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Long-term Effects of Arsenic Accumulation in Rainbow Trout, *Salmo gairdneri*

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The toxicity of arsenic and its compounds to aquatic organisms is well documented. Acute exposure has been shown to result in reduced growth or increased mortality in aquatic organisms. Acute toxicity levels have been established for several species of fish. Minnows exposed to 20 ppm As (sodium arsenite - $NaAsO_2$) and 250 ppm arsenic, as sodium arsenate in water, survived for a maximum of 36 and 16 hours respectively (Grindley 1946). Gilderhus (1966) obtained a 96 h LC50 value for sodium arsenite at 12°C as 25.6 ppm for rainbow trout, Salmo gairdneri, 34 ppm for bluegills (Lepomis macrochirus). A number of studies have been carried out on the effects on fish of long term exposure to sublethal concentrations of various forms of arsenical herbicides. The "safe" concentrations recommended for one of the earliest common aquatic herbicide, sodium arsenite, varied from 2-10 ppm (Surber 1931; MacKenthun 1955). It was reported that these levels were not toxic in fish.

The above-mentioned studies indicate that the rainbow trout (Gilderhus 1966; Hale 1977) and other fish species (Sorensen 1976) had very high 96 h LC50 values, suggesting that fish are relatively tolerant of arsenic. However, very little has been done on the long-term effects or toxicity of arsenic ingested with the diet in spite of many reports (Woolson 1975) that indicated a contamination of aquatic food chain by arsenic.

The few studies (Lawrence 1958; Gilderhus 1966) on the long-term effects of arsenic had reported that 4 ppm sodium arsenite in water reduced the survival and growth of bluegills, Lepomis macrochirus. Studies of Speyer (1975) and Speyer and Leduc (1975) noted a decrease in body and fat weight in yearling rainbow trout exposed to different concentrations of arsenic trioxide in water for 21 days. Sublethal concentrations of arsenic were shown to affect conditioned response in goldfish, Carassius auratus (Weir and Hine 1970). These authors reported that exposure to 0.1 ppm As in water for 48 hours caused up to 48% impairment in the

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avoidance reaction of goldfish to light and electric shock.

The primary objective of this study was to determine the accumulation kinetics of arsenic and its effect on certain physiological parameters such as growth and haematology.

MATERIALS AND METHODS

Rainbow trout, Salmo gairdneri, were obtained from Thistle Springs Trout Farm, Ashton, Ontario. All test fish were fed the control diet and acclimated to experimental conditions for at least two weeks prior to the start of the experiment. A day prior to the start of the experiment, 5 fish were randomly selected and sacrificed for establishing baseline blood haemoglobin, hematocrit and tissue arsenic levels. At the beginning of the experiment, 96 rainbow trout of 29.2 ± 0.1 g wet weight were selected and randomly alloted to 4 groups of 24 in 120-litre aquaria. One group served as the control and the other three were to be exposed to 10, 20 and 30 mg As kg^{-1} of diet. A flow rate of approximately 12 litres h^{-1} was maintained in each aquarium. Following the allocation to the test groups, each fish was anaesthetized in MS 222 and tagged (through the musculature anterior to the dorsal fin, using 5 mm x 3 mm fingerling tags supplied by Floy Tag Manufacturing Ltd., Seattle, Washington), blotted dry and weighed. Subsequent weighings were carried out at 2-week intervals and the fish ration adjusted accordingly.

After 2, 4 and 6 weeks of exposure, 4 fish were sacrificed (all the fish in each aquarium having been weighed) from each of the four test groups for blood haemoglobin (Hb) hematocrit (PCV) and tissue arsenic residues. Blood haemoglobin (cyanmethemoglobin method) and hematocrit were determined separately for each fish, using standard techniques (Blaxhall 1972). Arsenic residue analysis was also carried out separately on muscle from each fish, and on pooled samples of gill, skin and liver.

At the end of the experiment, all surviving fish in each test group were weighed. Blood haemoglobin content was measured on 5 fish from the surviving fish in each test group. All the remaining fish were frozen pending tissue arsenic residue analysis.

The four fish that were sacrificed from each test group after 2, 4, 6 and 8 weeks of exposure were thawed out for analysis. Arsenic analysis was carried out on freeze-dried muscle. An 8 to 10-gram muscle fillet was weighed out from each fish, homogenized with its own weight of water, and freeze-dried to constant weight. Liver, gill and skin were freeze-dried and later ground up using a mortar and pestle. After drying, samples were stored in dessicators ($CaCl_2$) until used. Arsenic residue analysis was carried out by Professor J.G. Dick, Dept. of Chemistry, Concordia University, Montreal. The method used was the flame atomic absorption technique involving the generation of arsine and its injection by argon gas entrainment into a hydrogen-air flame (Dick et al. 1976). Arsine was generated from wet-ashed samples by treatment with

hydrochloric acid and sodium borohydride. This technique overcomes the arsenic absorbance inhibition known to result from phosphate interference when the carbon or graphite furnace methods were used (Dick et al. 1976). The general conditions maintained during the various analytical procedure were outlined earlier (Dick et al. 1976).

The diet used for this study was Purina Trout chow. The chow was ground to a powder in a waring blender. Sodium arsenite solution was prepared in distilled water. Sodium arsenite was introduced into the diet by mixing 5 parts (by weight) of the sodium arsenite solution with four parts of dried chow and one part of the binding agent, gelatin. The mixture was poured into a shallow tray, put in the refrigerator where it set and was then cut into small pieces which were frozen until used.

The diets consisted of the control which contained no arsenic, and three others containing 10, 20 and 30 mg As kg^{-1} dry weight of diet. These levels are equivalent to a daily dose of 0.2, 0.4 and 0.6 mg As kg^{-1} fish wet weight respectively at a feeding rate of 2% (dry weight of food to wet weight of fish) body weight per day.

RESULTS AND DISCUSSION

The initial mean wet weight of the test groups were 29.19 ± 1.13 for the control and 29.58 ± 1.03, 29.07 ± 1.03 and 29.22 ± 1.04 for the 10, 20 and 30 mg As kg^{-1} diet groups respectively. Growth was calculated from the sum of the individual wet weight of the remaining fish at 2, 4, 6 and 8 weeks and expressed as percent wet weight gain. The results show that the percent wet weight gains in control group increased from 21.6% at 2 weeks to 79.3% at 8 weeks (Fig. 1). The growth of fish that were exposed to 10 and 20 mg As kg^{-1} diet were not different from those for the control. However, the weight gains of fish exposed to 30 mg As kg^{-1} diet were significantly less than those for the control throughout the duration of exposure ($P < 0.05$).

Haemoglobin and hematocrit for the control group did not deviate much from the baseline values throughout the test period (Table 1). Blood of fish exposed to 10 mg As kg^{-1} diet exhibited no loss in haemaglobin content up until 8 weeks of exposure when the level dropped to a significantly low level of 9.30 g/100 ml blood ($P < 0.05$). Similarly, the 20 and 30 mg As kg^{-1} diet exposure group showed slight deviations from the baseline level up until the sixth week. After 8 weeks of exposure, the haemoglobin levels showed a significant ($P < 0.05$) reduction of 20.5% for the 20 mg As kg^{-1} diet group and 29% for the 30 mg group.

Fish exposed to 10 mg As kg^{-1} diet showed a decrease in hematocrit after 4 and 6 weeks of exposure, followed by an increase at 8 weeks. The 30 mg As kg^{-1} diet group had consistently lower hematocrit.

The baseline mean corpuscular haemoglobin concentration (MCHC) was

Table 1. Mean haemoglobin (g/100 ml blood), haematocrit and mean corpuscular haemoglobin concentration (MCHC) of rainbow trout exposed to arsenic for 2 to 8 weeks at 10±1°C.

Dose of arsenic mg kg^{-1} diet	Weeks of exposure			
	2	4	6	8
		Haemoglobin (Hb)[a]		
0	10.55±0.22	10.92±0.23	10.35±0.98	10.75 ±0.22
10	11.72±0.64	10.32±0.39	9.93±0.84	9.30*±0.62
20	11.20±0.43	11.08±0.70	10.71±0.15	8.43*±0.23
30	10.73±0.45	10.66±0.56	9.83±1.00	7.56*±0.53
		Haematocrit (Hct)		
0	37.68±1.22	35.40±0.51	33.60±1.21	37.60 ±1.63
10	37.25±2.01	34.60±1.32	31.40*±2.43	38.40 ±1.57
20	36.00±1.78	35.00±1.05	32.75*±0.48	35.25 ±1.55
30	32.25±2.10	31.80*±1.49	32.80*±1.65	33.50*±1.26
		MCHC[b]-Hb/Hct x 100		
0	28.00±1.00	31.00±1.00	31.00±2.00	29.00 ±1.00
10	31.00±1.00	30.00±2.00	32.00±2.00	24.00*±1.00
20	32.00±1.00	35.00±3.00	33.00±1.00	25.00 ±1.00
30	34.00±2.00	34.00±2.00	30.00±2.00	22.00*±2.00

[a]Baseline values determined before fish were alloted to the exposure groups were 10.60±0.44, 36.45±0.81 and 29.00 for haemoglobin, haematocrit and MCHC respectively.

[b]MCHC = Mean corpuscular haemoglobin concentration.

*Values significantly different from the baseline at $P < 0.05$ - Student t test.

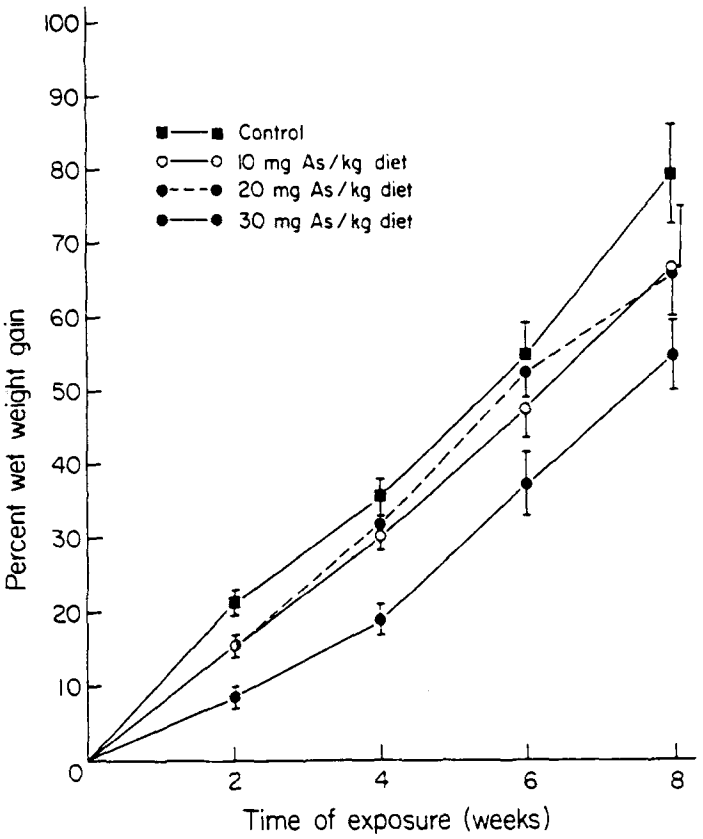

Fig. 1 Percent wet weight gain of control and rainbow trout exposed to 10, 20 and 30 mg As kg^{-1} diet for 8 weeks at 10°C. Means ± S.E. of 24, 19, 14, and 9 fish at 2, 4, 6 and 8 weeks respectively are presented.

29%. The control group did not show any significant difference from the baseline mean throughout the duration of the experiment. However, the MCHC was significantly reduced in all treated groups after 8 weeks of exposure to arsenic. The values were 25%, 25 and 22 for 10, 20 and 30 mg As kg^{-1} diet groups respectively.

In general, the levels of arsenic accumulated in tissues were directly related to levels of arsenic to which the fish were exposed (Table 2). Among the tissues analysed, the liver accumulated the highest concentration of arsenic during the 8 weeks of exposure. The trend is not quite clear as to the relationship between the amount of arsenic accumulated and duration of exposure since the liver for each of the test groups attained its highest concentration of arsenic at different times.

The skin had the next highest level among the tissues examined. The concentrations of arsenic in the skin of the 10 mg kg^{-1} diet group reached 1.05 µg g^{-1} after two weeks of exposure and showed only an insignificant ($P < 0.05$) deviation from this value at other time periods. The 20 mg As kg^{-1} diet group increased to 1.54 µg g^{-1} at 4 weeks after which it declined. The skin of fish exposed to 30 mg kg^{-1} diet, like liver, contained higher levels of arsenic than the other two groups.

The residue level in the gills of fish exposed to 10 and 30 mg As kg^{-1} diet increased to a maximum at two weeks and showed a decline thereafter. However, the 20 mg group seemed to show a different trend. This difference could have been due to an unusually high level in one of the tissues that made up the pooled sample.

The muscle of fish exposed to the lowest level of arsenic attained maximum concentration of arsenic of 1.35 µg g^{-1} after 4 weeks of exposure. The levels in the muscles of fish from this group analysed at subsequent time periods were not significantly different ($P < 0.05$). Arsenic levels in the 20 mg As kg^{-1} diet group increased to 1.59 µg g^{-1} after 4 weeks and declined. Similarly, muscle of fish from the highest exposure group contained 2.40 ± 0.44 µg g^{-1} arsenic after two weeks of exposure, but decreased to significantly lower levels ($P < 0.05$) for the remaining duration of exposure.

Arsenic fed in the diet to rainbow trout was shown to impair growth, primarily at high concentration. As can be seen from Fig. 1, growth of fish exposed to 10 mg As kg^{-1} diet for 8 weeks was not impaired, but those exposed to 20 and 30 mg As kg^{-1} diet for the same period showed significantly reduced growth relative to the control, suggesting a dose-dependent effect. A number of studies have demonstrated a somewhat similar toxic effect of arsenic on other fish species. It is reported (Lawrence 1958) that two applications of 4.0 ppm sodium arsenite made two months apart in experimental outdoor ponds reduced bluegill (Lepomis macrochirus) production by about 42% compared to the control. Benthic invertebrates were also reduced by 34%. Two applications of 8.0 ppm applied one month apart reduced the number of fish by an

Table 2. Residue[a] of arsenic in tissues (μg g^{-1} dry wt) of rainbow trout, Salmo gairdneri, exposed[b] to 10, 20 and 30 mg As kg^{-1} diet for 8 weeks at 10±1°C. Means ± S.E. of 4 samples for muscle and single pooled samples for other tissues are shown.

Exposure groups mg kg^{-1} diet	Weeks of exposure			
	2	4	6	8
		Muscle		
10	1.35±0.12	1.40±0.13	1.24±0.05	1.28±0.15
20	1.45±0.20	1.59±0.16	1.26±0.15	1.28±0.12
30	2.40±0.44	1.60±0.10	1.49±0.14	1.52±0.07
		Liver		
10	1.31	1.18	1.77	1.55
20	1.99	2.60	2.45	3.41
30	6.24	6.04	4.98	5.21
		Gills		
10	2.80	0.68	0.89	0.84
20	0.90	0.98	0.68	1.71
30	3.37	1.43	1.84	1.88
		Skin		
10	1.05	0.95	1.18	1.21
20	1.26	1.54	1.43	1.45
30	2.47	1.56	1.94	1.98

[a]No arsenic was detected in control tissues; detection limit = 0.14μg/g.

[b]The exposure levels of 10, 20 and 30 mg/kg diet are equivalent to 0.2, 0.4 and 0.6 mg/kg fish wet weight per day.

average of 65%. A later study (Gilderhus 1966) noted a similar effect on adult and immature bluegills (L. macrochirus). The marked reduction in the production of bluegills in the above studies must have been due to the fact that the fish accumulated arsenic from water directly via the gills as well as by consuming invertebrates.

A decrease in growth was reported in yearling rainbow trout that were exposed to 6.0 mg As/litre (as arsenic trioxide) for 21 days (Speyer 1975). Similar to the results of the present study, Speyer observed a growth impairment that was dose-dependent.

The results indicated that orally ingested arsenic caused a significant decrease in haemoglobin levels that was inversely related to the duration of exposure. The mean corpuscular haemoglobin concentration (MCHC) was also reduced after 8 weeks of exposure, suggesting that the primary effect of the arsenic on blood was a decrease in the haemoglobin in the red blood cells and not hemolysis of the cells. It is noted (Zharkova 1971; Speyer 1975) that arsenic caused a reduction in blood haemoglobin in rats and rainbow trout respectively. The growth impairment observed in the rats and trout in this study could have been due to inhibition of enzymes as well as to a reduced oxygen-carrying capacity of blood. This could have resulted in an inefficient utilization of assimilated food.

In the present study arsenic accumulation in rainbow trout tissues increased with increasing levels of arsenic in the diet. The liver, which has always been identified as a target organ in arsenic poisoning (Gilderhus 1966; Bencko and Symon 1970), contained the highest levels of arsenic. In the previously mentioned study of bluegills (Gilderhus 1966), a high-level of 11.6 ppm arsenic in the liver of fish exposed to 4 ppm arsenic for 16 weeks was reported. This level is much higher than the 6.24 ppm maximum for the present study. The marked difference may be due to the fact that the bluegills were exposed to arsenic in the food for a shorter duration. High levels of arsenic in the liver have been associated with functional change. Oxygen consumption in homogenized liver tissue of hairless mice exposed to 50 mg As/litre in drinking water for 256 days was markedly suppressed compared to the control (Bencko and Symon, 1969). Similarly, the high levels of arsenic observed in the liver of rainbow trout exposed to arsenic in this study may have caused histological changes resulting in impairment of liver functions.

Residue levels of arsenic in the skin were next highest to those for the liver. The keratin of hair and nails is rich in disulphide, and it has been postulated that the arsenic is incorporated into the growing portion of the hair root and the nail base (Shapiro 1967). Similarly, arsenic could have been bound to sulfhydryl groups in the skin and scale of fish, resulting in the observed high levels.

Muscle tissue sampled in the present study did not accumulate as

much arsenic as the liver or skin. Muscle of the bluegill had arsenic residues lower (1.3 μg g^{-1}) compared to the liver (Gilderhus 1966).

The gills did not seem to accumulate any substantial amount of arsenic during the continuous exposure. This observation might be due to the fast elimination of arsenic via the gills (Oladimeji 1980).

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