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Polycyclic Aromatic Hydrocarbon Metabolism in Mullets, *Chelon labrosus*, Treated by Polychlorinated Biphenyls

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Contamination of the ocean by hydrocarbons has become a major environmental problem. Consequently, it is not surprising that residues of potentially toxic xenobiotics, such as benzo(a)pyrene (B(a)P), are present in marine species used as human food (Dunn and Stich 1976). The oxidative metabolism of aromatic hydrocarbons proceeds via intermediate arene oxides (Safe et al. 1978). Some of these metabolites are very reactive electrophiles and may interact with cellular macromolecules such as proteins, DNA and RNA (Kuroki et al. 1971). Thus, the ability of an organism to further metabolize arene oxides can be an important protective mechanism against the possible toxic effect of these molecules. The existence of hepatic cytochrome P-450 dependent monooxygenase activities in fish is now well established (Pohl et al. 1974, Bend et al. 1977). The exposure of fish to polychlorinated biphenyls (PCB) increases both the monooxygenase activities and the total amount of cytochrome P-450 in the microsomal fractions of fish liver (Lidman et al. 1976, Narbonne and Gallis 1979). The purpose of this study was to compare the toxication-detoxication balance in both control and PCB induced estuarine fish (Grey mullets).

MATERIALS AND METHODS

Mullets were caught in November in the Arcachon Bay and transferred to 20-L aquaria with filtered and aerated sea water at a temperature of 12°C. Under these conditions, fish were acclimated and starved for 10 days before the experiment. Then the fish weighing 135 ± 28g (mean ± SD) were selected into two groups of 5 animals which were injected ip either a solution of polychlorobiphenyle (Phenoclor DP6) in dimethylsulfoxide (10 mg/Kg body weight) or an equivalent volume of solvent. Sacrifice occured 4 days later.

Liver homogenization of each fish was prepared in 0.150 M KCl, 0.010 M $MgCl_2$ and 0.050 M Tris buffer at pH 7.4 with 4 passes of a teflon pestle in a Potter Elvehjeim homogenizer. Homogenates were centrifuged at 15,000 g for 10 min and supernatents were centrifuged at 105,000 g for 60 min to sediment microsomal fractions. All pellets were resuspended in the same buffer and protein concentrations were determined by the method of Lowry et al.(1951). The following assays were carried out in microsomal suspensions : aminopyrine N demethylase activity (APDM), using the colorimetric method described by Gilbert and Golberg (1965), benzopyrene monooxygenase activity (BaPMO) with a radiometric method using ^3H B(a)P as described by Depierre et al. (1975), epoxide hydrolase activity (EH) with ^3H styrene oxide by the radiometric method of Oesch et al. (1971). The cytochrome P-450 content was determined by the method of Omura and Sato (1964). The binding of ^3H B(a)P metabolites to DNA was estimated by the method of Alexandrov and Frayssinet (1974). Glutathione S transferase activity (GST) was measured in the cytosolic fraction with ^3H styrene oxide as substrate using the method of Marniemi and Parkki (1975).

^3H Benzopyrene and ^3H styrene oxide were obtained from Amersham and the cofactors from Sigma Chemical Company. Phenoclor DP6 was provided by Prodelec Company (France).

RESULTS AND DISCUSSION

The total body weight and length of fish were not modified by DP6 treatment (Table 1).

Table 1. Body and liver parameters in control and DP6 treated fish.

	Controls (a)	DP6 treatment (a)	Difference (%)
Fish number	5	5	
Fish weight (g)	153 ± 23	137 ± 19	
Fish length (cm)	25 ± 5	23 ± 5	
Liver weight (% body weight)	1.18 ± 0.16	1.35 ± 0.18	+ 14
Microsomal proteins (mg/whole liver /100g body weight)	47.0 ± 4.2	48.0 ± 3.8	
Cytochrome P-450 (pmoles/mg microsomal proteins)	226 ± 52	379 ± 69	+ 68 (b)

(a) : values are mean ± SD
(b) : significantly different from control group (t test $P<0.05$)

The liver somatic index and the microsomal protein content were not significantly modified by DP6 treatment (Table 1). However, the cytochrome P-450 content significantly increased in the treated group (+ 68%).

Table 2 shows the changes in mullet hepatic microsomal enzyme activities after the Phenoclor DP6 treatment. The mixed function oxygenase activities were enhanced by DP6 treatment (+ 129% and + 88% respectively for APDM and BaPMO activities). In contrast epoxide hydrolase and glutathione S transferase activities were not altered.

Some metabolites generated by MFO system activities can bind to DNA but the extent of this binding was not influenced by PCB treatment (Table 3).

Table 2. Effect of Phenoclor DP6 on drug metabolism activities in mullet liver.

Activities	Control (a)	DP6 (a)	Difference (%)
APDM pmoles/min / mg microsomal proteins	12.7 ± 1.8	29.0 ± 2.9	+ 129 (b)
BaPMO nmoles/min / mg microsomal proteins	1.2 ± 0.12	2.26 ± 0.22	+ 88 (c)
EH nmoles/min / mg microsomal proteins	4.02 ± 0.99	4.41 ± 0.54	+ 9.7
GST nmoles/min / mg cytosolic proteins	5.73 ± 0.79	5.69 ± 0.79	- 0.69

(a) : values are mean ± SD
(b) : significantly different from control ($P<0.02$) by t test
(c) : significantly different from control ($P<0.001$) by t test

Table 3. Effect of Phenoclor DP6 on BaP metabolites binding to DNA

	^{3}H B(a)P binding nmoles/g DNA (a)	Difference (%)
Control	23.6 ± 1.15	
DP6 treated	19.4 ± 1.2	- 17 (b)

(a) : values are mean ± SD
(b) : not significantly different (P 0.05) by t test

Clearly, the Phenoclor DP6 induces the cytochrome P-450 dependent MFO activities in mullet liver. This effect is similar to that reported previously (Narbonne and Gallis 1979).In the present study PCB increased the activity of both APDM and BaPMO. The cytochrome P-450 content was also enhanced. These data are in accord with previous results on fish and mammals (Alvares et al. 1973; Lidman et al. 1976; Addison et al. 1979). In contrast the enzymatic pathways of arene oxides metabolism including EH and GST did not significantly change after PCB treatment at the dose tested. James and Bend (1976) showed that Aroclor 1254 injected ip to Archosargus probatocephalus (100 mg/Kg) was a potent inducer of BaP hydroxylase (+ 448%) and cytochrome P-450 (+ 91%). In these experimental conditions, epoxide hydrolase and glutathione transferase activities were not significantly modified. Bend et al. (1977) showed no alteration in these two enzyme activities after a treatment by 3-methyl cholanthrene. The biological significance of these observations were not clear for these authors. However, the induction of the metabolic pathways leading to the formation of potentially toxic arene oxides without concomitant induction of the detoxication pathways might increase the susceptibility of fish to xenobiotic cytotoxicity or carcinogenicity.

The metabolic activation of B(a)P toxicity can be estimated by in vitro measurement of ^{3}H B(a)P metabolites binding to DNA in presence of microsomal enzymes. Gelboin (1969) suggested that the B(a)P binding to DNA was related to hydroxylase enzyme activities in rats. In the present study benzo(a)pyrene binding to DNA and EH activity were not modified by DP6 treatment whereas B(a)P monooxygenase activity was induced. Thus, in this case the toxication seems to be linked to EH activity. In the metabolism of benzopyrene it appears that some epoxide dihydrodiols metabolites are ultimate carcinogens which bind to cellular macromolecules (Sims et al. 1974). Arene oxides are hydrated in dihydrodiols by EH activity. These metabolites can be further transformed in diol epoxides by the MFO system. Thus, EH activity seems to be a limiting factor for the toxication pathways of xenobiotics in fish.

The results reported here show that chemicals which induce hydroxylase activities in fish may not necessary influence the overall toxicity of hydrocarbons or related chemicals.

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