Bull. Environm. Contam. Toxicol. 23,338-343 (1979)

In Vivo and *In Vitro* Effect of Phenoclor DP6 on Drug Metabolizing Activity in Mullet Liver

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USHIO and DOGUCHI (1977) have shown, by using experimental diets in 1972-1973, that the average daily intake of polychlorinated biphenyls (PCB) in the japonese population was 3.5 g per capita. The higher dietary levels of PCB were attributed to marine animal products in the Japonese diet. It is worldwide acknowledged that PCB is found in fish and wildlife (KOEMAN et al. 1969, LINKO et al. 1974, SIMS et al. 1977, WOOLLARD and SETTLE, 1978), but in spite of the lively concern is known about xenobiotic metabolism in fish. Originally it was believed that fish laked the enzymes necessary for xenobiotic metabolism (BRODIE and MAICKEL, 1962), but more recently numerous studies have proven the existence of fish mixed function oxidase systems (MFO) similar to mammalian mixed function oxidase systems (BACKER et al. 1963, POTTER and O'BRIEN 1964, CREAVEN et al. 1967, LINDMAN et al. 1976). Fish MFO appear to be capable of catabolizing many of the same drugs catabolized by mammalian MFO (BEND et al. 1973, LINDMAN et al. 1976, STOTT and SINNHUBER 1978) Furthermore, fish MFO drug metabolism has been shown to be inductible by PCB compounds (AHOKAS et al. 1975, LINDMAN et al. 1976).

Polychlorinated biphenyls are protent inducers of cytochrome P_{450}, drug hydroxylation and demethylation (LITTERST et al. 1972, BRUCKNER et al. 1974, PARKKI et al. 1977), and have been reported to possess properties of phenobarbital and polycyclic classes of inducers (ALVARES et al. 1973, VAINIO 1974, GOLDSTEIN et al. 1978). This paper describes certain *in vivo* and *in vitro* effects of Phenoclor DP6 on MFO activities in hepatic microsomes of estuarine fish.

MATERIALS AND METHODS

Two groups of mullet of the species *Chelon labrosus* were used. Fish were kept in 20 l tanks containing seawater at 11-12°C reviewed every 48 hrs, and were fed a dry diet ("Aqualim" mullet chow). All fish were acclimated to this environment prior to the initiation of exposure to contaminent. Fish of the treated group were fed a diet containing 50 µg/g of Phenoclor DP6 (PRODELEC Co, France) and fish of the untreated group were fed a control diet. After eight days of experimental feeding, all fish were sacrificed, their livers excised and

0007-4861/79/0023-0338 $01.20

homogenised in phosphate buffer (0.1 M, pH 7.4) and cell fractionation was carried out as previously described (see NARBONNE and BOURDICHON, 1978). All manipulations were conducted in a refrigerated room at 4°C. The liver homogenate was centrifuged at 10.000 x g for 10 minutes to remove nuclei and mitochondria. The remaining supernatant was centrifuged at 105.000 x g for 60 minutes. The microsomal pellet was resuspended in the phosphate buffer. Aminopyrine-N-demethylase activity was assayed using the method described by GILBERT and GOLDBERG (1965) and aniline hydroxylase activity was measured according to a method established by IMAI et al. (1966). Thirty minute incubation periods at 37°C were stopped by TCA precipitation and microsomal proteins were then solubilized in 0.66 N KOH and measured by the method established by LOWRY et al. (1951). The liver microsomal concentrations of cytochrome P_{450} and cytochrome b_5 were determined by OMURA and SATO's method (1964). For the in vitro studies, microsomal suspensions were made from the control fish livers and incubated with different concentrations of DP6, ranging from 10 to 1000 ppm solubilized in ethylene glycol monoethyl ether (EGME) at 1.66 % final concentration.

RESULTS AND DISCUSSION

The hepatic MFO activities of the fish fed a DP6 diet are given in table 1. Liver hydroxylase and demethylase activities as well as cytochrome P_{450} and cytochrome b_5 contents are significantly increased in treated fish, rising from 63 to 118 per cent with respect to control values. Aniline hydroxylase activity and cytochrome b_5 concentration are more sensitive to DP6 treatment than aminopyrine-N-demethylase and cytochrome P_{450}. However, as has been shown in rat liver studies, the last two microsomal parameters correlate very well. Table 2 shows that fish liver MFO activities are not significantly modified by Phenoclor DP6 in the in vitro experiments.

The results of the present study clearly indicate the ability of PCB to induce the fish hepatic drug metabolizing enzymes. Earlier studies (unpublished results) inducated an increase in liver weight and protein content within 30 days of installation of a similar DP6 diet. SASTRY and SHARMA (1978) have reported histopathological changes in livers of Chana punctatus injected with endrine, an organochlorine compound. Some of the most conspicuous early changes were structural disarrary and hepatic enlargement, pyknosis of nuclei, cytoplasmic granulation, and rupture of cell membranes. It should be pointed out that organochlorine compounds, such as DDT, act in a similar fashion in other vertebrates than fish (FITZUGH and NELSON, 1947; DURHAM et al., 1943). The changes noted in liver morphology have been correlated with the modification in MFO activities in mammals (BRUCKNER et al. 1974). LIDMAN et al. (1976) have reported significant increases in

TABLE 1

IN VIVO EFFECT OF PHENOCLOR DP6 ON MIXED FUNCTION OXIDASE ACTIVITY FROM MULLETS LIVERS

Parameters	Controls	DP6 treated	Difference (%)
Number of fish	5	5	
Weight of fish (g)	103 ± 19	138 ± 23	
Length of fish (cm)	23 ± 1	24 ± 2	
Aniline hydroxylase *	3.67 ± 0.42	6.56 ± 0.59	+ 78 **
Aminopyrine N demethylase *	2.67 ± 0.17	4.39 ± 0.08	+ 64 ****
Cytochrome b_5 *	0.038 ± 0.007	0.083 ± 0.009	+ 118 **
Cytochrome P_{450} *	0.212 ± 0.026	0.346 ± 0.008	+ 63 ****

* Aniline hydroxylase and aminopyrine N demethylase activities given in n moles/30 min/mg protein, Cytochrom b_5 and P_{450} concentration given in nMole/mg protein ± SE

** Significant ($P < 0,01$) difference relative to controls Student's t test for 9 degrees of freedom

**** Significant difference $P < 0,001$.

TABLE 2

IN VITRO EFFECTS OF PHENOCLOR DP6 ON MIXED FUNCTION OXIDASE ACTIVITY IN MULLET LIVERS

DP6 concentration ppm EGME concentration %	0 0	0 1.66	10 1.66	100 1.66	1000 1.66
Aniline hydroxylase *	2.48 ± 0.12	2.47 ± 0.16	2.32 ± 0.20	2.70 ± 0.19	2.39 ± 0.13
Aminopyrine demethylase *	3.70 ± 0.10	3.68 ± 0.08	3.79 ± 0.17	3.59 ± 0.19	3.66 ± 0.15

* Aniline hydroxylase and aminopyrine-N-demethylase activities given in nmoles/30 min/mg protein ; All differences relative to controls are not significative (Student's t test)

cytoplasm P_{450} and several drug metabolizing enzymes activities at 3 and 7 days post-oral dosage of 1000 mg PCB/kg in rainbow trout (S. Gardneri). GRUGER et al. (1977) have found hepatic aryl hydrocarbon hydroxylase to be induced in Coho Salmon exposed to 1 ppm PCB in their diet.

Their results are interesting when taken from the point of vue of PCB metabolism in fish versus other species. HUTZINGER et al. (1972) found PCB to be very little metabolized in brook trout as compared to in rats. HUTZINGER et al. (1972) found a nearly negligible level of PCB metabolism in brook trout as compared to that in rats.

MELANCON and LECH (1976) confirmed this general phenomenon, but discovered small amounts of conjugated metabolites in the bile of ^{14}C tetrachlorobiphenyl treated rainbow trout.

In some recent work (unpublished results) we found that the disappearance rate of DP6 from mullets tissues after removal of DP6 from the experimental diet was much lower than in rats. Moreover, we have shown (1979) that there is no selective metabolism of specific DP6 components in mullet metabolisms (NARBONNE, 1979) whereas there appears to exist such specific metabolism in the rat (NARBONNE and GILLET 1978).

In this paper we have shown that both mullets and rats possess inductible MFO enzymes and therefore the difference in PCB metabolism in these two vertebrates would not appear to be due to different activity levels of these drug metabolizing enzymes.

Furthermore, two researchers, HINZ and MATSUMURA (1977), have shown that there exists different PCB metabolism profiles among three different species of fish.

ACKNOWLEDGMENTS

I thank Michèle DAUBEZE for technical assistance. This work was supported by a grant from the Commission des Communautés Européennes n°151-77-1 Bruxelles.

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