Induction of Hepatic Microsomal Enzymes by Aroclor® 1254 in *Ictalurus punctatus* (Channel Catfish)

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CAMP et al. (1974) studied the absorption, distribution and physiological effects of Aroclor^R 1254 (PCBs) on Ictalurus punctatus (channel catfish). The study indicated that PCBs concentrate largely in the liver and brain with attendant signs of liver dysfunction. The hydrophobicity and the biomagnification of PCBs in the liver suggest that PCBs may have an effect on hepatic microsomal enzymes.

Previous studies (ADAMSON 1967; POHL et al. 1974) have shown that aquatic vertebrates possess microsomal enzymes and that the activity of these enzymes are considerably lower than those observed in mammals. Phenylbutazone, DDT (BUHLER 1966) and 3-methylcholanthrene (BEND et al. 1973) have been reported to induce microsomal enzymes in certain aquatic vertebrates. The induction of rat hepatic microsomal enzymes by PCBs has been well documented (LITTERST and VAN LOON 1972; BRUCKNER et al. 1973; JOHNSTONE et al. 1974), however, Aroclor^R 1254 does not appear to induce these enzymes in Raja erinacea (little skate) (BEND et al. 1973).

The purpose of this study was to determine the effects of Aroclor^R 1254 on hepatic microsomal enzyme activity and mitochondrial respiration in channel catfish. The interaction of exposure and retention time of Aroclor^R 1254 in channel catfish was also determined.

Materials and Methods

For the biological magnification and retention study, 40 channel catfish were divided into 2 groups. Each group was exposed to 2 ppm Aroclor^R 1254. Nineteen fish were exposed for 12 hours and 21 fish were exposed for 24 hours. At the end of the exposure period, 5 fish from each group were sacrificed and the brain, liver, and smooth muscle tissues of each fish were assayed for PCBs. To study the tissue depletion of PCBs, the remaining fish were placed in fresh water. From each treatment group, 6 fish were sampled at 168 hours, 5 fish were sampled at 336 hours and at 504 hours the remaining fish (3 fish from the 12-hours initial exposure group and 5 fish from the 24-hours initial exposure group) were sacrificed. The liver, brain, skeletal muscle and the fluid from the gall bladder of each fish were assayed for PCBs by a glc procedure described previously (CAMP et al. 1974).

Bulletin of Environmental Contamination & Toxicology,
Vol. 16, No. 4 © 1976 by Springer-Verlag New York Inc.

For the microsomal enzyme study, 19, mixed-sex, channel catfish weighing 318±49 g (SD) were placed in aquaria 1 day prior to exposure. Aroclor^{R} 1254 was dissolved in Corexit 7664 (Enjay Co.) (ZITKO 1970) and added to the water of 10 principal fish to give a concentration of 1 ppm. An equivalent amount of Corexit 7664 was added to the water containing 9 control fish.

After 96 hours, the spinal cord of each fish was pithed and the liver was immediately removed. A liver homogenate of each fish was prepared by macerating the tissue in 0.05% KCl in 0.1 M phosphate buffer at pH 7.4 (0.5 g/ml) with 4 passes of a teflon pestle in a Potter-Elvehjem homogenizer. The homogenate was centrifuged at 10,000 x g and the supernatant phase assayed for analine hydroxylase and aminopyrine N-demethylase activity (MAZEL 1971). All reagents used in the assay were dissolved in 0.1 M phosphate buffer (pH 7.4). The incubation mixture consisted of 1.0 ml cofactor solution (2 mM NADP, 10 mM glucose-6-phosphate and 25 mM $MgCl_2$), 0.1 ml glucose-6-phosphate dehydrogenase (25 units/ml) and 0.5 ml 10,000 x g supernatant phase (0.5 g liver/ml). The aminopyrine N-demethylase incubation mixture also contained 0.1 ml semicarbazide HCl (45 mM). The assay solutions were equilibrated at 29 C for 5 minutes, and then the reactions were initiated by adding 0.5 ml resublimed analine HCl (10 mM) for the hydroxylase assay or 0.5 ml aminopyrine (10 mM) for the N-demethylase assay.

The analine hydroxylase activity was determined by measuring the amount of p-aminophenol produced during a 20 minute incubation period. The reaction was stopped by adding 0.5 ml of 33% TCA to the incubation mixture. A 2.0 ml aliquot of the TCA supernatant fraction was made basic with 0.5 ml of 20% Na_2CO_3 and the blue, phenol indophenol complex was formed by adding 1.0 ml of 2% phenol in 0.2 N NaOH. The absorbance of the colored complex was measured on a Beckman DBG using scale expansion (full scale deflection was equivalent to 10% T).

The N-demethylase reaction was allowed to proceed for 30 minutes before terminating with 1.0 ml of 15% $ZnSO_4$. The increased concentration of the end product, formaldehyde, was determined by a dilution modification of the Nash reaction (NASH 1953).

Protein content of the 10,000 x g supernatant phase was determined by a modification of the Lowery method (MILLER 1959). Bovine serum albumin was used as the standard.

PCBs were extracted from a 0.5 ml sample of the 10,000 x g supernatant phase by vortexing with 1.0 ml of

30 ppb aldrin in nanograde hexanes for 30 seconds. One to 5 µl of the hexane layer was injected on a glc column of 3% OV-17 on Gas Chrom Q at 185 C. The chromatograms were quantitatively analyzed as described previously (CAMP et al. 1974).

Mitochondria from fish and rat liver were isolated according to the procedure of SCHNEIDER and HOGEBOOM (1950) using 0.25 M sucrose, 1.0 mM EDTA Na and 1.4 mM tris buffer (pH 7.3). Respiration of mitochondria was measured at 25 C in a volume of 3.0 ml with a YSI Model 53 Biological Oxygen Monitor equipped with a Clark type polarographic electrode. Succinate and α-ketoglutarate were used as substrates for the mitochondrial preparations.

Results and Discussion

Table 1 shows the biological magnification of PCBs in various tissues of channel catfish after 12 and 24 hours of exposure to 2 ppm Aroclor^R 1254, and the rate of post-exposure PCB elimination and redistribution. Statistical analysis by the student t test showed no difference in the amount of PCBs in the tissues after 12 hours of exposure when compared with the same tissues after 24 hours of exposure. There was no evidence that the PCBs absorbed in either exposure group were eliminated or redistributed from any of the tissues at any time during the 480-hour post-exposure period. The data indicates that at a 2 ppm exposure level, a maximum concentration of PCBs was obtained in all tissues tested after exposure for 12 hours and that the level of PCBs was maintained for at least 480 hours post-exposure. The greatest magnification of PCBs was in the liver and brain (high lipid content) with much less concentrating in the skeletal muscle (low lipid content). The gall bladder also contained a significant amount of PCBs.

The effects of PCBs on hepatic microsomal enzyme activity from fish exposed 96 hours to 1 ppm of Aroclor^R 1254 are presented in Table 2. Both aminopyrine N-demethylase and aniline hydroxylase activities were significantly increased in the principals. N-Demethylase activity increased 68.4% and hydroxylase activity increased 47.5% over the control fish. BRUCKNER et al. (1973) found a 47.3% increase in aminopyrine N-demethylase and a 235% increase in N-acetyl-p-aminophenol hydroxylase activities in rats given 100 mg/kg Aroclor^R 1254 ip. Other investigators (LITTERST et al. 1972) have reported a 233% increase in aminopyrine N-demethylase activity in rats treated with Aroclor^R 1254.

TABLE 1

Biological Magnification and Retention of PCBs in Ictalurus Punctatus (channel catfish) Exposed to 2 ppm of Aroclor^{R} 1254 for 12 and 24 Hours

	Fish Group Exposed 12 Hours Hours After Exposure				Fish Group Exposed 24 Hours Hours After Exposure			
Tissue	12	168	336	504	24	168	336	504
	ppm $\bar{x}$ ± SD							
Brain	86±51	86±49	99±50	102±74	179±125	74±30	45±40	79±92
Liver	121±109	87±62	93±50	61±47	201±179	76±50	80±92	108±66
Skeletal muscle	15±9	8±4	6±4	9±8	28±21	13±12	7±8	8±3
Gall bladder	---	27±19	29±8	114±78	---	51±33	111±37	87±14
Fish wt., g	7.0±1.9	5.4±2.2	5.2±2.2	3.6±1.4	7.4±4.7	6.7±3.6	3.8±1.7	4.0±0.8
# Fish	5	6	5	3	5	6	5	5

TABLE 2

Effect of Aroclor^{R} 1254 on Microsomal Enzyme Activity of Fish Exposed to PCBs for 4 Days (1 ppm)

	# Specimen	Aniline Hydroxylation nm pAP[a] per g liver	Aniline Hydroxylation nm pAP[a] per mg protein	Aminopyrine N-Demethylation nm HCHO[b] per g liver	Aminopyrine N-Demethylation nm HCHO[b] per mg protein
Fish (Control)	9	11.7±6.3	0.141±0.073	207.5±108.6	2.50±1.26
Fish (Principal)	10	16.9±3.9[c]	0.208±0.056[c]	343.7±103.06[d]	4.21±1.42[d]
Rat	2	151.4±98.2	1.36±0.725	1264.8±250.2	11.74±0.68

[a]nmoles p-Aminophenol per 20 min.; [b]nmoles HCHO per 30 min.; [c]Values statistically different from control at the $P < 0.05$ level as determined by student t tests; [d]Values statistically different from control at the $P < 0.02$ level as determined by student t tests.

TABLE 3

10,000 x g Supernatant Phase PCB Concentration and Effect of Aroclor^{R} 1254 on Liver Weight and Protein Content in Fish Exposed to PCBs for 4 Days (1 ppm)

	# Specimen	Liver weight g	10,000 x g protein[a] mg per g liver	PCB Concentration µg per g liver
Fish (Control)	9	2.61±0.69	82.2±6.6	< 0.25
Fish (Principal)	10	3.36±0.96[b]	83.2±9.3	24.0±23.2

[a]Concentration of protein in 10,000 x g supernatant phase of liver homogenate; [b]Value statistically different from control at the $P < 0.05$ level as determined by student t test.

It has been demonstrated that microsomal enzymes react with physiological steroids to form physiologically inactive hydroxylated products. Induction of microsomal enzymes accelerate hydroxylation of endogenous steroid hormones and thus alter the physiologic action of these steroids (CONNEY 1971). CAMP et al. (1974) have demonstrated a decrease in blood cortisol levels in channel catfish treated with PCBs. The observed reduction in blood cortisol of fish could be attributed to increased biotransformation of cortisol. In man, it has been shown that the conversion of cortisol to 6-β-hydroxycortisol increases with an increase in microsomal enzyme activity (CONNEY 1971). Physiologically, cortisol increases the mobilization of fatty acids from adipose tissue. A decrease in cortisol level could reduce the elimination of fatty acids containing PCBs and thus cause the observed long retention time of PCBs in channel catfish.

PCB induction of microsomal enzymes could decrease the physiological concentration of other steroids. A decrease in steroid sex hormones could interfere with reproductive processes in fish.

The analine hydroxylase activity of the control fish in this study (0.035 μmoles/g liver/h) compares favorably with the analine hydroxylase activity in channel catfish reported by Buhler (0.026 μmoles/g liver/h) (ADAMSON 1967). However, there is considerable difference in Buhler's value for aminopyrine N-demethylase activity (0.009 μmoles/g liver/h) in channel catfish and the activity observed in the current study (0.415 μmoles/g liver/h). These values were reported by ADAMSON (1967) and sufficient information on the assay procedure was not presented to determine the reason for this large discrepancy in N-demethylase activities.

There was a significant increase in the average weight of livers from fish exposed to the PCBs without a concomitant increase in the protein content (Table 3). This effect was observed by BRUCKNER et al. (1973) in rats given Aroclor^{R} 1242.

All fish treated with Aroclor^{R} 1254 showed a significant PCB concentration in the liver 10,000 x g supernatant phase (Table 3). There was no correlation between either of the microsomal enzyme activities tested and the concentration of PCBs in the individual liver 10,000 x g supernatant phase. PCBs (< 0.25 μg/g liver) could not be detected in the liver 10,000 x g supernatant phase of the control fish. Using glc, the PCB profile obtained from the tissue of fish treated with

Aroclor^{R} 1254 showed no change from the profile of the standard Aroclor^{R} 1254.

In contrast to the observation of PARDINI (1971) on the inhibition of mitochondrial succinoxidase, we did not observe an inhibition of mitochondrial respiration employing succinate or α-ketoglutarate as substrates for non-phosphorylating fish liver mitochondria. If a change in oxygen utilization did occur, the change was an increase in oxygen uptake. In this study, we were unsuccessful in isolating coupled-phosphorylating fish liver mitochondria with the isolation procedure using 0.25 M sucrose. However, we did study the effects of Aroclor^{R} 1254 on phosphorylating rat liver mitochondria. At a concentration of 152 ppm Aroclor^{R} 1254, we did not observe an inhibition of cellular respiration or an uncoupling of oxidative phosphorylation. From this study it must be concluded that Aroclor^{R} 1254 at moderate concentrations does not effect electron transport or oxidative phosphorylation in liver mitochondria and therefore, does not interfere with the aerobic synthesis of ATP.

Acknowledgement

This work was supported in part by The Texas Agriculture Experiment Station, Project No. H-6032 and Sea Grant Account No. 53277, Texas A&M University System.

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