Bull. Environm. Contam. Toxicol. 21, 162-169 (1979)

Arsenic Accumulation, Tissue Distribution and Cytotoxicity in Teleosts Following Indirect Aqueous Exposures

Elsie M. B. Sorensen[1], Roland E. Henry[2], and Ruben Ramirez-Mitchell[3]

[1]Department of Zoology, [2]Nuclear Reactor Laboratory, Department of Mechanical Engineering, [3]Cell Research Institute, University of Texas at Austin, Austin, Tex. 78712

Arsenic is a contaminant of marine and fresh waters and it is accumulated by organisms inhabiting these waters. As early as 1926, CHAPMAN reported arsenic levels of 8.5, 7.0, and 3.5 ppm (wet weight) in plaice, sole, and "dab", respectively, from waters containing 0.28 ppm of the metalloid. In 1941, ELLIS et al. presented arsenic uptake levels for 15 fresh-water species; average arsenic levels in whole and eviscerated fish were 0.75 and 0.48 ppm (wet weight), respectively. No attempt was made to establish tissue distribution patterns for arsenic in these fish except for those made in large-mouthed black bass which accumulated 0.66 ppm (wet weight) in the whole body, 0.50 ppm in the eviscerated portions, and 6.06 ppm in the liver. Other tissues were not considered and the concentration of arsenic in the water was not determined. WINDOM et al. (1973) found that 9 species of marine finfish (exposed at an unknown concentration of arsenic) contained an average ($\pm$ standard deviation) arsenic concentration of 12.1 $\pm$5.55 ppm (dry weight) in the liver (or 3.67 $\pm$1.68 ppm, wet weight, based on a conversion factor of 3.3-1 calculated from ELLIS et al. (1941) data). Muscle and spleen averaged 6.03 and 3.67 ppm (dry weight).

Such investigations are incomplete, but do indicate that low arsenic exposure levels can result in 12- to 30- fold elevations in whole fish (CHAPMAN 1926) and that the liver appears to be a target organ in teleosts, as it is in mammals (HARVEY 1975). Little has been reported regarding concentration factors, uptake and excretion routes, organ distribution patterns, or the major organ of impact for teleosts in either field or laboratory situations. To answer some of these questions, fish were exposed to arsenic under controlled, experimental conditions and sacrificed at specific intervals for measurement of progressive arsenic concentration within various critical organs. Since cells of the liver (SORENSEN 1976a) and kidneys (BROWN et al. 1976) are affected morphologically in cases of arsenic poisoning, samples of each were processed for optical microscopy.

0007-4861/79/0021-0162 $01.60

Materials and Methods

Green sunfish (Lepomis cyanellus Raf.) were collected, maintained, and treated as previously reported (SORENSEN 1976c).

All specimens were within specimen size range limits for bioassays (DOUDOROFF et al. 1951). The mean fresh weight (± standard deviation) was 36 ± 13 g; mean total length was 13 ± 2 cm. After acclimation, specimens were placed in individual compartments of aquaria containing 60 ppm arsenic (as sodium arsenate, $Na_2HAsO_4.7H_2O$) for 0, 2, 4, and 6 days--at which time fish were removed at random and sacrificed. The 0d-60 ppm specimens will be referred to as "control" animals.

At sacrifice, the spinal cord was severed with one, dorsal-to-ventral cut. The left lateral body wall was incised caudally and retracted for removal of the liver, kidneys, gall bladder (containing bile), ovaries or testes, and spleen. The left operculum was retracted and the entire set of gill arches and filaments removed for the gill sample. To reduce possible leaching of arsenic and contamination, each organ (except the gall bladder) was gently blotted with paper tissue before it was placed in preweighed 2-dram polyethylene vials. The vials were weighed to the nearest 0.0001 g; all concentration data was calculated on the basis of fresh weight.

The total quantity of arsenic per g of tissue was determined using neutron activation analysis (NAA), as previously described (SORENSEN 1976b).

Fresh liver and kidney samples were removed as soon as possible and processed for optical microscopy (OM). Tissue was fixed in Bouin's solution, washed 24 hr in running water, dehydrated in an ethanol and amyl acetate series and embedded in paraffin. Sections about 4 µm thick were cut on a Leitz microtome and stained with hematoxylin and eosin (H&E) for examination on a Zeiss GFL Standard Microscope. A minimum of one thousand nuclei from proximal convoluted tubules and liver parenchymal cells from each treatment were examined for the presence of intranuclear inclusions.

Results and Discussion

The gall bladder and bile accumulated the highest concentrations of arsenic during the 6-day exposure interval; levels increased from about 35 to 78 to 159 ppm during the 2, 4, and 6-day exposures, respectively (Table 1). Arsenic concentrations were significantly higher ($p<0.001$) after 4 than 2 days exposure and after 6 than 4 days exposure ($p<0.05$). Since numerous metals

(lead (KLAASSEN 1974a), mercury, copper, and magnesium (CIKRT 1972)) are known to be removed by the biliary-fecal route, these elevations in the concentration of arsenic in gall bladder-bile samples are probably associated with the bile and not the wall of the gall bladder. KLAASSEN'S (1974b) data on biliary excretion of arsenic in mammals following a single intravenous injection, showed that arsenic was excreted in the bile by an active transport mechanism but that arsenic absorption from the intestine after biliary excretion resulted in a redistribution in tissues other than the liver and intestine. Such redistribution would minimize the overall benefit of biliary excretion of arsenic to the organism and could be a factor resulting in the higher organ burdens in green sunfish observed following increasing exposure times in the present experiments.

Liver, spleen, and kidney tissue concentrated more arsenic as exposure time increased (Figure 1). Both liver and spleen arsenic burdens doubled in 4 days but did not differ significantly thereafter; whereas arsenic levels in the kidney did not double until 6 days with no significant differences observed prior to this time. Similar increases have been observed in rats for the liver and spleen, but not the kidney, at 2 hr and 7 days after a single 1 mg/kg intravenous injection of arsenic (KLAASSEN 1974b).

The liver binds arsenic "avidly" (KLAASSEN 1974b) and is generally considered one of the major target organs in cases of arsenic poisoning (HARVEY 1975, KODAMA et al. 1976). High levels accumulate and cause a number of functional and histological changes. KODAMA et al. (1976) has reported significant increases in glutamic-oxalacetic transaminase, lactic dehydrogenase, and thymol turbidity. Histological changes, on the other hand, range from fatty infiltration, central necrosis, cirrhosis, and vacuolation of the cytoplasm of hepatic cells (HARVEY 1975, GILDERHUS 1966) to marked edema and generalized capillary dilation (FINNER and CALVERY 1939).

The kidney also accumulates arsenic and is considered to play a major role in the metabolism and excretion of arsenic. Morphological changes (i.e., swollen mitochondria and increased numbers of autophagic lysosome-like bodies) and functional changes (i.e., decreased state 3 respiration and respiratory control ratios) have been reported for rats (BROWN et al. 1976). HARVEY (1975) reported the occurrence of transient effects following therapeutic administration of arsenicals to humans; these included glomerular dilation with proteinuria and tubular necrosis or degeneration resulting in cast production, mild albuminuria, and hematuria.

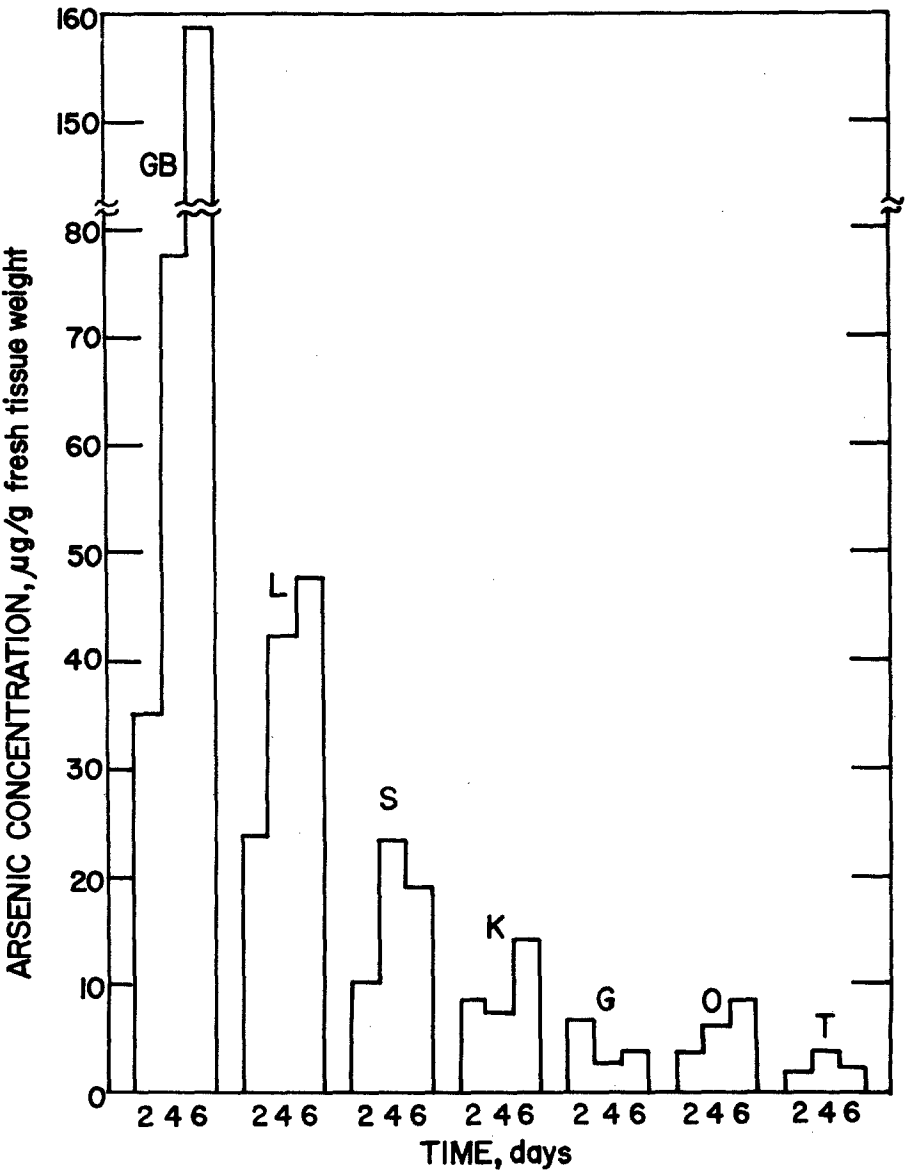

Figure 1. Mean arsenic concentration in bile and gall bladder (GB), liver (L), spleen (S), kidney (K), gill filaments and arches (G), ovaries (O), and testes (T) of L. cyanellus exposed to 60 ppm arsenic and 20°C for 2, 4, and 6 days.

The spleen is a storage site for arsenic and, with the exception of pronounced capillary bed damage (HARVEY 1975), no detailed studies on morphological or functional changes as a result of arsenic poisoning have been reported.

Although no significant differences were observed in arsenic accumulation in the gill, a noticeable decrease from 6.8 to 3.8 ppm was observed between 2 and 6 days of exposure. Arsenic has been reported to cause sloughing of external epidermal layers, including the gill (SORENSEN 1976b)--possibly leading to the coughing reflex, which was observed during exposures, and a subsequent reduction in arsenic levels in the gill tissue.

Ovaries and testes were not observed to accumulate arsenic to significantly different levels during the 6-day exposure period (Table 1) although ovaries show an increase of from 3.5 to 8.5 ppm during the exposure period. KLAASSEN (1974b) reported a ten-fold decrease in testicular levels of arsenic between 2 hr and 7 days following a single intravenous injection of 1 mg arsenic/kg in the rat.

Intranuclear inclusions were observed by optical microscopy in 68.8, 72.0, and 75.5% of all nuclei examined for the 2, 4, and 6 day exposure times, respectively, (Figure 2b-2d); however, 20.5% of the hepatocyte nuclei from control fish (Figure 2a) were found to contain nucleoli of sufficient size and staining intensity to be indistinguishable from smaller intranuclear inclusions. Corrected values for the percentage of nuclei containing inclusions would therefore be 48.3, 51.5, and 55.0% following 2, 4, and 6 days exposure, respectively. Corresponding neutron activation analysis data show that these livers contained averages of 23.8, 42.3, and 47.4 ppm arsenic for the same exposure intervals (Table 1).

No histological changes were observed in proximal convoluted tubules of the kidneys from these specimens after mean arsenic accumulation of 8.1, 7.2 and 14.2 ppm for 2, 4, and 6 days, respectively. After arsenic accumulation resulting in kidney burdens of 84 to 85 ppm (wet weight) following drinking water exposures of rats, BROWN et al. (1976) found only minor ultrastructural changes (i.e., swollen mitochondria and increased numbers of dense autophagic lysosome-like bodes). The lower resolving power of light microscopy combined with the 6- to 10-fold reduction in organ burdens observed for green sunfish as compared with rats could be expected to make such minor changes in mitochondria and lysosome-like bodies undetectable.

TABLE 1

Mean arsenic concentration values ($\bar{x}$) for tissues taken from Lepomis cyanellus following 2, 4, and 6 days exposure to 60 ppm arsenic at 20°C. Standard error of the mean ($s_{\bar{x}}$), total number of analyses made (n), and significance level (sign.) as determined using t-test (n.s., not significant; *, $p<0.05$; **, $p<0.01$; and ***, $p<0.001$). Superscript 1 refers to t-test comparisons made between mean levels for 2 and 4 days or 2 and 6 days exposure; superscript 2 designates significance levels of 4-day versus 6-day mean arsenic concentrations.

		Tissues						
Exposure Time		Gall Bladder (plus bile)	Liver	Spleen	Kidney	Gill	Ovary	Testes
2 days	$\bar{x}$	35.1	23.8	10.1	8.1	6.8	3.5	2.1
	$s_{\bar{x}}$	4.1	2.8	1.8	2.4	2.3	1.3	1.4
	n	10	7	10	14	10	2	8
	sign.	-	-	-	-	-	-	-
4 days	$\bar{x}$	77.7	42.3	23.4	7.2	2.7	6.2	3.7
	$s_{\bar{x}}$	32.0	13.9	13.3	2.9	1.7	3.1	0.4
	n	10	6	10	13	10	2	8
	sign.	$***^1$	$**^1$	$***^1$	$n.s.^1$	$n.s.^1$	$n.s.^1$	$n.s.^1$
6 days	$\bar{x}$	158.7	47.7	18.9	14.2	3.8	8.5	2.3
	$s_{\bar{x}}$	78.2	6.0	8.8	1.8	2.1	5.8	1.5
	n	10	7	10	14	10	2	7
	sign.	$\frac{***^1}{*^2}$	$\frac{***^1}{n.s.^2}$	$\frac{***^1}{n.s.^2}$	$\frac{*^1}{*^2}$	$\frac{n.s.^1}{n.s.^2}$	$\frac{n.s.^1}{n.s.^2}$	$\frac{n.s.^1}{*^2}$

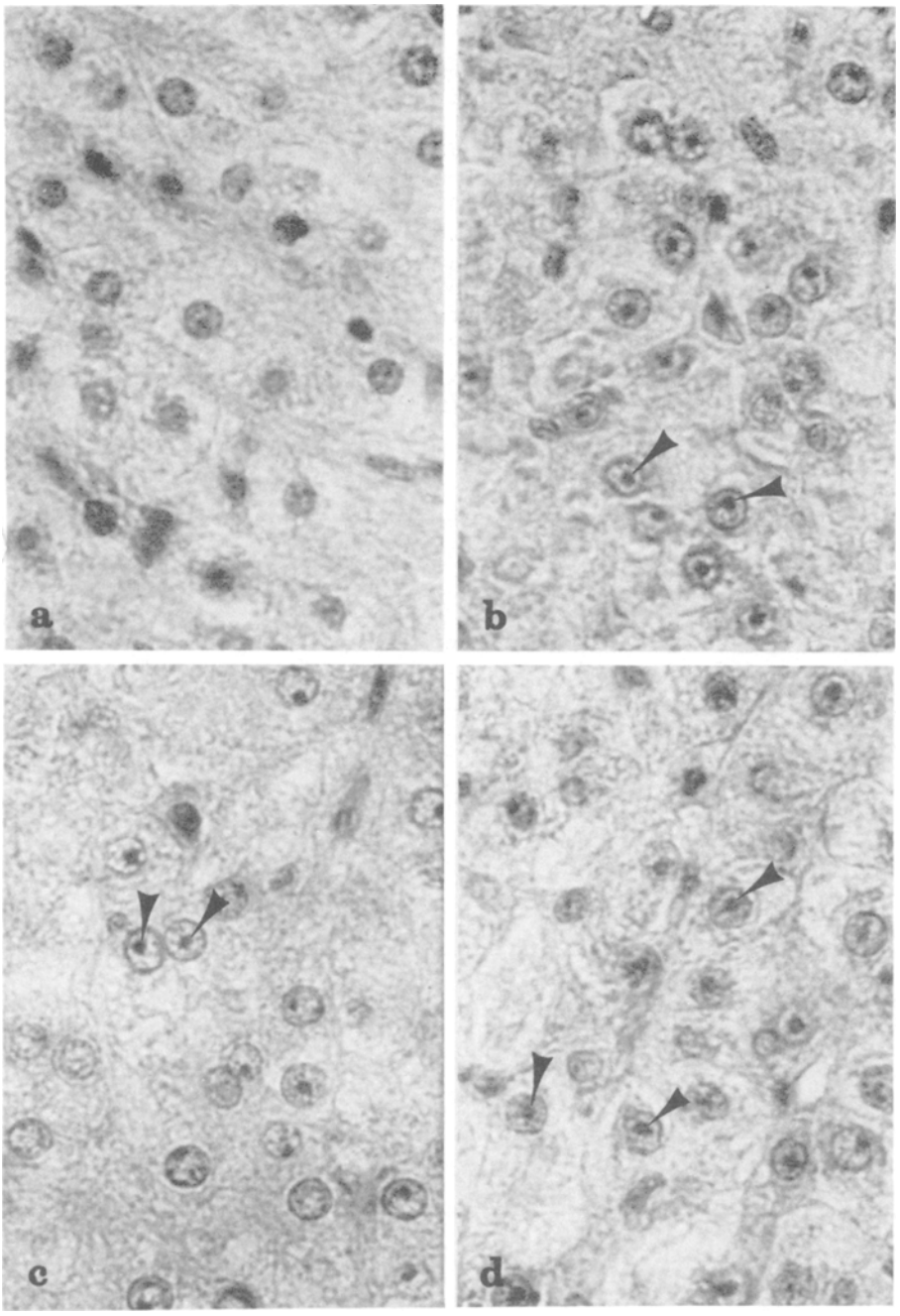

Figure 2. Liver from green sunfish exposed to 60 ppm arsenic for (a) 0, (b) 2, (c) 4, and (d) 6 days. Arrows indicate the presence of previously reported nuclear inclusions (SORENSEN 1976a), H&E x 800.

References

BROWN, M. M., B. C. RHYNE, R. A. GOYER, and B. A. FOWLER: J. Tox. Environ. Health 1, 505 (1976).

CHAPMAN, A. C.: Analyst 51, 548 (1926).

CIKRT, M.: Brit. J. Indust. Med. 29, 74 (1972).

DOUDOROFF, P., B. G. ANDERSON, G. E. BURDICK, P. S. GALTSOFF, W. B. HART, R. PATRICK, E. R. STRONG, E. W. SURBER, and W. M. VAN HORN. Sewage Ind. Wastes 23, 1380 (1951).

ELLIS, M. M., B. A. WESTFALL, and M. D. ELLIS: Indus. Engr. Chem. 33, 1331 (1941).

FINNER, L. L. and H. O. CALVERY: Arch. Path. 27, 433 (1939).

GILDERHUS, P. A.: Trans. Amer. Fish. Soc. 95, 289 (1966).

HARVEY, S. C.: In The pharmacological basis of therapeutics, 5 ed., pp. 924-930, L. S. Goodman and A. Gilman (ed.). London: The MacMillan Co. (1975).

KLAASSEN, C. D.: Tox. Appl. Pharma. 29, 434 (1974a).

KLAASSEN, C. D.: Tox. Appl. Pharma. 29, 447 (1974b).

KODAMA, Y., N. ISHINISHI, E. KUNITAKE, T. INAMUSU, and K. NOBUTOMO: In Effects and dose-response relationships of toxic metals, pp. 464-470, G. F. Nordberg (ed.). Amsterdam: Elsevier Sci. Pub. Co. (1976).

SORENSEN, E. M. B.: J. Fish Biol. 8, 229 (1976a).

SORENSEN, E. M. B.: Arch. Envir. Cont. Tox. 4, 8 (1976b).

SORENSEN, E. M. B.: Bull. Environ. Cont. Tox. 15, 756 (1976c).

WINDOM, H., R. STICKNEY, R. SMITH, D. WHITE, and F. TAYLOR: J. Fish. Res. Bd. Can 30, 275 (1973).