Polychlorinated Biphenyls: Effect of Long-Term Exposure on ATPase Activity in Fish, *Pimephales Promelas**†

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Evidence for the environmental contamination with polychlorinated biphenyls (PCBs) has been widely reported and is documented by a recent review (1). Several workers have reported the toxicity of these chemicals to several organisms but there exists a lack of evidence for the physiological action of PCBs. We have recently reported that, in vitro, PCBs inhibit Mg^{2+} ATPase and $Na^+ - K^+$ ATPase activity (2) and more specifically oligomycin-insensitive Mg^{2+} ATPase activity in fish (Blue Gill) tissue homogenates (3).

The present work was initiated to complement the in vitro studies with information on chronic exposure effects of PCBs, in vivo, on ATPases activity in fish.

EXPERIMENTAL

Fat head minnows, Pimephales promelas, were chronically exposed to Aroclors® 1242 and 1254 at the National Water Quality Laboratory, Environmental Protection Agency, Duluth, Minnesota, for several months. Chronic exposure studies were conducted under the direction of Dr. Allen Nebeker, NWQL. Samples of fish were obtained at a point in the experiment where reduction in the number of fish exposed to each specific concentration of PCB was warranted. The serial dilution dosing apparatus used was developed by Mount and Brungs (4,5).

Newly hatched fish (less than 24 hours old) were selected using twenty fish for each concentration of both PCBs in duplicate. They were exposed in January, 1971 and dissected in May for enzyme preparations. They were fed twice daily on brine shrimp, Daphnia, frozen and dried trout fry granules, during the exposure period.

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*Paper No. 7729 Scientific Journal Series, Institute of Agriculture, University of Minnesota, St. Paul, Minnesota.

†This research was supported by U.S. Dept. of Interior Grant FWPCA 16030 ELZ.

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Bulletin of Environmental Contamination & Toxicology,
Vol. 7, No. 2/3, 1972 © Springer-Verlag New York Inc.

MONS 086121

The water used in the tanks was untreated from Lake Superior, except for the chemicals which were dissolved in acetone. The added acetone was equivalent to 40 ppm. Fish exposed to acetone alone were used as controls. It was found that fat head minnows will tolerate 15,000 ppm of acetone. However, 100% mortality occurred within 4 - 10 days at concentrations of PCBs of 25 and 75 ppb. At low concentrations the mortality was much less with about 50% mortality in five months at 8.3 ppb. The water temperature was maintained at 22°C initially, increased at 24°C at maturity (2 mos.) and to 26°C at four months.

The fish tissues were dissected, homogenized and fractionated by centrifuging at 900g for ten minutes followed by 13,000 g for 20 minutes and the latter sediment was resuspended in 0.32 M sucrose 1 mM EDTA and 10 mM imidazole according to the procedure reported by Koch (6). The B fraction (suspension of the 13,000 g sediment) contained mitochondria and nerve ending particles. Each preparation was appropriately diluted and the samples were quick frozen in liquid nitrogen and stored at -20°C until the ATPase assay.

ATPase activity was determined by a continuous method described by Pullman et al. (7) and Fritz and Hamrick (8) and as reported by Yap and Cutkomp (9). Total ATPase is measured when Mg^{2+}, Na^{+}, K^{+} were present in the reaction mixture. Mg^{2+} ATPase activity was measured when 1 mM ouabain was present in the reaction mixture. Ouabain is a cardiac glycoside which specifically inhibits the Na^{+} - K^{+} ATPase activity (10). Na^{+} - K^{+} ATPase activity is the total ATPase activity minus Mg^{2+} ATPase activity. Mg^{2+} ATPase was further separated into oligomycin sensitive (mitochondrial) and oligomycin - insensitive portions by adding 0.03 µg oligomycin (oligomycin A 15% and B 85%) per ml reaction mixture (11-13).

The 3 ml reaction mixture used contained: 4.3 mM ATP, 5 mM Mg^{2+}, 100 mM Na^{+}, 20 mM K^{+}, 135 mM imidazole buffer (pH 7.5), 0.2 mM NADH, 0.5 mM PEP (phosphoenol - pyruvate), 0.02 per cent BSA (bovine serum albumin), approximately 9 units of pyruvate kinase and 12 units of lactic dehydrogenase and 100 µl of homogenate fraction. The reaction temperature was maintained at 37°C. Absorbance changes were measured at 340 nm over a period of 15 minutes using a Beckman DU spectrophotometer with constant temperature control.

Protein determinations followed the procedure developed by Lowry et al. (14) with absorbance measured at 660 nm using a Spectronic 20 colorimeter.

RESULTS AND DISCUSSION

Results of the ATPase determinations obtained from chronically treated fish are presented in Table 1 for Aroclor 1242, and in Table 2 for Aroclor 1254. All fish showed responses of ATPase activities to exposure to the Aroclors compared to control fish. As with earlier in vitro studies (3) both inhibition and stimulation responses were observed for the different tissues tested and

TABLE 1

Per cent inhibition of activity of ATPases by Aroclor 1242 treated fat head minnows. Fish brain, kidney and liver homogenates were used following a 4 month exposure period.

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TABLE I

Per cent inhibition of activity of ATPases by Aroclor 1242 treated fat head minnows. Fish brain, kidney and liver homogenates were used following a 4 month exposure period.

CONCENTRATION (ppb)	PER CENT INHIBITION*								
	BRAIN			KIDNEY			LIVER		
	$Na^+ - K^+$	Oligomycin** Sens. Mg^{2+}	Oligomycin Insens. Mg^{2+}	$Na^+ - K^+$	Oligomycin Sens. Mg^{2+}	Oligomycin Insens. Mg^{2+}	$Na^+ - K^+$	Oligomycin Sens. Mg^{2+}	Oligomycin Insens. Mg^{2+}
0.93	21.1	28.1	15.0	20.5	56.0	22.7	+41.6	0.8	7.9
2.8	4.9	53.2	2.3	36.2	75.4	20.7	+40.7	40.5	3.7
8.3	2.9	43.6	+0.7	18.3	45.2	35.0	42.4	40.7	15.5
CONTROL*** Sp. Act. ±S.E.	34.3 ±1.8	5.2 ±1.1	12.6 ±0.45	39.3 ±2.02	9.0 ±1.85	18.4 ±1.35	3.6 ±0.61	7.6 ±1.6	8.2 ±1.9

* (+) Values represent increased enzyme activity.

** Sens. (sensitive) Insens. (insensitive).

*** Specific activity calculated as u moles Pi mg^{-1} protein hr^{-1}. Each value represents the mean from three separate determinations.

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TABLE 2

Per cent inhibition of activity of ATPases by Aroclor 1254 treated fat head minnows. Fish brain, kidney and liver homogenates were used following a 4 month exposure period.

CONCENTRATION (ppb)	PER CENT INHIBITION*								
	BRAIN			KIDNEY			LIVER		
		Oligomycin**			Oligomycin			Oligomycin	
	$Na^+ - K^+$	Sens. Mg^{2+}	Insens. Mg^{2+}	$Na^+ - K^+$	Sens. Mg^{2+}	Insens. Mg^{2+}	$Na^+ - K^+$	Sens. Mg^{2+}	Insens. Mg^{2+}
0.31	+20.3	31.3	+21.8	+20.0	+53.7	+14.3	-	-	-
0.93	+11.2	35.2	+ 9.8	14.3	+ 7.3	+ 4.9	+20.8	+37.8	+11.4
2.8	+ 3.2	35.8	+ 5.5	5.1	3.3	+22.5	1.0	20.3	20.8
8.3	5.2	7.5	2.2	30.7	+ 4.2	+31.6	4.4	30.7	+ 2.2
CONTROL***	32.6	5.2	12.5	31.4	7.9	12.6	5.9	7.9	9.5
Sp. Act. ±S.E.	± 1.95	± 0.34	± 0.68	± 2.45	± 2.05	± 3.85	± 2.0	± 2.31	± 3.61

* (+) Values represent enzyme activation.
** Sens. (sensitivity) Insens. (Insensitive).
*** Specific activity calculated as μ moles Pi mg^{-1} protein hr^{-1}. Each value represents the mean from three separate determinations.

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Although d sults, the to indicate "attack" of responses c activities study; also required to The prolind that kidney from visual tissues had

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variations occurred between the two Aroclors. Brain was the only tissue that showed only inhibition of oligomycin sensitive Mg^{2+} ATPase after exposure to both Aroclors (Tables 1,2). The maximum inhibitory effect on the ATPases occurred with Aroclor 1242 on kidney tissue (Table 1). It was not possible to establish a dose effect of PCBs (2,3) because of an apparent greater sensitivity at exposure level 2.8 ppb than at higher or lower exposure levels (Table 1).

There appears to be a difference in the response of the ATPase activities to Aroclors 1254 and 1242 between in vivo and in vitro exposure of the enzymes. In vitro, on blue gill fish (3), 1254 and 1242 were both quite inhibitory to the ATPases for all tissues tested. Some stimulation of mitochondrial Mg^{2+} ATPase activity was observed for muscle and kidney at the lower concentrations tested (0.5 to 1.0 ppm). However, Aroclors 1221 and 1268 caused definite stimulation of mitochondrial Mg^{2+} ATPase activity in most of the tissues tested in vitro (3). In the present preliminary in vivo exposure study Aroclor 1254 caused stimulation of Mg^{2+} ATPase activity, as was observed in vitro (3). However, in vivo both oligomycin sensitive (mitochondrial) and insensitive activities showed stimulation in kidney and liver tissue (Table 2). No stimulation of oligomycin insensitive activity was observed in vitro (3).

In general, when stimulation occurred it was more prominent at lower concentrations and more erratic. Stimulation also occurred more often with those Aroclors which were the poorest inhibitors (in vitro 1221 and 1268, in vivo 1254) of Mg^{2+} ATPases and even of $Na^{+} - K^{+}$ ATPase. In addition, the compound (1242) showing greatest inhibition of Mg^{2+} ATPases, both in vivo (Table 1,2) and in vitro (3), corresponded to those Aroclors (1242, 1248) which were most toxic under chronic toxicological test conditions (Dr. Nebeker, personal communications). However, with Aroclor 1242, in vivo, greater inhibition of oligomycin sensitive than insensitive Mg^{2+} ATPases was observed (Table 1), while in vitro findings showed oligomycin insensitive activity somewhat more inhibited than mitochondrial activity by 1242 (3). $Na^{+} - K^{+}$ ATPase sensitivity to the Aroclors varied considerably for the different tissues under in vivo exposure (Tables 1,2). Further studies will be required to determine the significance of $Na^{+} - K^{+}$ ATPase responses to Aroclors.

Although differences did occur between in vivo and in vitro results, there appears to be sufficient evidence from these studies to indicate that ATPase inhibition may be the specific site of "attack" of PCB's in fish tissues. The apparent differences in responses of oligomycin sensitive and insensitive Mg^{2+} ATPase activities between in vivo and in vitro results requires further study; also more detailed study (in vivo) on individual tissues is required to determine if there is a "target" tissue for PCB attack. The preliminary results, reported here, would seem to indicate that kidney tissue was most sensitive to Aroclor 1242. However, from visual observations during dissection both liver and kidney tissues had undergone considerable degradation at 8.3 ppb

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level of exposure for both Aroclor 1242 and 1254. Also in vitro findings (3) showed muscle tissue to be highly sensitive to PCB's. We were unable to confirm this finding because of technical problems in differential centrifugation of the tissue homogenates.

ACKNOWLEDGEMENTS

We are indebted to NWQL, Duluth, Minn. for supplying the fish used in this study. In particular we are grateful to Dr. Allen Mebaker who made arrangements to obtain the fish from his controlled study and to Dr. Glenn Christensen in whose laboratory initial sample preparation was conducted.

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