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In Vitro Effects of Phenoclor DP6 on Drug Metabolism in Rat Liver

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Polychlorinated biphenyls (PCBs), common environmental pollutants (RISEBROUGH et al. 1968), are protent inducers of drug hydroxylation (BRUCKNER et al. 1973, VAINIO 1974, GOLDSTEIN et al. 1975), demethylation (LITTERST et al. 1972, BRUCKNER et al. 1974, TURNER et GREEN 1974), glucuronidation (VAINIO 1974, GOLDSTEIN et al. 1975) and glutathione conjugation (ECOBICHON and COMEAU 1974, 1975, JOHNSTONE et al. 1974). PCBs have been reported to possess properties of phenobarbital and polycyclic hydrocarbon classes of inducers (ALVARES et al. 1973). More recently, GOLDSTEIN et al. (1978) have shown that these properties were due to the presence of dibenzofurans in commercially prepared PCB mixtures. However, the biochemical mechanisms by which PCB causes liver microsomal enzyme induction are unknown.

In previous studies we have demonstrated liver hypertrophy and microsomal membrane proliferation including enzyme induction in rats fed Phenoclor DP6 diets (NARBONNE and DAUBEZE 1978, NARBONNE and BOURDICHON 1978). Moreover, the incorporation of labeled aminoacids into the liver microsomal proteins was increased both *in vivo* and *in vitro* (NARBONNE 1979a, NARBONNE 1979b). The induction of synthesis of microsomal proteins by PCB suggests that there exists an indirect mechanism for mixed function oxydase (M.F.O.) activity induction. Thus we have demonstrated that DP6 has no direct effect on *in vitro* proteosynthesis activity (NARBONNE 1979 b). In view of the possibility that a direct relation might exist between the increased microsomal enzyme activity and the binding of DP6 to the microsomal membranes sites, we have expanded our study to the *in vitro* activity of M.F.O. in the presence of various amounts of DP6.

METHODS

Rat liver was homogenized and fractionated according the procedure previously described (NARBONNE and BOURDICHON 1976). The suspension of the microsomal fraction used contained nearly 2 mg of protein per ml. The enzyme activities were assayed according to GILBERT and GOLDBERG (1955) for aminopyrine-N-demethylase and to IMAI et al. (1966) for aniline hydroxylase. Each measurement was performed using four separate preparations. The final volume of the incubation mixture was 6 ml.

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First of all, we tried to find the best solvant of DP6. Dimethyl sulfoxyde (DMSO) and ethyleneglycol monomethyl ether (EGME) were tested at different concentrations. Secondly, the DP6 was added to the incubation mixture with 100 µl of EGME. After 30 minutes, incubations were stopped by TCA precipitation. The proteins were solubilized in 0.66 N KOH and measured by LOWRY's method (1951).

RESULTS AND DISCUSSION

Table 1 shows that aniline hydroxylase and aminopyrine N-demethylase activities were unaffected by either of the two solvents used DMSO or EGME. The signification of the t values were lower than the 0.05 confidence level.

In table 2 are summarized the effects of Phenoclor DP6 on in vitro MFO activities. The t test values show that these enzyme activities are not significantly modified by DP6. If we summarize briefly some of the factors which affect the observed activity of an enzyme system we could find that enzyme control mecanisms may be classified into two broad categories. Firstly, there are those factors that affect the enzymatic activity of specific molecules, and secondly, those factors that regulate the amount of enzyme protein. With respect to the former, the activity of an enzyme may be affected by the concentration of either substrate or product. The modification can be either "isosteric" (competitive) or "allosteric" (non competitive) (GELBOIN 1972). SCHMOLDT et al. (1977) have studied the in vitro metabolism of aminopyrine and aniline by rat liver microsomal monooxygenases in the presence of different polychlorinated biphenyl mixtures and some related hydroxybiphenyls. The tested PCB mixtures contained dichloro-, tetrachloro- or hexachlorobiphenyls. All PCB were competitive inhibitors of aminopyrine demethylation. Chlorinated 4-hydroxybiphenyls inhibited competitively the metabolism of both type I and type II substrates. These results are not in agreement with ours. However, it can be considered that DP6 is a mixture containing high chlorinated biphenyls (penta- to octochlorobiphenyls) little metabolized by rats (NARBONNE and GILLET 1978). HUTZINGER et al. (1974) have shown that monohydroxy compounds of chloro, dichlo and tetrachlorobiphenyls were found in rat species. Thus, the PCB competitive inhibition of metabolism of type I substrate seems to be due to the possibility of metabolization of the compound.

The present data suggest that the increased activity of MFO in DP6 fed rats is not due to a direct action of the chlorinated compounds on microsomal enzymes. The term "enzyme induction" can be used in these processes which increase the rate of synthesis of the microsomal enzyme as has been previously shown (NARBONNE 1979c).

TABLE 1

Effects of solvents on *in vitro* aniline hydroxylase and aminopyrine-N-demethylase activities from rat livers.

Solvent concentration (%)	0 (control)	0,83	1,66	4,16
A.H. [a]'				
E.G.M.E. [a]	9,27[b] ± 0,47	9,92 ± 0,16 (1,28)[c]	9,49 ± 0,11 (0,455)	9,29 ± 0,17 (0,039)
D.M.S.O. [a]	9,27 ± 0,47	9,25 ± 0,62 (0,025)	9,37 ± 0,19 (0,196)	-
A.P.D.M. [a]				
E.G.M.E. [a]	0,72 ± 0,020	0,769 ± 0,009 (1,19)	0,738 ± 0,032 (0,287)	0,714 ± 0,020 (0,20)
D.M.S.O. [a]	0,723 ± 0,020	0,734 ± 0,015 (0,267)	0,794 ± 0,022 (1,58)	-

a A.H., aniline hydroxylase; A.P.D.M., aminopyrine-N-demethylase; E.G.M.E., ethyleneglycol monomethyl ether; D.M.S.O., dimethyl sulfoxide.

b mean from four separate preparations. Specific activities (nMoles mg^{-1} protein 30 min^{-1}) ± S.E.

c t values from the Student's t test calculation.

TABLE 2

Effects of Phenoclor DP6 on *in vitro* aniline hydroxylase and aminopyrine-N-demethylase activities from rat livers

DP6 concentration ppm	0	0	1	10	100	1000
EGME concentration %	0	1,66	1,66	1,66	1,66	1,66
A.H. [a]	12,5[b] ± 0,3	12,9 ± 0,2 (0,879)	12,2 ± 0,8 (0,687)	12,9 ± 0,3 (0,857)	13,2 ± 0,5 (0,992)	13,4 ± 0,5 (1,33)
A.P.D.M. [a]	0,73 ± 0,03	0,79 ± 0,04 (1,09)	0,80 ± 0,05 (1,20)	0,78 ± 0,05 (0,958)	0,76 ± 0,05 (0,630)	0,76 ± 0,03 (0,753)

a - b - c See foot notes in table 1.

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