity was 163 mCi/mg. Stock solutions were made by placing a known amount of stable sodium selenite in a 7 liter mariotte bottle and adding to this 7 ml of radioactive selenite-75, taken from a stock solution consisting of 2 mCi/l. The total volume was brought to 7 liters by adding deionized water. The selenite-75 contributed less than 0.001 percent of the total amount of stable selenium present and was considered insignificant.

Sampling and Analytical Procedures

Water Samples

Water samples (200 ml) were collected weekly from each exposure tank, for all experiments except the static toxicity tests. Samples were placed in glass bottles and analyzed within 2-3 hours to minimize the loss of selenium due to adsorption (Table 10). Stable isotope analysis was conducted according to the colorimetric procedure (Cummins et al., 1965) described earlier (Section I). Radioactive water samples were analyzed by counting 10 ml of water in a gamma spectrometer.

Fish Samples

Eighteen sets of fish samples were collected during the 28 day static exposure of fathead minnows to selenite-75. Each sample set consisted of 4 fish which were collected on days 0.5, 1.5, 2.5, 3, 4.5, 5.5, 6.5, 7, 7.5, 9, 10, 11, 13, 14.5, 18, 23, 25, 28. During the period of elimination 8 sets of samples with 8 fish per sample were collected on days 1, 2, 4, 9, 16, 32, 67 and 94.

The fish samples were rinsed with tap water and the whole fish placed in counting vials. Standards which occupied approximately the same space and provided a constant geometry were used to determine the concentration of selenium in the fish. After the fish were counted they were scraped with a knife to remove the slime and then recounted to determine the concentration of selenium in the slime by difference.

During the period fathead minnows were exposed to stable and radioactive selenite, 10 sets of samples were collected on days 1, 2, 4, 8, 16, 24, 32, 64 and 96. Each sample consisted of 3 fish from each of the duplicate concentrations. Eight sets of samples were collected during the period of elimination on days 2, 4, 8, 12, 16, 32, 64 and 96.

Rainbow trout which died were rinsed with tap water, wrapped in aluminum foil and frozen until the completion of the experiment at which time they were analyzed for selenium content together with those fish which survived the exposure period. The 10 larger trout were dissected into the following tissues: brain, gill, head-tail, heart, entire intestine, kidney, liver, muscle, pyloric caeca and spleen. Blood samples were also collected from each fish prior to dissection by severing the caudal peduncle and quickly collecting three or four drops of blood in a counting vial. The smaller rainbow trout and fathead minnows were dissected to provide samples of muscle, gill, entire viscera, and remaining head and tail.

Fish tissues were weighed and placed in counting vials containing 5 ml of a 2:1 mixture of nitric and perchloric acid. The vials were capped, placed in a water bath at 60 C for 12 hours and then allowed to

cool to room temperature. The volumes were brought up to 10 ml with the acid mixture. Samples which were incompletely digested were reheated for an additional 2-3 hours. The samples were then counted and compared against a 10 ml standard.

Analytical Procedure

Radioisotope analysis was performed with a Nuclear-Chicago 512 channel gamma spectrometer equipped with an automatic sample changer and a sodium iodide, thallium activated detector. All samples were counted for selenium 75 activity (at the maximum energy peak of 0.465 MeV) with constant geometry, compared with calibrated standards and corrected for physical decay and instrument efficiency. Counting times sufficient to give 95 percent statistical reliability were used for all samples (Seelye, 1974). The results of all selenium analysis are reported as the concentration of the element selenium rather than the respective compounds.

RESULTS

Static Toxicity Tests

The toxicity of sodium selenite to fathead minnows was directly related to water temperature (Figure 4). The average 96 hour LC50 values for sodium selenite with fathead minnows at 13 C, 20 C and 25 C were 10.9, 6.7 and 2.8 mg/l, respectively (Table 11). These data are not conclusive, however, because the period of exposure was too short and the exposure concentrations were greater than the asmyptotic LC50 for sodium selenite.

Fathead minnows were also exposed to sodium selenate (15 C) and the average 96 hour LC50 was 11.8 mg/l. A comparison of the LC50 values for the two selenium compounds suggests that sodium selenite is more toxic than sodium selenate.

The results of static toxicity tests conducted with rainbow trout suggest that 96 hours is not a sufficient period of time to adequately determine the asmyptotic LC50 for sodium selenite. The average 96 hour and 120 hour LC50 values were 4.35 mg/l and 2.72 mg/l, respectively (Table 11). A further decrease in the LC50 value would be expected if the tests were conducted for a longer period of time. The above data also suggest that rainbow trout are more sensitive to sodium selenite than fathead minnows.

Sodium selenite, at concentrations ranging from 1 to 40 mg/l, had no effect on the hatchability of fathead minnow eggs with an average of 99.1 percent of the eggs hatching (Huckabee and Griffith, 1974).

Table 11. The 96 hour LC50 values, confidence intervals and slope values for sodium selenate and sodium selenite determined with fathead minnows and rainbow trout by static toxicity tests.

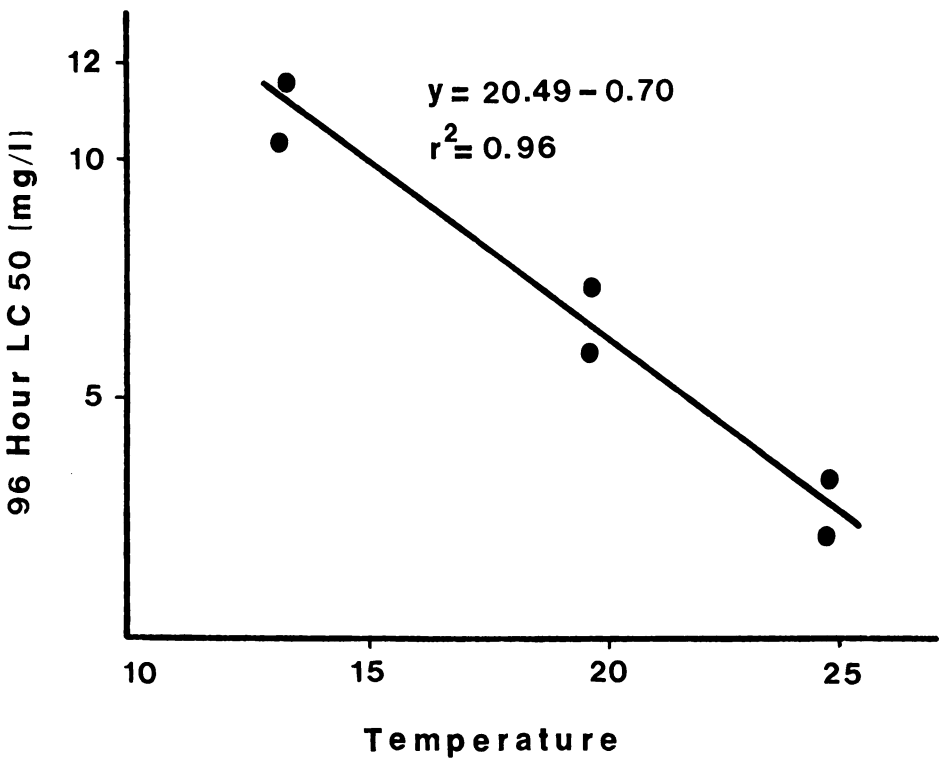
Compound	Species	Test Temperature (C)	Replicate	96 hr LC50 (mg/l)	95% Confidence Interval	Slope (s)
Sodium selenate	Fathead minnow	15	A	11.80	9.92-14.04	1.50
"	"		B	11.00	9.73-12.43	1.28
"	"		C	12.50	10.59-14.75	1.44
				$\bar{x}$ 11.76		
Sodium selenite	Fathead minnow	13	A	10.50	8.57-12.86	1.39
"	"		B	11.30	9.42-13.56	1.46
				$\bar{x}$ 10.90		
"	Fathead minnow	20	A	6.00	5.17-6.96	1.27
"	"		B	7.40	6.17-8.88	1.20
				$\bar{x}$ 6.70		
"	Fathead minnow	25	A	3.40	2.88-4.01	1.22
"	"		B	2.20	1.47-3.30	2.19
				$\bar{x}$ 2.80		
"	Rainbow trout[a]	15	A	4.50	3.98-5.08	1.23
"	"		B	4.20	3.78-4.66	1.24
				$\bar{x}$ 4.35		

Table 11 (cont'd)

Compound	Species	Test Temperature (C)	Replicate	~~96~~ 120 hr LC50 (mg/l)	95% Confidence Interval	Slope (s)
Sodium selenite	Rainbow trout[b]	15	A	2.70	2.35-3.11	1.33
"	"		B	2.75	2.57-2.94	1.12
				$\bar{x}$ 2.73		

[a,b]LC50 values calculated from the same toxicity test at 96 and 120 hours.

Figure 4. Relationship between temperature and the static 96 hour LC50 for juvenile fathead minnows exposed to sodium selenite.


12
10
5
96 Hour LC 50 (mg/l)
y = 20.49 - 0.70
r2 = 0.96
10
15
20
25
Temperature

and Griffith (1974) and Niimi and LaHam (1975) have also reported that selenium has no effect on the hatchability of carp and zebrafish eggs at concentrations up to 5 mg/l and 10 mg/l, respectively. The concentration of selenium in this experiment did, however, significantly reduce the incubation time of the eggs at concentrations of 15 mg/l and higher (Table 12) and caused a significant reduction in the post-hatch median survival time at 1 mg/l and higher (Table 13). Niimi and LaHam (1975) did not find an increase in mortality until zebrafish larvae were exposed to at least 3 mg/l of selenium dioxide (SeO_2). This compound in water would also form selenite (H_2SeO_3) suggesting that zebrafish are not as sensitive to selenite as are fathead minnow larvae. The toxicity curve, presented in Figure 5, is not asmyptotic with the time axis suggesting that additional mortality of the fathead minnow larvae would occur if concentrations less than 1 mg/l had been used.

Continuous Flow Toxicity Tests With Fish

Fathead minnows were exposed to sodium selenate and sodium selenite for 48 days and the LC50 values were found to be 2.48 mg/l and 1.08 mg/l, respectively (Table 14). Both values are considerably lower than the comparative static LC50 values and they agree with the initial finding that sodium selenite is more toxic than sodium selenate. This has also been demonstrated by Franke and Moxon (1936) using rats and by Kumar and Prakash (1971) with blue-green algae. The extended period of time during which mortality occurred (Figures 5 and 6) indicates that selenium is accumulative as has been suggested by Gortiner and Lewis (1939) and Niimi and LaHam (1975). This same phenomenon has also been described by Pickering (1972) with cadmium and bluegills.

Table 12. Percentage hatch of fathead minnow eggs exposed to sodium selenite. Initial exposure began at 2 days age (50 eggs per concentration).

Selenium Concentration (mg/l)	% Hatch, Hours After Exposure					Median Incubation Time (hrs)
	48	72	84	96	120	
Control	0	0	4	50	100	96
1	0	0	0	28	100	99
5	0	0	0	48	100	98
10	0	0	0	40	96	97
15	0	48	100			73
20	0	84	100			57
25	0	84	96			57
30	0	44	100			74
40	56	100				47

Table 13. Percent mortality and median survival time (MST) of fathead minnow fry exposed to sodium selenite (50 fry per concentration).

Selenium Concentration (mg/l)	Hours After Hatch												Post-hatch MST (hrs)
	12	24	36	48	60	84	92	108	132	180	187	204	
Control	8	--	12	12	12	12	12	16	16	36	48	100	188
1	8	--	12	12	16	24	32	40	56				120
5	8	--	12	12	12	48	96	100					85
10	4	--	20	28	36	92	92	100					62
15	20	44	--	68	100								33
20	12	48	--	92	100								25
25	52	84	--	100									11
30	28	96	100										14
40	48	--	100										12

Table 14. The LC50 values, confidence intervals and slope values of two selenium compounds with three species of fish and one invertebrate determined by continuous flow toxicity tests.

Compound	Species	Number of Days	LC50 (mg/l)	95% Confidence Interval (mg/l)	Slope (s)
Sodium selenate	Fathead minnow	48	2.00	1.56-2.56	1.66
"	*Hyallela azeteca*	4	0.76	0.49-1.19	2.11
Sodium selenite	Fathead minnow	48	1.08	0.99-1.18	1.20
"	Bluegill	48	0.40	0.35-0.46	1.16
"	Rainbow trout	48[a] 96[b]	0.50 0.28	0.47-0.53 0.26-0.30	1.10 1.07
"	Rainbow trout (fry)	21	0.46	0.43-0.49	1.19
"	Coho salmon (fry)	43	0.16	0.15-0.17	1.15

[a,b]Both values were calculated from the same test after 48 and 96 days of exposure.

Figure 5. Median survival time of fathead minnow larvae exposed to sodium selenite. The line was fitted by eye.

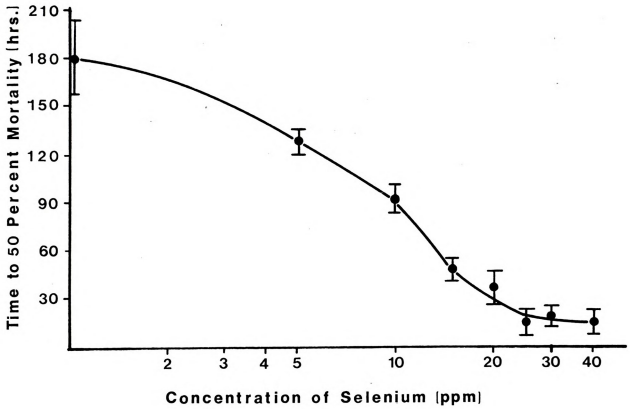
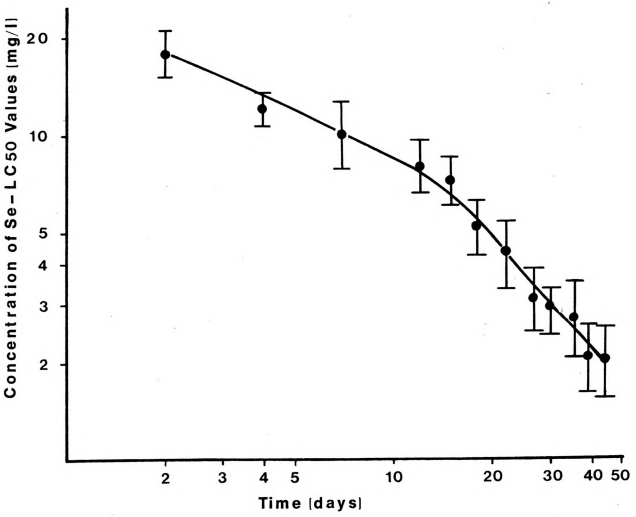

Time to 50 Percent Mortality (hrs.)
210
180
150
120
90
60
30
2
3
4
5
10
20
30
40
Concentration of Selenium (ppm)

Figure 6. The effect of time on the toxicity of sodium selenate to juvenile fathead minnows. The line was fitted by eye.


Concentration of Se-LC50 Values [mg/l]
20
10
5
4
3
2
2
3
4
5
10
20
30
40
50
Time [days]

To determine the toxicity of selenium to another species of fish, bluegills were exposed to sodium selenite for 48 days and the LC50 value was found to be 0.40 mg/l. This value is lower than the 48 day LC50 value obtained for fathead minnows suggesting that of the two species bluegills are the most sensitive. The above tests were terminated when it appeared that no additional mortality would occur, however, the toxicity curves (Figures 6 and 7) are not asmyptotic with the time axis suggesting that 48 days is not a sufficient length of time to determine the asmyptotic LC50 of selenium.

To further investigate this and to determine the toxicity of sodium selenite to an additional species of fish, rainbow trout were exposed to sodium selenite for 96 days. The LC50 values after 48 and 96 days were 0.50 mg/l and 0.29 mg/l, respectively (Table 14). The LC50 was decreased by almost one half by extending the period of exposure an additional 48 days. These data suggest that the length of time required to determine the asmyptotic LC50 should definitely be longer than 48 days and probably longer than 96 days (Figure 8). For the three species tested, the data indicates that fathead minnows are the least sensitive and rainbow trout are the most sensitive to sodium selenite.

During the course of conducting acute toxicity tests with both selenium compounds a series of symptoms were observed in the fish which lasted for a period of one to two weeks prior to death. The first symptom observed was a pronounced swelling of the abdomen followed by an obvious swelling of the entire mid-region of the fish. This condition was followed by exopthalmia and the distension of scales along the lateral line. Slight to severe hemmorrhage along the ventral midline and in the branchiostegal regions was frequently observed

Figure 7. The effect of time on the toxicity of sodium selenite to juvenile fathead minnows. The line was fitted by eye.

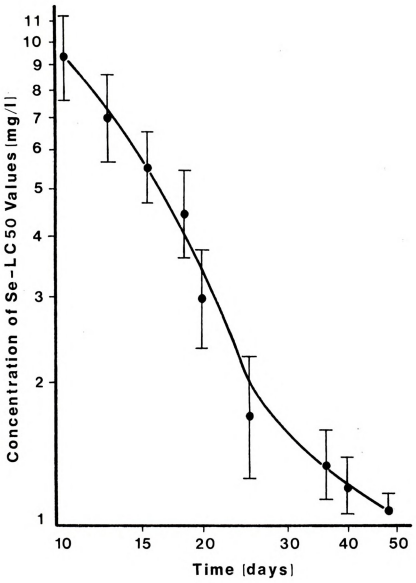
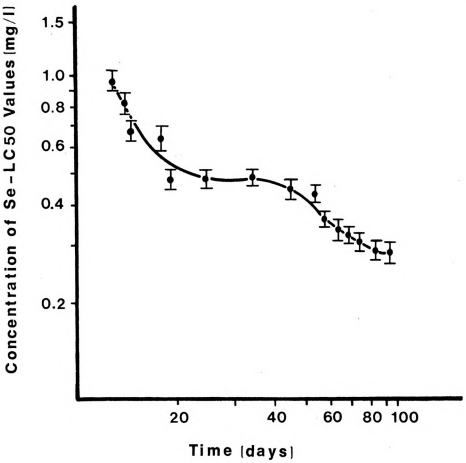

Concentration of Se-LC 50 Values (mg/l)
11
10
9
8
7
6
5
4
3
2
1
10
15
20
30
40
50
Time (days)

Figure 8. The effect of time on the toxicity of sodium selenite to fingerling rainbow trout. The line was fitted by eye.


Concentration of Se-LC50 Values (mg/l)
1.5
1.0
0.8
0.6
0.4
0.2
20
40
60
80
100
Time (days)

prior to death. Post mortem examination revealed additional signs of hemmorrhage in the lining of the peritoneum and accumulation of excess fluids in the peritoneal cavity. The liver and spleen appeared pale and the intestines and stomach appeared in a degenerative state.

The appearance of these symptoms was directly related to the concentration of selenium in the water and the length of the exposure period with the fish at the highest concentrations being the first to show signs of selenium poisoning. These symptoms were most pronounced in the fathead minnows with approximately 90 percent showing at least the initial symptom of abdomenal swelling. This was true for both sodium selenate and sodium selenite. Bluegills generally showed abdomenal swelling, but less than 50 percent showed any signs of hemmorrhage. Rainbow trout fingerlings and fry and coho fry showed the same symptoms, but to a lesser degree with only approximately 30 percent of the fish showing signs of hemmorrhage.

Rainbow trout eggs, in the eyed-up stage, were exposed to sodium selenite. After 10 days of exposure hatching was complete and ranged from 92-97 percent with no significant differences between any of the concentrations. The period of exposure was terminated 21 days after hatching was complete because of excessive mortality of the control fish. This mortality was presumably due to the failure of the fry to begin feeding. The LC50 based on the 21 days of exposure was found to be 0.46 mg/l. Because of the short period of exposure this value underestimates the toxicity of sodium selenite to rainbow trout fry. The growth of the rainbow alevin-fry was adversely affected during the 21 day exposure period. A significant reduction in both the length and weight of the fish occurred at concentrations of 0.25 mg/l Se and greater.

Seventy-five 2-day old coho salmon alevin were placed in each of six tanks and exposed to sodium selenite for 43 days. The test was terminated at this time because 50 percent mortality had been exceeded at all exposure concentrations, except the control which had 17 percent mortality. The estimated 43 day LC50 value is 0.16 mg/l. While this is lower than any of the previously determined LC50 values, it does not properly reflect the toxicity of sodium selenite to coho salmon. Had the coho larvae been exposed to lower concentrations over an extended period of time the asmyptotic LC50 would have been lower. Fathead minnow larvae were also exposed to sodium selenite, however, the data are inclusive because of the high mortality in the control fish, but it does suggest that the LC50 for fathead minnow larvae is less than 0.1 mg/l.

Invertebrate Exposure

Immature amphipods were exposed to sodium selenate in a continuous flow system for 96 hours with the resulting LC50 value of 0.76 mg/l. The 4 and 14 day LC50 values for the same species using sodium selenite was determined in an earlier study (unpublished) and was found to be 0.34 mg/l and 0.07 mg/l. These values once again reflect the greater toxicity of sodium selenite and they suggest that amphipods may be somewhat more sensitive to selenium than fish. However, this may not be true because 50 percent mortality was exceeded at all exposure concentrations with the coho larvae. It is quite probable that the asmyptotic LC50 for coho larvae is smaller than the observed LC50 value (0.16 mg/l) and it may be as small as the 14 day LC50 for amphipods.

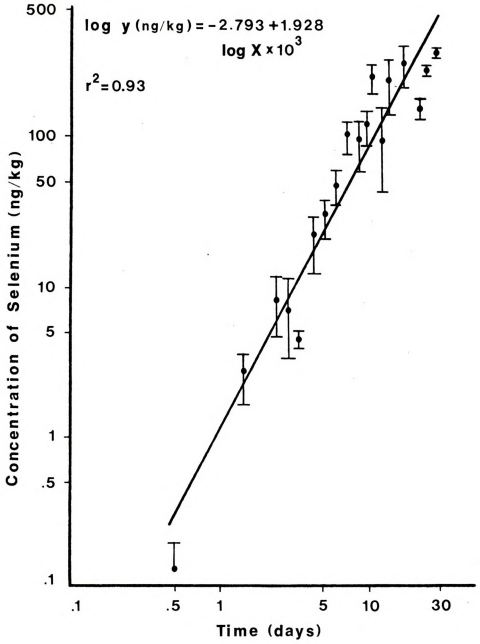
A chronic bioassay conducted with *Daphnia magna* in an earlier set of experiments (unpublished) revealed the maximum acceptable toxicant concentration for sodium selenite to be 0.28 mg/l. The 14 day LC50 was determined to be 0.43 mg/l. These values are in general agreement with the LC50 values determined for bluegills and rainbow trout in this experiment (Table 14) and suggest that daphnia are no more sensitive to selenium than fish.

Uptake of Selenium - Static Exposure

The uptake of selenium occurred in a linear manner throughout the entire 28 day period suggesting that a longer period of exposure is needed for equilibrium to be reached in the fish. Equilibrium, according to Macek (1975), may be defined as that time during the period of exposure where means obtained at three successive sampling periods do not statistically differ from each other and therefore indicate that the rate of elimination equals the rate of accumulation. The uptake data are presented using a log transformation of the tissue concentrations to demonstrate the initial rapid period of accumulation (Figure 9). A log transformation of both the tissue concentration (ng/kg) and the number of days ($x10^3$) was used to facillate the calculation of an equation for the rate of accumulation. The mathematical expression employed to characterize the rates of uptake and elimination for this and all subsequent experiments was a simple linear regression of selenium concentration on a wet weight basis against the exposure time in days. The uptake and elimination data are described by the general regression equation of the form:

$$Y = a + b\ (x)$$

Figure 9. The accumulation of selenium by juvenile fathead minnows during static exposure to selenite-75.


log y (ng/kg) = -2.793 +1.928
log X x 10^3
r^2 = 0.93
Concentration of Selenium (ng/kg)
500
100
50
10
5
1
.5
.1
.1
.5
1
5
10
30
Time (days)

where: Y = the concentration of total selenium residue in the fish tissue

a = the y-intercept of the regression line

b = the rate of uptake or elimination (slope)

x = the exposure time in days.

The y-intercept, slope and coefficient of determination (r^2) for the rate of accumulation using the described log-log conversion are -2.793, 1.298 and 0.931, respectively. The rate of accumulation of selenium may be expressed using the slope value, as 1.928 ng/Kg of body weight per (log) day ($x10^3$).

Bioconcentration factors were determined by dividing the maximum residue concentration (mg/Kg) by the mean concentration (mg/l) of selenium in the water during the total period of exposure. The maximum bioconcentration factor determined during this experiment was 4,443. This occurred on day 28 when the average whole body selenium residue in the fish was 368.7 ng/Kg. This large bioconcentration factor suggests that selenium is readily taken up by fish when present in the water in extremely small amounts.

Of the total amount of selenium in the fish only 6.36 ± 0.70 percent occurred in the slime. This low percentage of selenium in the slime plus the large bioconcentration factor suggests that selenium is not merely adsorbed on the fish but is absorbed across the gill membranes, assuming that uptake via the gastrointestinal tract is minimal.

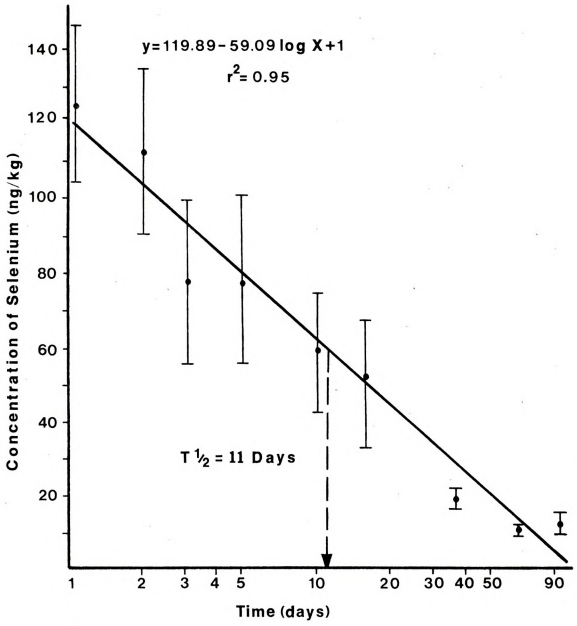
Fish which were used to measure uptake and elimination were kept in separate tanks and on day 28 when the last set of samples for uptake and the first set of samples for elimination were analyzed the fish collected for measurement of elimination were found to contain significantly

less selenium than the fish sampled for uptake (Figures 9 and 10). This difference is due to the fact the average concentration of selenium in the water was 0.083 ± 0.001 ng/l for the tanks designated for the uptake study and only 0.075 ± 0.005 ng/l in the tanks designated for measurement of elimination rates. The different selenium concentrations in the two sets of tanks resulted from the fact that during the period of uptake 18 sets of fish samples were collected from the tanks designated for measurement of accumulation, but no fish were collected from the tanks designated for measurement of elimination rates.

Elimination was measured over a 96 day period and occurred in a curvilinear manner. In order to describe the rate of elimination a log transformation of the time axis (days) was used so that a straight line could be fitted to the data by linear regression analysis (Figure 10). The y-intercept, slope and coefficient of determination for this regression is 119.89, -51.09 and 0.95, respectively. Calculation of the biological half-life, the time for 50 percent of the selenium residue to be eliminated, was done graphically and by substituting 50 percent of the calculated initial body burden as ng/Kg into the regression equation. The half-life was found to be 10.3 days. Using the calculated negative slope for the regression equation, the rate of elimination may be expressed as 59.09 ng/Kg of body weight per (log) day.

The amount of selenium remaining in the fish after 96 days was 13.05 ng/Kg, 10.3 percent of the initial amount. At this time the elimination curve was asmyptotic with the time axis and no further significant elimination of selenium was expected.

Figure 10. The elimination of selenium by juvenile fathead minnows after static exposure to selenite-75.


y=119.89-59.09 log X+1
r²= 0.95
T ½ = 11 Days
Concentration of Selenium (ng/kg)
140
120
100
80
60
40
20
1
2
3
4
5
10
20
30
40
50
90
Time (days)

Uptake, Distribution and Elimination of Selenium - Continuous Flow Exposure System

The mean concentrations of selenium in the duplicate tanks during the periods of exposure were compared by Students t test (P = 0.01) and no significant difference was found. The average concentrations of selenium in the water during the period of exposure were 11.57 ± 0.42 µg/l, 24.42 ± 0.84 µg/l and 50.57 ± 1.49 µg/l. These concentrations were selected because it was thought that information on the exposure of fish at natural levels would be of aid in evaluating the concentrations found in the muscle tissue of fish collected from Lake Erie (Table 5). The concentrations of selenium used in this experiment were not lethal to the fathead minnows although there was some initial mortality, less than 5 percent in all treatments and controls.

No significant difference was found between replicate fish samples collected during the periods of accumulation and elimination. The data was combined and the results are presented as the mean of six samples for each fish at each sampling date.

The accumulation of selenium occurred in a curvilinear manner in the whole fish and all tissues with a rapid period of accumulation occurring in approximately the first 8 days and a slower rate of accumulation occurring during the remaining 88 days. The data for the whole fish and individual tissues are presented using a log transformation of the tissue concentrations in order to provide an accurate description of the initial rapid period of accumulation (Figures 11-15). In each tissue it appears that the equilibrium concentration was being approached after 96 days of exposure. However, the data does not adequately meet the previously stated definition of equilibrium

Figure 11. The accumulation of selenium in the viscera of adult fathead minnows.

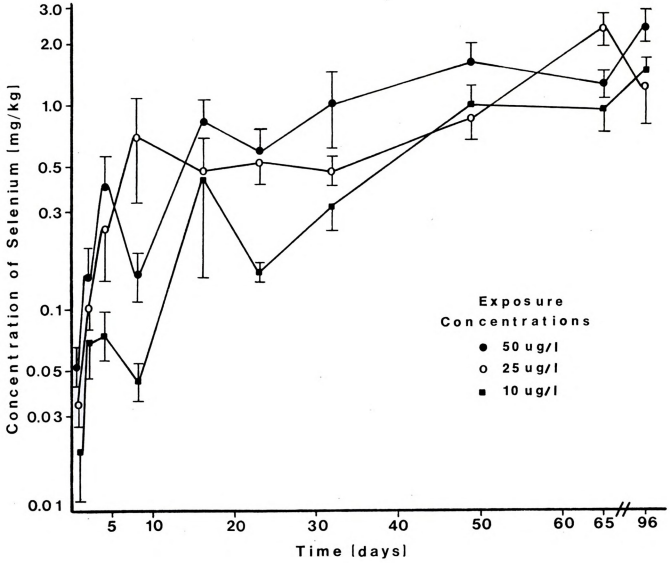

Concentration of Selenium [mg/kg]
3.0
2.0
1.0
0.5
0.3
0.1
0.05
0.03
0.01
5
10
20
30
40
50
60
65
96
Time [days]
Exposure
Concentrations
50 ug/l
25 ug/l
10 ug/l

Figure 12. The accumulation of selenium in the gills of adult fathead minnows.

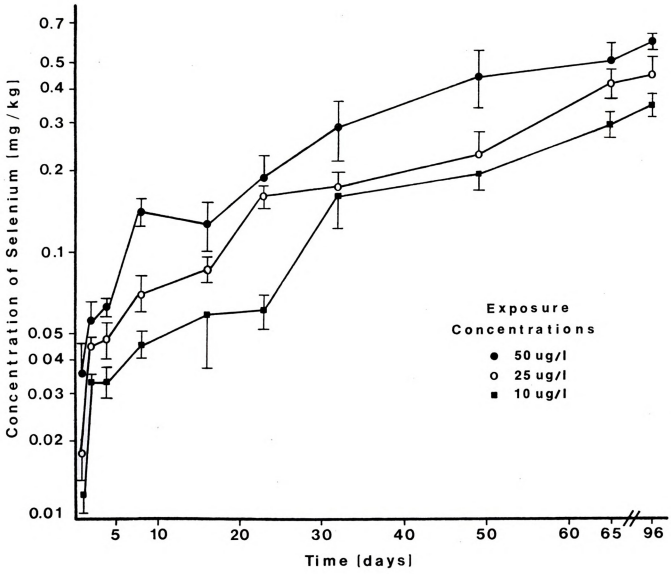

Concentration of Selenium [mg/kg]
0.7
0.5
0.4
0.3
0.2
0.1
0.05
0.04
0.03
0.02
0.01
5
10
20
30
40
50
60
65
96
Time [days]
Exposure
Concentrations
50 ug/l
25 ug/l
10 ug/l

Figure 13. The accumulation of selenium in the head and tail of adult fathead minnows.

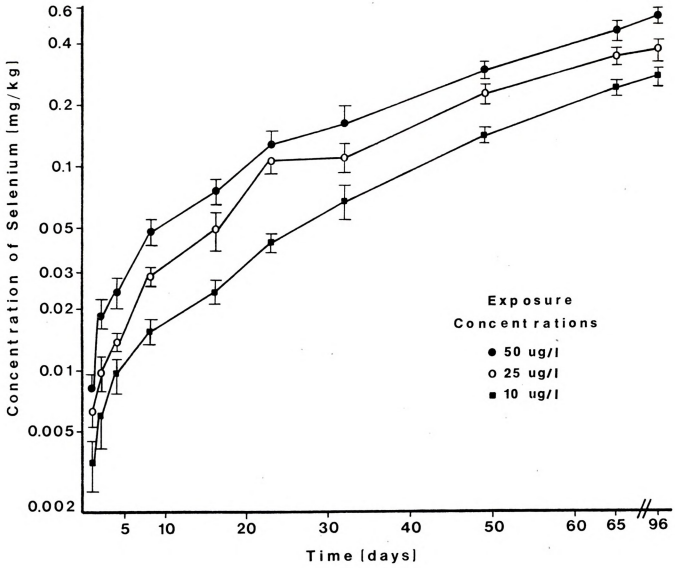

Concentration of Selenium [mg/kg]
0.6
0.4
0.2
0.1
0.05
0.03
0.02
0.01
0.005
0.002
5
10
20
30
40
50
60
65
96
Time [days]
Exposure
Concentrations
● 50 ug/l
o 25 ug/l
■ 10 ug/l

Figure 14. The accumulation of selenium in the muscle of adult fathead minnows.

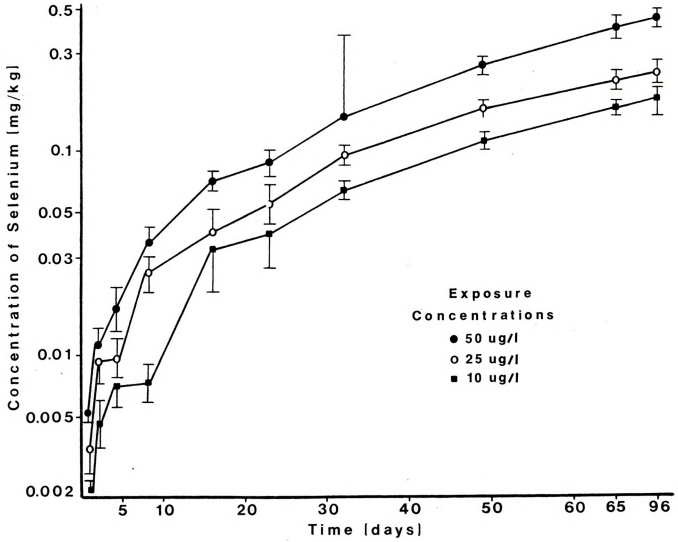
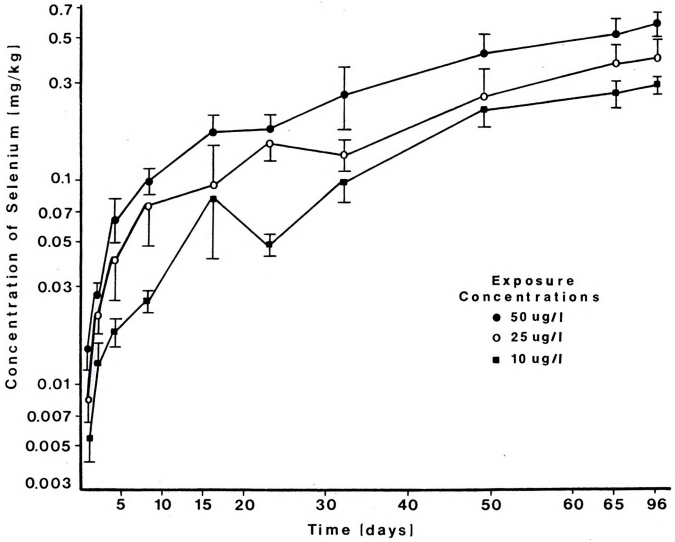

0.5
0.3
0.1
0.05
0.03
0.01
0.005
0.002
Concentration of Selenium [mg/kg]
5
10
20
30
40
50
60
65
96
Time [days]
Exposure
Concentrations
● 50 ug/l
o 25 ug/l
■ 10 ug/l

Figure 15. Whole-body accumulation of selenium by adult fathead minnows.


Concentration of Selenium [mg/kg]
0.7
0.5
0.3
0.1
0.07
0.05
0.03
0.01
0.007
0.005
0.003
5
10
15
20
30
40
50
60
65
96
Time [days]
Exposure Concentrations
● 50 ug/l
o 25 ug/l
■ 10 ug/l

concentration and therefore suggests that the time for true equilibrium to occur is longer than 96 days. It does appear, however, that if one additional set of samples had been collected at the sampling interval of 32 days, that equilibrium would have occurred at 128 days. A log transformation of both the tissue concentration and the sampling time was used to calculate a regression equation which accurately describes the rates of accumulation for each tissue. Based on this log-log transformation the rate of accumulation of selenium for the various tissues can be expressed by their respective slope values which are presented in Table 15 together with the confidence intervals on the slope values, y-intercepts and coefficients of determination.

The viscera consistently accumulated a greater amount of selenium than the other tissues regardless of exposure concentration. The largest concentration of selenium that occurred was 2.44 mg/Kg in the viscera of fish exposed to 50 µg/l for 96 days. The viscera also showed the largest amount of variability. Part of this variability may be due to the amount of selenium adsorbed on the food and would reflect the individual feeding habits of the fish. Sandholm et al. (1973) have demonstrated that a commercial fish food (Tetra Min) is capable of adsorbing selenite-75 from water in significant amounts over a 24 hour period. To reduce this variability and to minimize the uptake of selenium via the gastrointestinal tract the fish were fed only once a day. The food was usually consumed within a few minutes after feeding. The possibility that some unknown amount of selenium may have been adsorbed through the gastrointestinal tract, however, cannot be entirely dismissed.

Table 15. Accumulation of selenium in fathead minnows exposed to three concentrations of selenium for 96 days. Regression values were obtained by plotting log concentration (µg/Kg) against log days.

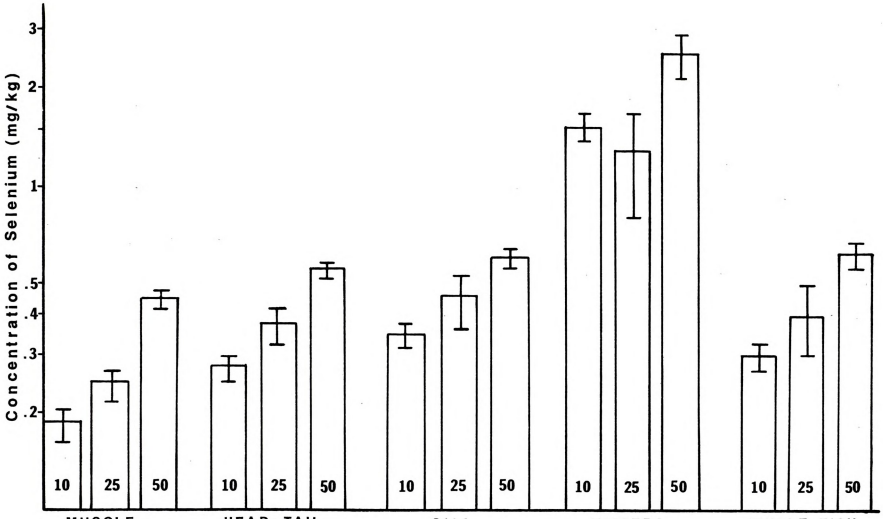
Tissue	Exposure Concentration (µg/l)	Y Intercept (log µg/Kg)	Rate of Accumulation (slope)	95 Percent Confidence Interval on Slope	Coefficient of[a] Determination (r^2)	Bioconcentration Factor
Muscle	10	0.185	1.067	±0.091	0.972	18.0
"	25	0.541	0.951	±0.032	0.983	9.8
"	50	0.642	1.021	±0.198	0.991	8.8
Gill	10	1.120	0.673	±0.283	0.916	34.0
"	25	1.294	0.655	±0.398	0.956	17.6
"	50	1.498	0.632	±0.459	0.965	11.6
Viscera	10	1.314	0.886	±0.427	0.848	149.9[b]
"	25	1.705	0.789	±0.538	0.832	93.0
"	50	1.777	0.798	±0.553	0.888	48.7
Head-tail	10	0.414	0.981	±0.147	0.964	27.0
"	25	0.673	0.958	±0.213	0.978	14.8
"	50	0.870	0.929	±0.269	0.985	10.8
Whole fish	10	0.757	0.865	±0.243	0.953	29.2
"	25	1.024	0.816	±0.317	0.972	15.6
"	50	1.241	0.804	±0.383	0.964	12.0

[a]Coefficient of determination for the linear regression of y on x.
[b]Calculated after 65 days of exposure instead of 96 days.

The maximum concentration of selenium in the gill, head-tail, muscle and whole fish was 0.58, 0.54, 0.44 and 0.60 mg/Kg, respectively, and occurred after 96 days of exposure to 50 µg/l of selenite (Figure 16). The largest concentrations of selenium were consistently found in the tissues of the fish exposed to 50 µg/l Se and they were significantly greater than the residue levels in the tissues of the fish exposed to 10 µg/l Se. These data suggest that tissue residue levels are directly related to the exposure concentration. However, by using the previously described log-log transformation for the data in Figures 11-15, it was found that the three regression lines for each tissue were not significantly different. Therefore, there is not enough evidence to definitely conclude that exposure concentration influences the tissue concentration, but the data do suggest this. This view is further supported by comparing the whole-body accumulation of selenium by the fish in both this and the previous experiment (Figures 9 and 15).

The concentration of selenium in the muscle of fish exposed to 10, 25 and 50 µg/l for 96 days was 0.18, 0.25 and 0.44 mg/Kg, respectively. These values are smaller than the residue levels found in most of the fish (0.74 mg/Kg) analyzed from Lake Erie even though the exposure concentrations were somewhat larger than the reported levels of selenium in Lake Erie waters (Tables 2 and 5). On the basis of this experiment it does not appear that the concentration of selenium (as selenite) in the water can entirely account for the residue levels in the Lake Erie fish. Data supporting this view has also been reported by Sandholm et al. (1973).

Figure 16. The average concentration of selenium (± 1 S.E.) in the tissues and whole-body of adult fathead minnows after 96 days of exposure to sodium selenite at concentrations of 10, 25 and 50 µg/l.


Concentration of Selenium (mg/kg)
3
2
1
.5
.4
.3
.2
10
25
50
MUSCLE
HEAD–TAIL
GILL
VISCERA
WHOLE FISH

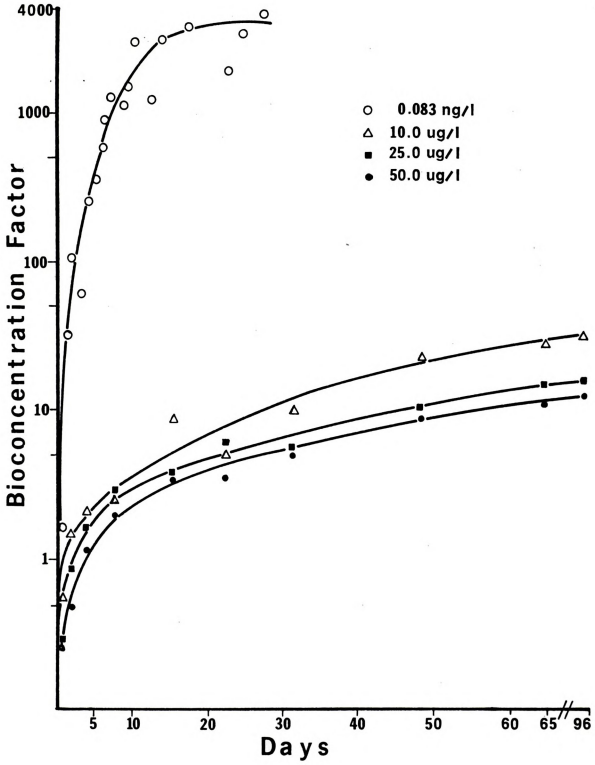

4000
1000
100
10
1
Bioconcentration Factor
0.083 ng/l
10.0 ug/l
25.0 ug/l
50.0 ug/l
5
10
20
30
40
50
60
65
96
Days

Figure 18. The elimination of selenium by the viscera of adult fathead minnows.

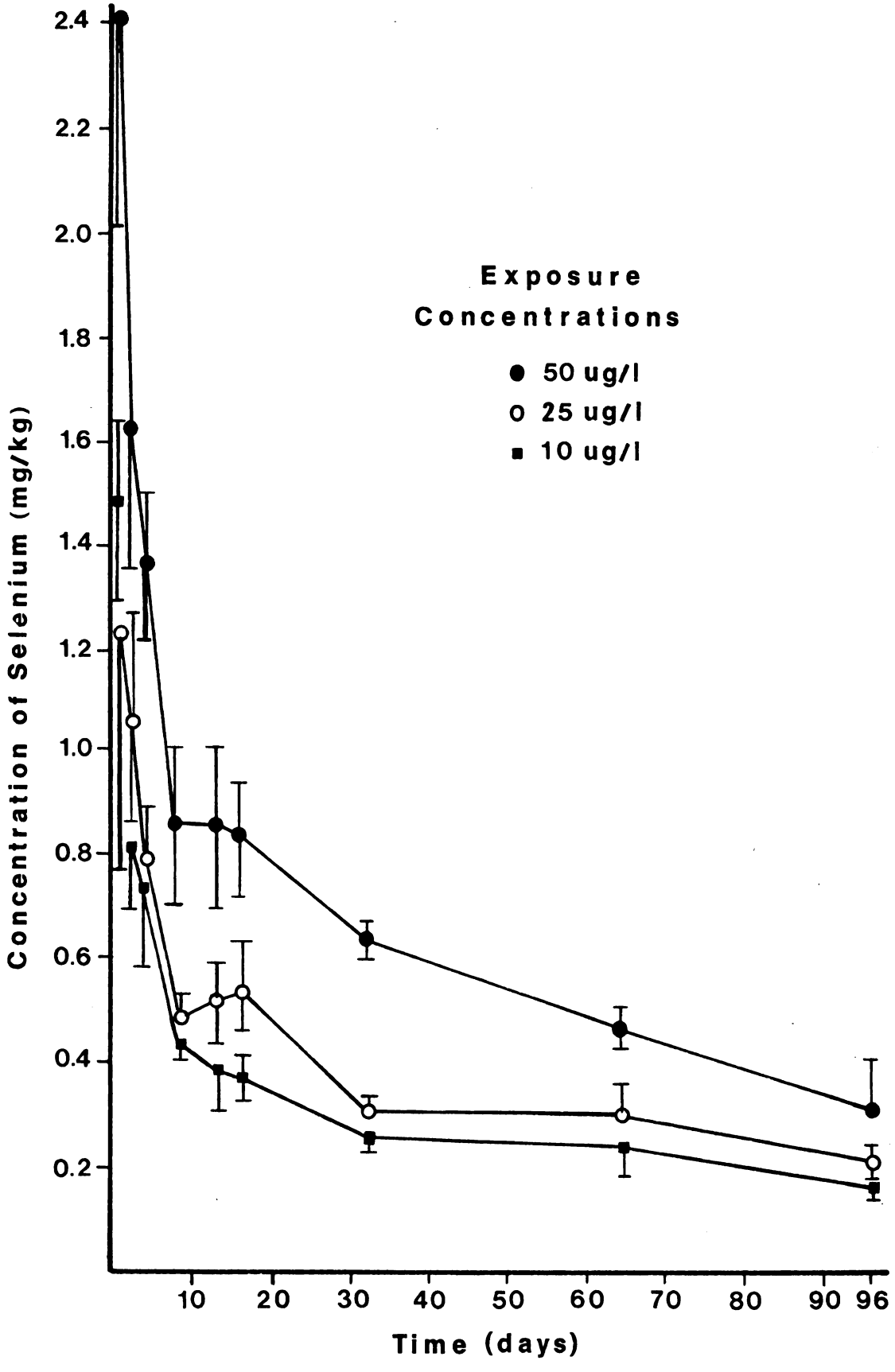

Exposure
Concentrations
● 50 ug/l
o 25 ug/l
■ 10 ug/l
Concentration of Selenium (mg/kg)
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
10
20
30
40
50
60
70
80
90
96
Time (days)

Figure 19. The elimination of selenium by the gills of adult fathead minnows.

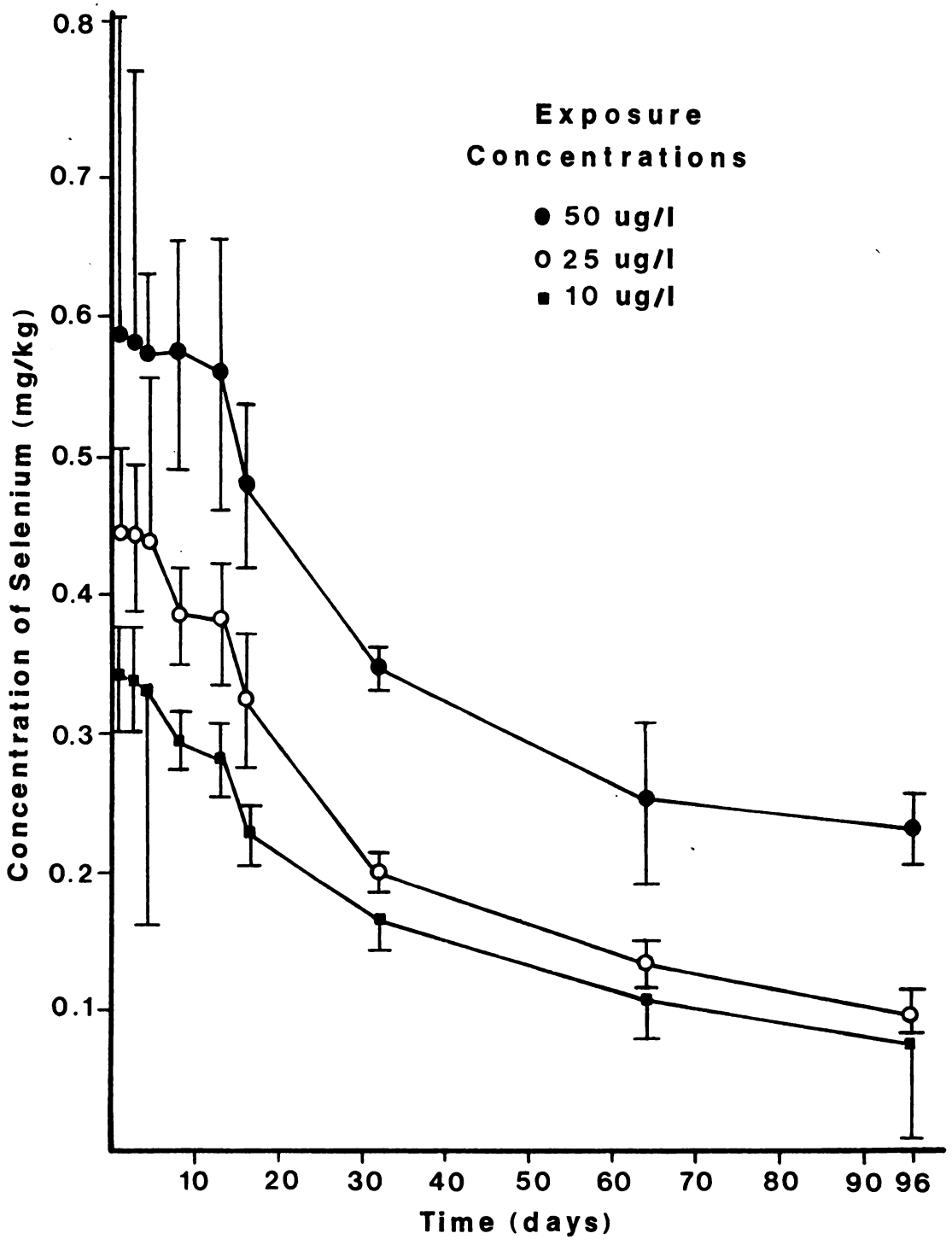

Exposure
Concentrations
● 50 ug/l
o 25 ug/l
■ 10 ug/l
Concentration of Selenium (mg/kg)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
10
20
30
40
50
60
70
80
90
96
Time (days)

Figure 20. The elimination of selenium by the head and tail of adult fathead minnows.

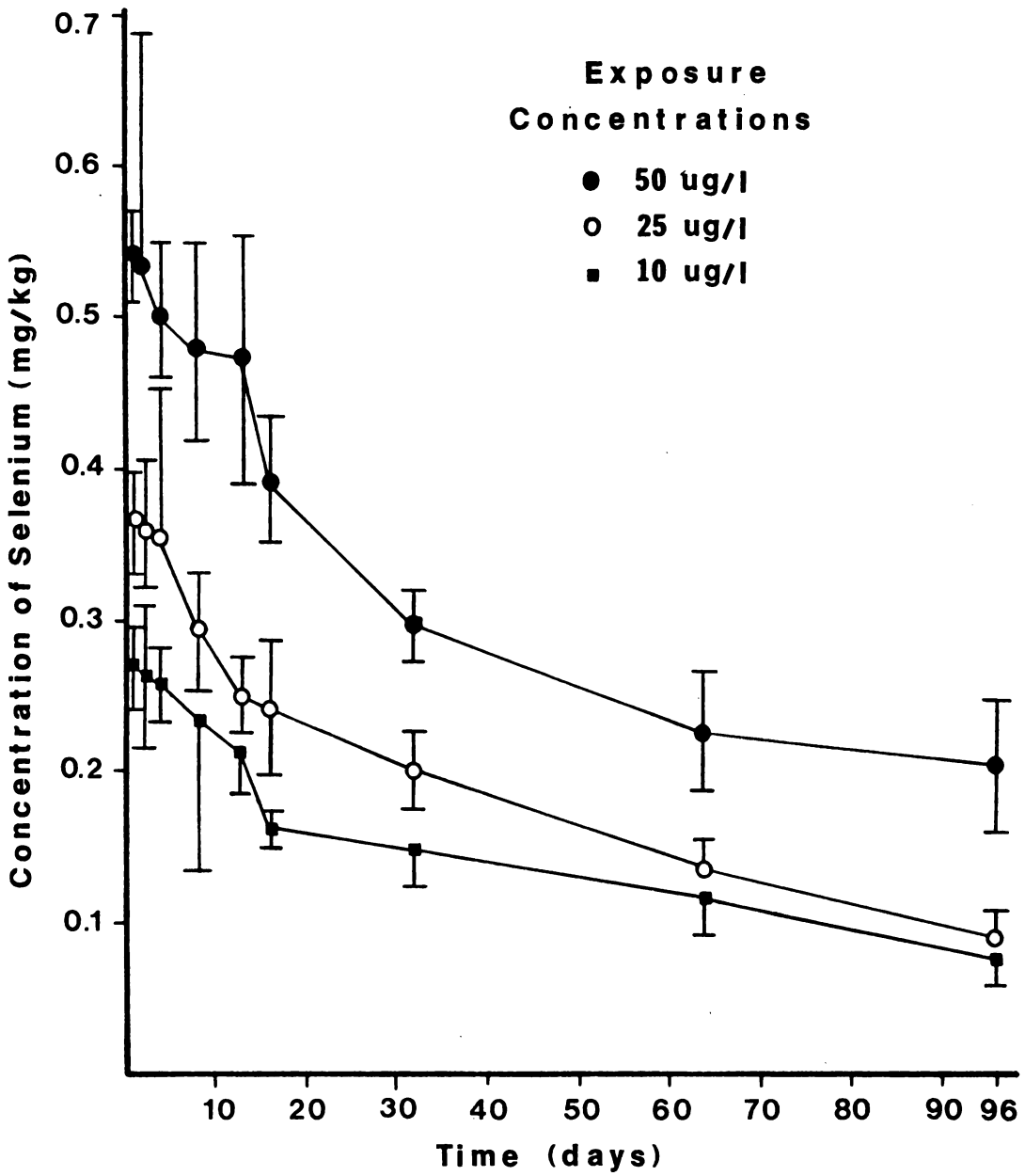

Exposure
Concentrations
50 ug/l
25 ug/l
10 ug/l
Concentration of Selenium (mg/kg)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
10
20
30
40
50
60
70
80
90
96
Time (days)

Figure 21. The elimination of selenium by the muscle of adult fathead minnows.

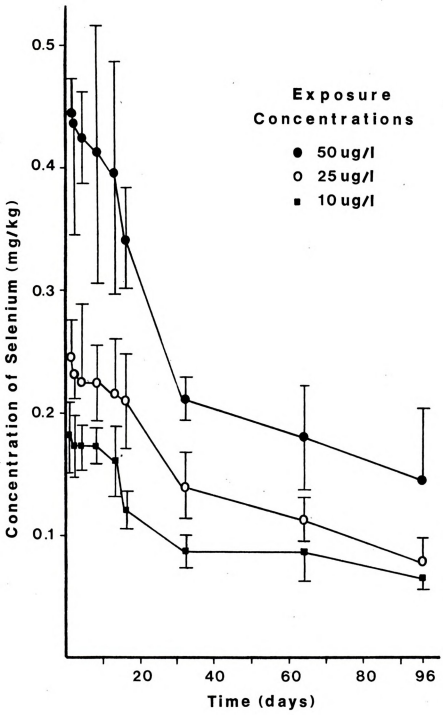
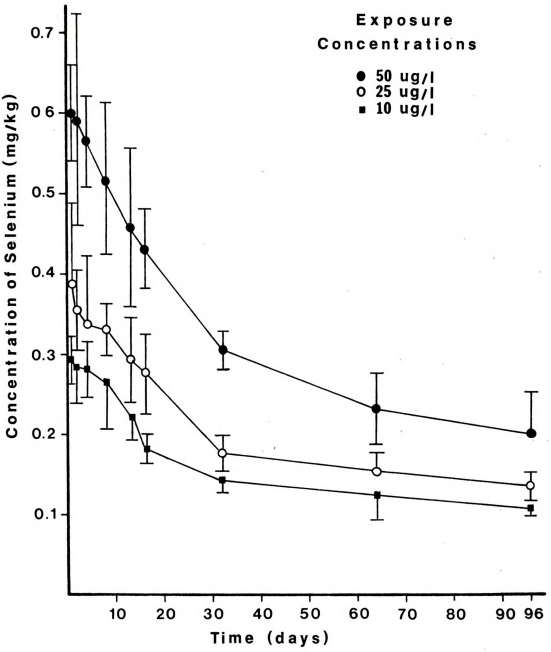

Exposure
Concentrations
50 ug/l
25 ug/l
10 ug/l
Concentration of Selenium (mg/kg)
0.5
0.4
0.3
0.2
0.1
20
40
60
80
96
Time (days)

Figure 22. Whole-body elimination of selenium by adult fathead minnows.


Exposure
Concentrations
● 50 ug/l
○ 25 ug/l
▪ 10 ug/l
Concentration of Selenium (mg/kg)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
10
20
30
40
50
60
70
80
90
96
Time (days)

The rate of elimination of selenium from the viscera was found to be significantly faster than the other tissues. The viscera had an average half-life of 5.1 days as compared to half-lives generally in excess of 50 days for the other tissues (Table 16). This suggests that the rate of exchange of selenium between the internal organs, including the liver, spleen and kidney, with the plasma is more rapid and complete than in the muscle and gill. The elimination of selenium from the muscle and whole fish, as depicted by their half-lives, appears to be inversely related with the exposure concentration. This relationship is not apparent in the other tissues, however, a similar relationship has been reported by Lopez et al. (1969) for sheep after administering selenite-75 both orally and intravenously at four different concentrations.

Accumulation and Distribution of Selenium in Rainbow Trout

The largest concentration of selenium occurred in the viscera followed by the gill, head-tail and muscle. This same relative distribution was also found in fathead minnows in the previous experiment. The exposure concentrations of this experiment were approximately one order of magnitude larger than were used in the previous experiment and the residue levels in the trout remaining alive at the end of the exposure period were significantly higher than found in the fathead minnows (Table 17). This is consistent with the previous data which suggest that the accumulation of selenium is directly related to the exposure concentration. However, the average concentration of selenium in the tissues of the trout exposed to 0.41 mg/l and collected alive was

Table 17. Average concentration of selenium in the tissues of rainbow trout exposed to four concentrations of selenium. Standard errors are in parentheses.

Exposure Concentration (mg/l)	Average Time of Exposure (days)	Fate	Number of Fish	Mean Concentration of Selenium in the Tissues (mg/Kg)				
				Viscera	Gill	Head-tail	Muscle	Whole Fish
0.95	14.0 (13-15)[a]	Dead	17	8.90 (0.69)	5.50 (0.51)	3.31 (0.22)	1.57 (0.12)	2.83 (0.14)
0.57	18.8 (14-19)	Dead	17	8.40 (0.58)	4.49 (0.66)	3.90 (0.26)	2.00 (0.14)	3.20 (0.19)
0.41	75.5 (47-94)	Dead	8	19.49 (3.22)	4.35 (0.74)	2.32 (0.16)	1.58 (0.19)	3.49 (0.38)
0.41	96.0	Alive	12	15.93 (1.71)	2.92 (0.29)	1.65 (0.14)	0.81 (0.11)	2.46 (0.18)
0.31	70.0 (50-82)	Dead	20	16.28 (0.91)	3.05 (0.20)	1.90 (0.10)	1.27 (0.08)	2.75 (0.08)
0.31	96.0	Alive	7	19.26 (2.38)	4.42 (0.40)	1.93 (0.17)	0.98 (0.07)	3.25 (0.25)

[a]Range of days of exposure.

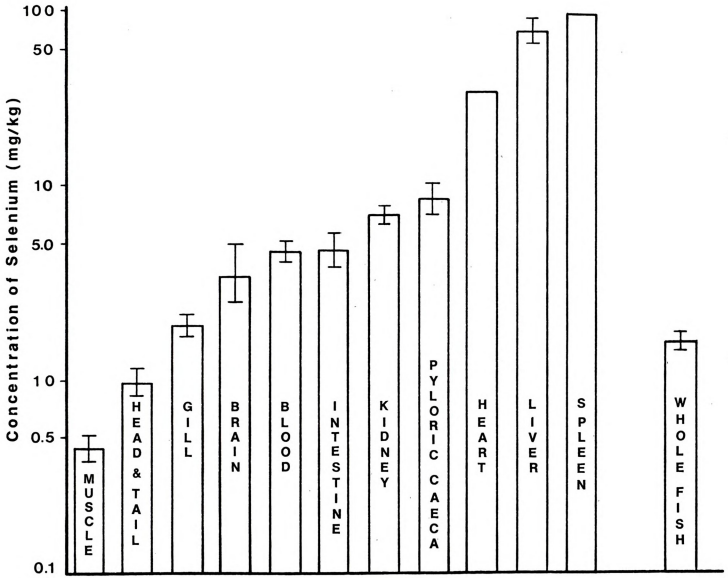
lower than the concentration in the trout exposed to 0.31 mg/l, although the difference is not significant.

The concentration of selenium in the tissues of the dead fish were generally higher than in the fish remaining alive at the end of the experiment, except for the fish exposed to 0.31 mg/l. At all concentrations the muscle contained significantly less selenium than any other tissue and the muscle of fish collected alive contained significantly less than the muscle of the fish collected dead. The average concentrations of selenium in the muscle of the fish collected alive and dead were 1.61 ± 0.18 mg/Kg and 0.90 ± 0.11 mg/Kg, respectively.

The bioconcentration factors in the trout exposed to 0.31 mg/l for 96 days were 62.1, 14.3, 6.3, 3.2 and 10.5 for the viscera, gill, head-tail, muscle and whole fish, respectively. The maximum whole-body bioconcentration factors obtained by fathead minnows in the previous two experiments and the rainbow trout in this experiment were 4,443, 29.2 and 10.5, respectively, and the exposure concentrations were 0.083 ng/l, 50.6 µg/l and 0.31 mg/l, respectively. These data clearly show the inverse relationship between bioconcentration factor and exposure concentration and they demonstrate that bioconcentration factors may vary by several orders of magnitude as a direct result of different exposure concentrations.

The largest concentration of selenium in the tissues of six rainbow trout (12 cm) exposed to a mixture of radioactive and stable selenite (0.22 mg/l) for 48 days occurred in the spleen followed by the liver and heart (Figure 23). Because of the minute size of the spleen and heart these tissues were analyzed collectively and it was

Figure 23. The average concentration of selenium (± 1 S.E.) in the tissues and whole-body of rainbow trout after 48 days of exposure to sodium selenite at a concentration of 0.22 mg/l.


Concentration of Selenium (mg/kg)
100
50
10
5.0
1.0
0.5
0.1
MUSCLE
HEAD & TAIL
GILL
BRAIN
BLOOD
INTESTINE
KIDNEY
PYLORIC CAECA
HEART
LIVER
SPLEEN
WHOLE FISH

not possible to calculate standard errors. Previous studies by Hopkins et al. (1966), Lindberg and Siren (1963), Rosenfeld (1964), Rosenfeld and Eppson (1964) and Wright (1965) using other species including mice, rats, dogs and swine have not demonstrated such high levels of selenium in the spleen and heart. They have found the greatest accumulation of selenium in the liver, kidney and lungs. The large concentrations of selenium in the liver and spleen of the fish in this experiment suggest that selenium is actively metabolized and detoxified in these organs. They also provide an explanation for the large concentrations of selenium found in the viscera of the fathead minnows and rainbow trout in the previous experiments.

The muscle of the rainbow trout was found to contain significantly less selenium than the other tissues with an average concentration of 0.44 ± 0.05 mg/Kg. A comparison of the average whole-body content of selenium (17.23 µg/fish) with the average total amount of selenium in the muscle (3.39 µg) shows that only 19.7 percent of the whole-body selenium content is contained in the muscle. This indicates that approximately 80 percent of the whole-body selenium content would be lost in the process of cleaning fish for consumption. The data from the previous experiments with rainbow trout and fathead minnows indicates that the muscle contains 20 and 27 percent, respectively, of the total amount of selenium in the whole fish. These data point out that the muscle would be the most important tissue for analysis when conducting routine environmental surveys of the selenium content in fish.

DISCUSSION

Toxicity Tests

The results clearly show that the toxicity of selenium was greater in the continuous flow tests than in the static tests. The extended period of time during which mortality occurred demonstrates the accumulative nature of selenium and indicates that 96 hours is not a sufficient length of time to determine the asmyptotic LC50 for either sodium selenate or sodium selenite. Tests conducted with fathead minnows showed a reduction in the LC50 values from 11.8 and 10.9 mg/l at 4 days to 2.0 and 1.1 mg/l at 48 days for sodium selenate and sodium selenite, respectively. Rainbow trout exposed to sodium selenite had 48 and 96 day LC50 values of 0.50 mg/l and 0.28 mg/l, respectively. These data indicate that at least a 96 day period of exposure is needed to determine the asmyptotic LC50 for inorganic selenium compounds.

The results of the static toxicity tests indicate that selenium is more toxic at higher temperatures, however, the data are not conclusive because of the short periods of exposure. Brown (1973) has suggested that increasing the temperature might decrease the toxicity of many chemicals if the test is conducted over a long period of time and at concentrations near the asmyptotic LC50. He suggests that higher temperatures at these concentrations will increase the metabolic rate and will in fact favor the detoxification mechanisms in fish and therefore fish will actually be more sensitive to many chemicals at lower temperatures.

The acute toxicity of selenium at concentrations ranging from 0.16 mg/l to 2 mg/l (Table 14) as determined with several species of fish in this study, is not in agreement with previously reported lethal levels of 2-5 µg/l for adult goldfish (Ellis et al., 1937), 3 µg/l for larval zebrafish exposed to selenium dioxide for 10 days (Niimi and LaHam, 1975) or 12 µg/l for goldfish exposed to selenium dioxide for 7 days (Weir and Hine, 1970). The above values were all higher than the LC50 values obtained in this study by continuous flow tests and the difference appears to be due to the short periods of exposure. The 96 hour LC50 values obtained in the present study using static toxicity tests more closely approximate the previous values and provide further evidence that previous studies have underestimated the toxicity of selenium. It is also important to point out that the LC50 values obtained in this study were for a water hardness of 330 mg/l $CaCO_3$ as compared to 50 mg/l $CaCO_3$ for the tests conducted by Niimi and LaHam (1975) and Weir and Hine (1970). If soft water had been used in the present study the LC50 values would be expected to be even smaller.

The use of long term chronic bioassays, where a species of fish is exposed to a chemical throughout its entire life cycle, has been suggested as a way of determining the maximum acceptable toxicant concentration (MATC) or "safe" concentration of a chemical and as a way of providing criteria for the establishment of water quality standards for the protection of fish popualtions (McKim and Benoit, 1971; Mount and Stephan, 1967, 1969). As an alternative to a long term chronic test, Eaton (1974) has suggested the use of a partial chronic test where only the fish larvae are exposed to the chemical

for a period of 30 to 60 days. This suggestion is based on the fact that the larvae were found to be the most sensitive stage in the life cycle of the bluegill when exposed to cadmium. Similar findings have also been reported by Akiyama (1970) for *Oryzais latipes* with mercury, by Benoit (1975) using bluegills and copper, and Hazel and Meith (1970) using copper and king salmon, by McKim and Benoit (1971, 1974) using brook trout and copper and by Skidmore (1965) using zinc and zebrafish.

In order to determine the concentration of selenium which might be considered "safe" for salmonid fishes, coho salmon larvae were exposed to sodium selenite for 43 days and the LC50 value was calculated to be 0.16 mg/l. Although this is the lowest LC50 obtained in this study, it does not properly reflect the toxicity of sodium selenite to coho larvae because 50 percent mortality was exceeded at all test concentrations. It does, however, provide a relative indication of the toxicity of sodium selenite and suggests that a "safe" concentration would be less than 0.1 mg/l. This view is supported by the 14 day LC50 (0.07 mg/l) obtained for amphipods exposed to sodium selenite. While no "safe" concentration of selenium can be predicted by these data, they do suggest that concentrations in excess of 0.07 mg/l would result in damage to both fish populations and their food species.

Uptake, Distribution and Elimination Studies

Uptake studies with selenite-75 suggest that the accumulation of selenium by fish is directly related to the exposure concentration. The accumulation of selenium in all tissues occurred in a curvilinear manner with the rate of accumulation becoming asmyptotic with the time axis after 96 days of exposure. The data suggest that an equilibrium

concentration of selenium in the fish would be reached in approximately 128 days.

The distribution of selenium in both rainbow trout and fathead minnows showed the viscera to have the largest concentration of selenium followed by the gill, head-tail and muscle. Analysis of the visceral organs of six larger rainbow trout revealed that the large concentrations of selenium in the viscera of these fish was due to the high selenium content of the spleen, liver and heart and to a lesser extent the kidney and pyloric caeca (Figure 23).

The results from the uptake experiments suggest that the muscle would be the tissue of choice for conducting routine environmental surveys of the selenium content in fish. This conclusion is based on the fact that the concentration of selenium in the muscle has been shown to be consistantly less than in any other tissues examined, the concentration of selenium in the muscle of fish which died as a result of exposure to selenite was always in excess of 1 mg/Kg, sample variation of muscle tissue concentrations was small, the half-life of selenium in the muscle was relatively long (63 days) and the muscle is the tissue most often consumed by the public. However, if the purpose of the survey was to determine if fish were accumulating selenium as a result of accidental selenium contamination of an area, then an analysis of the liver content in addition to the muscle would be of value because the liver appears to accumulate selenium more rapidly than the muscle. The liver would be a better tissue to analyze for this purpose than either the spleen or heart because it is large enough in most fish to allow for two or more analysis whereas the spleen and the heart would not.

Fathead minnows were exposed to concentrations of 10, 25 and 50 µg/l because it was thought that information on the exposure of fish at natural levels or slightly higher would be of aid in evaluating the concentrations found in the muscle tissue of fish collected from Lake Erie (Table 5). The concentration of selenium in the muscle tissue of the fathead minnows after 96 days, regardless of exposure concentration, was less than the average concentration of selenium in the muscle of the fish collected from Lake Erie. These data suggest that the residue levels in the fish of Lake Erie cannot be entirely accounted for by the concentration of selenium (as selenite) in the water and suggest that the concentration of selenium in the diet significantly affects the residue levels in the tissues.

Sandholm et al. (1973) found that the residue levels in fish more than doubled when the fish were exposed to selenite-^{75} in both the food (zooplankton) and the water as compared to water alone. They also reported that during a 72 hour period of exposure the concentration of selenite in the zooplankton (*Daphnia pulex*) was greater than in the fish exposed to selenite in the water only. The phytoplankter, *Scenedesmus dimorphus*, appeared to be incapable of accumulating selenite although it did accumulate significant amounts of selenium as organic selenomethionine. The concentration of selenium in several samples of zooplankton collected from Lake Erie (Table 14) was somewhat less than the mean concentration of selenium in the Lake Erie fish, however, Copeland et al. (1973) have reported the residue level of Lake Michigan zooplankton to be as high as the average concentration found in the fish. These studies corroborate the data obtained in this study and suggest that the concentration of selenium in the diet of

fish in natural systems plays an important part in determining the residue levels in fish.

The elimination of selenium from fish appears to occur at a slower rate than from other species. Blincoe (1960) reported that after 300 hours less than 15 percent remained in rats given intravenous injections of sodium selenite. Lopez et al. (1969) reported the half-life of selenite-75 administered to sheep both orally and intravenously, at concentrations similar to this experiment, to be 21 and 52 days, respectively. The comparative half-life for whole-body loss of selenium from adult fathead minnows in this study was 63 days.

This relatively long half-life suggests that selenium is fairly persistent in spite of the fact that the maximum whole-body bioconcentration factor obtained in this same experiment was only 29.2. In comparison, Macek et al. (1970), Bransen et al. (1974) and Hansen et al. (1971) have reported bioconcentration factors of 10,000, 9,550 and 37,000 for dieldrin, tetrachlorobiphenyl and arochlor 1254, respectively. The respective half-lives for these compounds were 40, 30 and 42 days. While these studies showed bioconcentration factors several orders of magnitude larger than obtained in this study, their respective half-lives were smaller. This data suggests that the use of bioconcentration factors to describe the relative persistence of selenium in fish is of little value.

The results of the uptake studies clearly indicate that bioconcentration factors for selenium are inversely related to the exposure concentration. Macek (1975), however, after testing 50 herbicides, concluded that the bioconcentration factors were independent of the exposure concentration. This may be true for some groups of chemicals,

but it is obviously not true for selenium. When bioconcentration factors are used to describe the relative propensity for accumulation or the persistence of a chemical they should be accompanied by the exposure concentration.

The average concentration of selenium in the muscle tissue of rainbow trout which died as a result of exposure to sodium selenite was 1.61 mg/Kg. The relevancy of this data to natural systems is somewhat questionable due to the fact that the residue levels in some of the fish collected from Lake Erie (Table 5) are as high as those found in the dead trout in this experiment (Table 17). This would suggest that many of the fish in Lake Erie are dying of selenium poisoning. There is no data to support this view. The discrepancy may be partially explained by the fact that part of the selenium accumulated by fish in natural systems may be through the food chain as organically bound selenium rather than as selenite or perhaps by the fact that some species of fish are capable of accumulating selenium to a greater extent than other species. A very important consideration is the effect of competing elements on the uptake of selenium, especially mercury, cadmium, arsenic and copper. It appears possible that in the presence of these metals selenium can be accumulated to a greater extent without injuring the fish. The importance of the antagonistic effect of these metals on the toxicity of selenium has been recently stressed by Frost (1970) and Hill (1974). Ganther et al. (1974) have reported that methylmercury is capable of reducing the toxicity of sodium selenite to Japanese quail. They also fed tuna fish high in mercury and selenium to Japanese quail and found a reduction in the toxicity of methylmercury. They attributed it to the presence of

selenium. Their data indicates that on a molar basis the increment of selenium in tuna fish is in an approximate 1:1 ratio with the increment in mercury. They also found that the selenium content of high-mercury tuna was greater than the content of low-mercury tuna and suggested that tuna accumulate additional amounts of selenium as they accumulate mercury. Koeman et al. (1975) have also reported a 1:1 Hg/Se molecular increment ratio in the livers of marine mammals and they found an almost perfect linear correlation between the concentration of mercury and selenium in the liver. It cannot be said that mercury is the primary factor controlling the accumulation of selenium in fish, but the above studies do suggest that mercury is capable of influencing the accumulation of selenium. Further investigation of this relationship is necessary before the toxicity of either of these elements to fish and other organisms can be fully understood and assessed.

The biochemical role of selenium in the aquatic environment cannot at this time be accurately assessed due to the scarcity of information. Available data does suggest that the actions of selenium are in many ways similar to those of mercury (Niimi and LaHam, 1975). The results of this study indicate that selenium is highly toxic to aquatic organisms and that it is accumulative. Fleming and Alexander (1972) have demonstrated the methylation of inorganic selenium salts into organoselenium compounds by microorganisms and Shrift (1964) and Frost (1972) have proposed a possible cycle for selenium. These similarities with mercury suggest that organic selenium compounds, like organic mercury compounds, may be more toxic than the inorganic salts.

This has not been demonstrated for aquatic organisms, but it has been suggested for domestic animals (Muth and Binns, 1964; Nelson et al., 1933). Studies on the toxicity of organoselenium compounds and their accumulation in aquatic organisms are definitely needed. Identification of the types of selenium compounds present in aquatic systems and the changes that they undergo as a result of microbial action are needed before the existence of a selenium cycle in the aquatic environment can be confirmed.

Chronic toxicity studies with both cold and warm water species of fish are needed to establish water quality criteria for the protection of fish populations. Additional studies on those factors which affect selenium toxicity such as pH, water hardness, organic and inorganic complexes and the interactions with other metals are needed to accurately assess the toxicity of selenium in natural systems.

APPENDIX

APPENDIX A

ADDITIONAL DATA

Table A1. Determination of the loss of selenium, due to volitilization during digestion, from the muscle of fish which had been exposed to selenite-75.

Sample No.	Concentration of Selenium (mg/Kg)		Percent Deviation
	Initial	Final	
1	1.72	1.75	+1.86
2	1.95	1.81	-7.27
3	2.17	2.08	-3.91
4	1.80	1.70	-5.86
5	1.86	1.86	0.00
6	1.84	1.84	0.00
7	1.63	1.58	-2.82
8	1.86	1.74	-6.12
9	1.58	1.60	+1.14
10	1.89	1.83	-3.38
Mean±S.E.	1.83±0.05	1.78±0.05	-2.78±1.03

Table A2. Percent recovery of selenium from fish muscle.

Selenium Added (µg)[a]	Percent Recovery
5.0	101.6
5.0	105.0
5.0	103.0
5.0	101.4
5.0	106.6
Mean±S.E.	103.0±1.0

[a]Selenium was added to walleye muscle and the samples were analyzed by the method of Cummins et al. (1965.)

Table A3. Determination of the loss of selenium, due to volitilization, from *Daphnia magna* which had been exposed to selenite-75 and dried at 60 C for 30 hours.

Sample No.	Counts Per Minute		Percent Deviation
	Initial	Final	
1	9561.05	9181.52	-3.98
2	6301.60	6167.12	-2.15
3	9593.15	9226.69	-3.82
4	14141.65	13580.22	-3.97
5	11294.95	10709.87	-5.18
6	8816.90	8567.38	-2.83
Mean±S.E.			-3.65±0.43

Table A4. Determination of the loss of selenium, due to volitilization, from the muscle of fish which had been exposed to selenite-75 and then dried at 60 C for 30 hours.

Sample No.	Concentration of Selenium (ppm)		Percent Deviation
	Initial	Final	
1	1.89	1.88	-0.53
2	1.75	1.62	-7.43
3	1.57	1.54	-1.91
4	1.57	1.57	0.00
5	1.79	1.83	+2.23
Mean±S.E.	1.71±0.06	1.69±0.07	-1.53±1.62

Table A5. Comparison of the selenium content in water samples (2 ml) using radioisotope and stable analysis.

Sample No.	Selenium Concentration (ppm)		Percent Deviation
	Radioisotope Analysis	Stable Analysis	
1	2.50	2.50	0.00
2	2.50	2.50	0.00
3	2.39	2.38	-0.42
4	2.51	2.45	-2.39
5	2.51	2.45	-2.39
6	2.51	2.60	+3.58
7	2.51	2.62	+4.20
Mean±S.E.	2.49±0.02	2.50±0.03	1.85±0.99

Table A6. Concentration of selenium in fish collected from six locations in western Lake Erie.

Species	Collecting Station	Collection Date	Tag No.	Length (cm)	Weight (gm)	Sex	No. of Analysis	ppm (Wet wt.)	ppm (Dry wt.)[a]
Yellow perch	1	6/25/73	166	19.0	80.5	M	2	1.21	5.43
"	"	"	167	19.0	73.1	M	2	1.42	6.37
"	"	"	168	17.5	59.4	M	2	0.72	3.23
"	"	"	169	19.0	78.2	M	2	0.94	4.22
"	"	"	170	18.0	65.5	M	2	0.97	4.35
"	"	"	171	20.0	86.9	F	2	1.33	5.96
"	"	"	172	18.5	71.3	F	2	1.21	5.43
"	"	"	173	18.5	69.8	F	2	1.26	5.65
"	"	"	174	18.5	66.9	M	2	0.93	4.17
"	"	"	175	15.5	58.6	M	2	1.15	5.16
"	"	"	143	10.5	13.4	F	2	0.84	3.77
"	"	"	144	9.0	9.4	M	2	0.86	3.86
"	"	"	145	10.0	10.0	M	2	0.94	4.22
"	"	"	146	11.0	16.4	F	2	0.68	3.04
"	"	"	147	10.0	12.0	M	2	0.79	3.54
"	"	"	148	10.5	14.3	F	2	0.78	3.48
"	"	"	149	9.5	9.5	M	2	0.54	2.42
"	"	"	150	10.5	12.5	F	2	0.61	2.74
"	"	"	151	9.5	10.3	M	2	0.41	1.84
"	"	"	152	10.0	12.3	-	2	0.27	1.21
"	2	8/16/72	8	18.0	----	-	1	0.65	2.91
"	"	8/29/72	18	18.0	----	-	1	1.44	6.46
"	"	"	19	21.0	----	-	1	1.22	5.47
"	"	"	20	18.0	----	-	1	0.79	3.54
"	"	"	21	18.5	75.2	-	1	0.59	2.65
"	"	4/18/73	64	19.5	90.0	F	1	1.54	6.91
"	"	"	65	18.5	82.3	F	1	0.46	2.06
"	"	"	66	18.5	69.9	F	1	0.92	4.13
"	"	"	67	20.5	103.6	M	1	0.21	0.94
"	"	"	68	20.5	88.2	F	1	0.27	1.21

Table A6 (cont'd)

Species	Collecting Station	Collection Date	Tag No.	Length (cm)	Weight (gm)	Sex	No. of Analysis	ppm (Wet wt.)	ppm (Dry wt.)[a]
Yellow perch	2	4/18/73	69	18.0	61.5	M	1	0.20	0.90
"	"	"	70	18.5	65.1	M	1	1.99	8.92
"	"	"	71	19.0	70.6	M	1	0.10	0.45
"	"	"	72	17.5	65.1	M	1	0.18	0.81
"	"	"	73	17.5	59.2	M	1	1.48	6.66
"	"	"	74	20.0	85.7	M	1	0.88	3.95
"	"	"	75	19.0	75.2	M	1	1.11	4.98
"	"	"	76	19.5	73.3	M	1	0.92	4.13
"	"	"	77	19.5	69.7	M	1	1.37	6.14
"	"	"	78	19.0	71.6	M	1	0.10	0.45
"	"	"	79	24.0	186.1	F	1	0.10	0.45
"	"	"	80	25.5	199.7	F	1	0.10	0.45
"	"	"	81	20.0	96.1	F	1	0.37	1.66
"	"	"	82	19.0	76.8	M	1	1.37	6.14
"	"	"	83	18.0	62.2	M	1	0.21	0.94
"	5	8/16/72	6	21.5	124.0	F	2	0.16	0.72
"	"	9/30/72	52	19.5	81.0	F	1	1.56	7.00
"	"	"	58	15.5	47.5	M	1	0.69	3.09
"	"	"	59	17.0	57.4	M	1	1.15	5.16
"	"	"	60	19.0	94.0	M	1	0.41	1.84
"	"	"	61	19.5	79.1	F	2	0.32	1.44
"	"	"	62	17.0	54.7	M	2	0.48	2.15
"	"	4/18/73	103	19.0	70.8	M	3	0.48	2.15
"	"	"	104	19.0	14.2	M	2	0.40	1.79
"	"	"	105	17.5	74.0	M	2	0.48	2.15
"	"	"	106	18.0	62.1	M	2	0.40	1.79
"	"	"	107	18.0	66.7	M	2	0.49	2.20
"	"	"	108	18.0	67.9	M	2	0.61	2.74
"	"	"	109	18.5	64.7	M	2	0.52	2.33
"	"	"	110	11.0	69.5	F	2	0.35	1.60

Table A6 (cont'd)

Species	Collecting Station	Collection Date	Tag No.	Length (cm)	Weight (gm)	Sex	No. of Analysis	ppm (Wet wt.)	ppm (Dry wt.)[a]
Yellow perch	6	9/30/72	32	23.0	141.8	-	1	1.02	4.57
"	"	"	33	21.0	117.3	-	1	0.28	1.26
"	"	"	34	19.0	101.9	-	1	0.92	4.13
"	"	"	35	19.0	78.8	-	1	0.51	2.29
"	"	"	37	18.0	66.2	-	1	1.04	4.66
"	"	5/29/72	53	19.5	74.8	-	1	0.91	4.08
"	"	"	54	19.0	72.8	-	1	0.60	2.69
"	"	6/25/73	153	19.0	78.6	F	4	0.53	2.38
"	"	"	154	16.5	49.4	F	2	0.17	0.76
"	"	"	155	15.0	37.0	F	3	0.40	1.79
"	"	"	156	16.5	50.7	M	2	1.15	5.16
"	"	"	157	18.0	72.5	M	5	0.43	1.93
"	"	"	158	11.0	16.4	-	2	0.10	0.45
"	"	"	159	10.0	10.2	-	2	0.10	0.45
"	"	"	160	10.5	12.3	-	3	0.41	1.84
"	"	"	161	10.0	11.2	-	2	1.08	4.84
"	"	"	162	10.5	12.3	-	2	0.44	1.97
"	"	"	163	11.0	15.0	-	2	1.36	6.10
"	"	"	164	10.0	11.4	-	2	0.85	3.81
"	L. Huron	9/30/73	176	27.0	293.4	M	2	0.50	2.24
"	"	"	177	19.5	100.4	F	3	0.51	2.29
"	"	"	178	18.5	86.2	F	3	0.67	3.00
"	"	"	179	14.5	36.7	M	1	0.68	3.05
"	"	"	180	14.5	33.9	M	2	0.46	2.06
"	"	"	181	11.5	15.5	F	1	0.65	2.91
Lake trout	"	"	182	24.0	116.0	M	3	0.73	3.30
Common shiner	2	4/18/73	92	12.0	15.3	-	2	0.41	1.67
"	"	"	93	11.5	14.0	-	2	0.37	1.51
"	"	"	94	11.0	10.9	-	2	0.37	1.51

Table A6 (cont'd)

Species	Collecting Station	Collection Date	Tag No.	Length (cm)	Weight (gm)	Sex	No. of Analysis	ppm (Wet wt.)	ppm (Dry wt.)[a]
Common shiner	2	4/18/73	95	11.5	12.5	-	2	0.48	1.96
"	"	"	96	11.0	13.3	-	2	0.20	0.82
"	"	"	97	12.0	17.4	-	2	0.28	1.14
"	"	"	98	11.0	13.8	-	2	0.49	2.00
"	"	"	99	11.0	11.4	-	2	0.74	3.02
"	"	"	100	11.0	12.6	-	2	0.47	1.92
"	"	"	101	10.5	11.3	-	2	0.65	2.65
"	"	"	102	14.0	49.5	-	2	0.39	1.59
"	5	"	120	11.5	15.8	-	2	0.62	2.53
"	"	"	121	11.5	14.4	-	2	0.41	1.67
"	"	"	122	11.5	14.2	-	2	0.37	1.51
"	"	"	123	11.0	13.4	-	2	0.33	1.35
"	"	"	124	12.5	19.1	-	2	0.67	2.73
"	"	"	125	11.0	13.7	-	2	0.48	1.96
"	"	"	126	10.5	11.6	-	2	0.32	1.31
"	"	"	127	11.0	12.0	-	2	0.27	1.10
"	"	"	128	11.0	13.0	-	2	0.46	1.88
"	"	"	129	11.5	13.6	-	2	0.39	1.59
Spottail shiner	1	6/25/73	133	11.5	11.6	-	3	0.60	2.45
"	"	"	134	11.5	14.8	-	2	0.49	2.00
"	"	"	135	12.5	17.5	-	2	0.63	2.57
"	"	"	136	11.5	13.9	-	2	0.30	1.22
"	"	"	137	10.5	13.4	-	2	0.46	1.88
"	"	"	138	11.5	14.4	-	2	0.41	1.67
"	"	"	139	11.0	11.8	-	2	0.76	3.10
"	"	"	140	12.0	12.1	-	2	0.76	3.10
"	"	"	141	10.5	10.4	-	2	0.40	1.63
"	"	"	142	10.5	11.8	-	2	0.39	1.59

Table A6 (cont'd)

Species	Collecting Station	Collection Date	Tag No.	Length (cm)	Weight (gm)	Sex	No. of Analysis	ppm (Wet wt.)	ppm (Dry wt.)[a]
Spottail shiner	6	6/25/73	197	11.0	13.4	-	2	0.72	----
"	"	"	198	10.5	11.0	-	2	0.77	----
"	"	"	199	10.5	12.7	-	2	0.41	----
"	"	"	200	11.5	10.9	-	2	0.92	----
"	"	"	201	11.5	12.6	-	2	1.54	----
"	"	"	202	11.0	12.4	-	1	1.43	----
Goldfish	4	8/17/72	13	20.0	160.1	-	3	1.60	6.96
"	5	9/30/72	48	18.5	122.3	-	1	1.11	4.83
Carp	5	8/16/72	5	22.5	170.2	-	1	1.39	6.04
"	"	9/30/72	47	28.0	294.1	-	1	0.74	3.22
"	"	4/18/73	115	24.0	184.2	M	2	0.96	4.17
"	6	"	88	25.5	195.2	M	2	0.60	2.61
"	"	"	89	27.0	258.4	M	2	0.61	2.65
"	"	"	90	26.0	244.9	M	2	0.63	2.74
White bass	2	4/18/73	87	28.5	281.3	F	2	0.80	4.17
"	5	"	111	28.5	288.8	M	1	0.65	3.39
"	"	"	112	28.0	227.8	M	1	1.20	6.25
"	"	"	113	29.5	311.3	F	2	0.62	3.23
Gizzard shad	5	4/18/73	116	18.0	51.0	-	2	0.66	3.35
"	"	"	117	18.5	61.2	-	2	0.57	2.89
"	"	"	118	19.5	67.2	-	2	0.84	4.26
"	"	"	119	19.0	57.0	-	3	0.84	4.26
Walleye	4	5/23/73	130	41.0	666.5	F	2	0.45	2.11
"	"	"	131	42.0	729.5	M	2	0.33	1.55
"	"	"	132	27.5	180.6	M	2	0.19	0.89

Table A6 (cont'd)

Species	Collecting Station	Collection Date	Tag No.	Length (cm)	Weight (gm)	Sex	No. of Analysis	ppm (Wet wt.)	ppm (Dry wt.)[a]
Walleye	5	5/23/73	55	14.5	26.1	M	1	0.30	1.41
"	"	"	56	16.0	33.4	M	2	0.92	4.32
"	"	"	57	14.0	20.5	M	1	0.61	2.86
"	"	4/18/73	114	23.0	103.9	M	2	0.84	3.94
Sheepshead	1	6/25/73	187	24.5	166.3	-	2	0.64	3.44
"	"	"	188	19.5	68.7	-	2	1.08	5.81
"	"	"	189	17.5	61.0	-	2	1.20	6.45
"	3	8/29/72	63	22.5	107.0	M	1	1.48	7.96
"	4	9/30/72	50	19.5	75.0	-	1	1.98	10.65
"	"	"	51	17.5	57.1	-	1	0.28	1.51
"	6	"	22	21.0	119.9	-	1	2.83	15.22
"	"	"	23	17.5	67.0	-	1	2.46	13.23
"	"	6/25/73	190	19.5	80.8	-	2	1.08	5.81
"	"	"	191	22.0	95.9	-	2	1.53	8.23
"	"	"	192	19.5	80.3	-	2	1.42	7.63
"	"	"	193	18.5	64.0	-	2	1.64	8.82
"	"	"	194	16.5	61.1	-	2	1.99	10.70
White sucker	2	4/18/73	84	34.5	469.2	M	2	0.65	3.32
"	"	"	85	23.5	133.3	M	2	0.53	2.70

[a]Dry weight values were obtained by multiplying wet weight values by the average percent moisture for each species.

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