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Long Term Effects of PCBs (Phenoclor DP5) on Rat Microsomal Enzymes, Liver, and Blood Lipids after Peri- and Postnatal Exposure

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PCBs have numerous toxic effects on laboratory animals (Safe 1984; Silberhorn et al. 1990) and are strongly retained in body fat because of their high liposolubility.

Previous work (Poul 1987) has shown that activities of some hepatic drug metabolizing enzymes and parameters of lipid metabolism were modified in adult rats (PND100), after exposure to PCBs (Phenoclor DP5) during lactation. Perinatal or early postnatal treatment with inducers, like phenobarbital and phenytoin, seems to induce permanent effects on hepatic microsomal enzymes in adults (Faris and Campbell 1983; Shapiro et al. 1986), even though the drugs have completely disappeared from the body.

Time course evolution of induction-related parameters and tissue residues of DP5, from weaning to PND100, have been studied (Poul 1991) : the effects observed in adult rats at PND100 could be residual aspects of the important changes induced before weaning by acute exposure via milk or consequences of the relative high concentrations of PCBs still present in tissues. So, assessment of permanent effects, such as imprinting of liver mixed function oxidases, induced by prenatal or postnatal administration of PCBs must be recognized only when these substances have been cleared away from the organism as completely as possible.

The present study was designed to investigate the effects of DP5, administered peri- and postnatally, on microsomal enzyme activities and in vitro genotoxic activation of 2-aminofluorene and on liver and blood lipids, in adult rats at PND180 and PND300. Tissue residues of Phenoclor DP5 were measured in liver, fat and brain at the same periods.

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MATERIALS AND METHODS

Male (320-350 g) and female (230-250 g) Sprague Dawley rats (Charles River France) were housed in an air conditioned room and allowed free access to a commercial diet (UAR A03) and water.

Virgin female rats were mated overnight with males. Vaginal smears were taken in the morning and the presence of sperm was considered as day 0 of gestation. After mating, females were randomly assigned to one of the three treatment groups :
- Control
- Phenoclor DP5: 50 mg/kg, every two days, from day 2 to 20 of lactation (DP5L)
- Phenoclor DP5: 50 mg/kg, every two days, from day 14 to 20 of gestation and from day 2 to 20 of lactation (DP5GL)

Phenoclor DP5 was dissolved in sunflower oil and administered by stomach tube in a volume of 1 ml/kg. On postnatal day 1 (PND1), offsprings were weighed, sexed and reduced to 10 pups, 5 males and 5 females when possible. They were weaned on PND21. Part of the control and DP5L group rats was sacrificed on PND180 and the other part on PND300. All the rats of the DP5GL group were sacrificed on PND300.

After a 12h starvation period, rats were lightly anaesthetized with ether, and after blood withdrawal, liver was perfused with cold 0.9% NaCl. Liver lipid extraction procedure, triacylglycerol, cholesterol and phospholipid estimation in blood and liver extracts, liver microsome preparation and microsomal enzyme activity dosage methods have been described elsewhere (Poul 1983). Aldrin epoxydase activity was estimated according to Kurihara et al.(1984). In vitro mutagenic assays were performed according to Maron and Ames (1983), on *Salmonella typhimurium* strain TA98 with 2-aminofluorene (5 µg per plate) and different concentrations of S9 liver fractions of control and DP5L rats killed at PND300.

For analysis of Phenoclor DP5, the whole brain and sample of liver (1g) and fat tissue (0.5g) were homogenized in a total volume of 50 ml of petroleum ether. After cleanup with sulfuric acid, the extracts were analyzed on a Varian 3500 gas-chromatograph with a 10 CPSil-19CB capillary column and 63Ni electron capture detector (Poul 1991). The lower limit of detection is 0.025ppm.

Results, presented as mean and mean standard deviation, were analyzed by one way analysis of variance or Student t test.

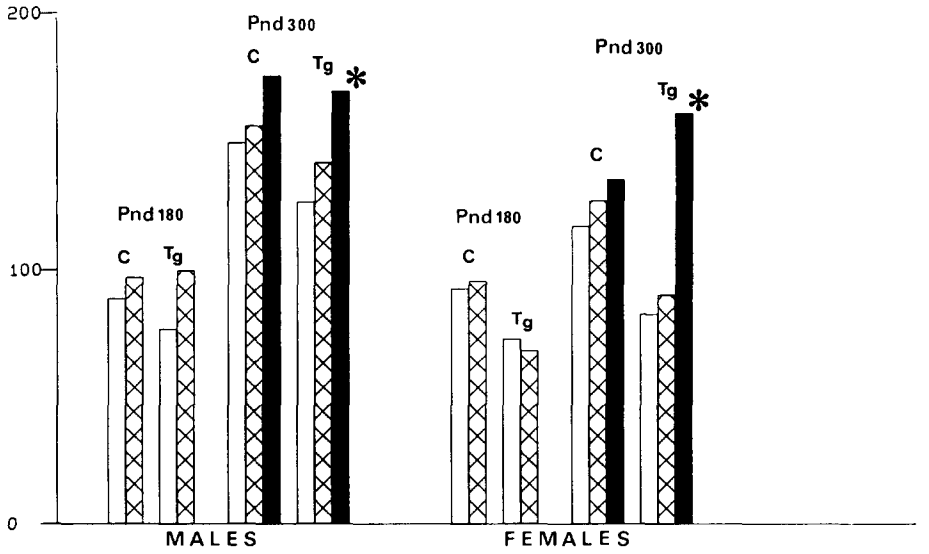

Figure 1. Effects of peri- and postnatal treatment with PCBs on blood lipids (mg/100ml) in adult rats.
(□):Control; (⊠):DP5L; (■):DP5GL. Asterisks indicate statistically different mean at P<0.05. TG = Triacylglycerols; C = Total cholesterol.

RESULTS AND DISCUSSION

DP5 administration to dams during the end of gestation (day 14 to 20) and the lactation (day 2 to 20) enhanced the rate of mortality of newborns before weaning (2% in control against 24.7% in DP5GL rats). These results confirmed the toxicity of PCBs during gestation. Tissue concentrations of DP5 are shown in Table 1.

Table 1. DP5 stores in offspring tissues (ppm)

	CONTR.	DP5L				DP5GL	
		PND180		PND300		PND300	
		Male	Female	Male	Female	Male	Female
LIVER	nd	0.09 ±0.03	0.29 ±0.03	0.16 ±0.06	0.12 ±0.02	0.12 ±0.02	0.22 ±0.02
BRAIN	nd	0.08 ±0.01	0.14 ±0.02	0.05 ±0.02	0.10 ±0.03	nd	0.08 ±0.03
FAT	0.60 ±0.06	10.2 ± 1.1	30.8 ± 3.3	7.5 ± 0.5	16.2 ± 1.2	7.9 ± 1.2	20.5 ± 1.6

nd : <0.025ppm

Table 2. Microsomal drug metabolizing enzyme activities in male offsprings

	Liver weight g/100g	Liver micros prot. mg/g	Cyt. P 450 nM/mg	A.P.D.M. nM/mn /mg	A.P.D.M. nM/mn /100g	A.H. nM/mn /mg	UDPGt nM/mn /mg	B.P.H. nM/mn /mg	B.P.H. nM/mn /100g	A.E. nM/mn /mg
PND180										
CONTROL (20)	2.62 ±0.06	22.1 ± 0.5	1.01 ±0.02	2.56 ±0.08	146.5 ± 4.7	0.56 ±0.02	16.8 ± 0.7	88.0 ± 2.4	5042 ± 156	3.92 ±0.15
DP5L (20)	2.72 ±0.07	21.3 ± 0.5	1.03 ±0.03	2.87* ±0.10	167.0* ± 8.3	0.56 ±0.03	16.6 ± 0.6	91.0 ± 2.8	5289 ± 237	4.14 ±0.15
PND300										
CONTROL (20)	2.60 ±0.09	20.4 ± 0.6	0.96 ±0.03	2.22 ±0.08	116.1 ± 5.6	0.54 ±0.03	14.9 ± 0.8	75.2 ± 3.6	3988 ± 297	3.26 ±0.18
DP5L (20)	2.77 ±0.09	20.5 ± 0.5	1.00 ±0.03	2.36 ±0.09	132.4 ± 6.9	0.55 ±0.02	14.0 ± 0.6	76.5 ± 4.8	4260 ± 257	3.43 ±0.21
DP5GL (14)	2.85 ±0.08	19.8 ± 0.6	0.95 ±0.03	2.52* ±0.11	140.3* ± 7.0	0.53 ±0.03	16.7 ± 1.1	91.2* ± 3.5	5088* ± 230	3.43 ±0.14

( ):number of rats per group; * :significantly different from respective control ($P<0.05$)
Enzyme specific activity is expressed as nM/minute/mg of microsomal proteins
Total enzyme activity is expressed as nM/mn/100 g body weight
APDM :aminopyrine-demethylase; AH :aniline-hydroxylase; UDPGt :UDPglucuronyl-transferase
BPH :benzo(a)pyrene-hydroxylase; AE :aldrin-epoxidase

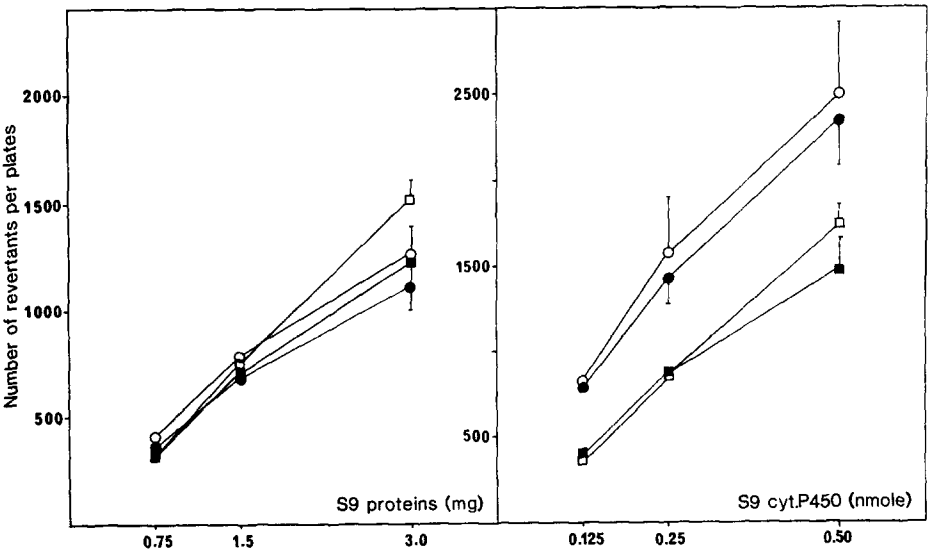

Figure 2. Genotoxic activation of Aminofluorene (Ames test) by S9 homogenates of DP5L treated rats.
(■): Male control; (□): Male DP5L; (●): Female control; (○): Female DP5L

Liver and brain had very low stores of DP5 as compared with fat tissue. This experiment and previous work (Poul 1991) indicate that DP5 elimination from the body proceeds slowly. Female rats had more elevated DP5 tissue levels than males in the three organs. Additional administration of PCBs during the late period of gestation did not seem to influence the tissue concentrations at PND300.

Effects of peri- and postnatal treatment with DP5 on blood lipids are shown in Figure 1. Treatment during lactation (DP5L group) had no effect in both sexes, but administration during the end of gestation and the lactation (DP5GL group) induced a significant rise in plasma triacylglycerols at PND300 in male (+34%) and especially in female (+95%) rats. Liver concentrations of all classes of lipids were the same in the three groups (results not shown).

Effects of DP5 administration on liver microsomal drug metabolizing enzymes are presented in Table 2 (males) and Table 3 (females). DP5 exposure during lactation (DP5L group) induced a transient increase of aminopyrine-demethylase in male rats at PND180, which was no longer detectable at PND300. In DP5GL group, specific and total activities of aminopyrine-demethylase and benzo(a)pyrene-hydroxylase for male rats, UDPG-transferase and benzo(a)pyrene-hydroxylase for female rats were slightly above control values at PND300 (increase ranging from 20 to 25%). Other enzyme

Table 3. Microsomal drug metabolizing enzyme activities in female offsprings

	Liver weight g/100g	Liver micros prot. mg/g	Cyt. P 450 nM/mg	AP.DM nM/mn /mg	A.H. nM/mn /mg	UDPGt		B.P.H.		A.E. nM/mn /mg
						nM/mn /mg	nM/mn /100g	nM/mn /mg	nM/mn /100g	
PND180										
CONTROL (20)	2.41 ±0.04	22.1 ± 0.5	0.72 ±0.02	2.35 ±0.10	0.59 ±0.02	11.1 ± 0.8	584 ± 43	25.8 ± 1.0	1371 ± 70	0.46 ±0.03
DP5L (20)	2.50 ±0.07	21.8 ± 0.5	0.72 ±0.02	2.39 ±0.08	0.59 ±0.02	12.0 ± 0.7	665 ± 56	27.9 ± 1.4	1539 ± 110	0.49 ±0.03
PND300										
CONTROL (20)	2.41 ±0.05	21.2 ± 0.5	0.77 ±0.02	2.67 ±0.08	0.69 ±0.01	10.9 ± 0.6	556 ± 33	26.5 ± 1.4	1346 ± 77	0.50 ±0.01
DP5L (20)	2.50 ±0.13	21.0 ± 0.4	0.76 ±0.02	2.68 ±0.08	0.67 ±0.02	10.6 ± 0.7	556 ± 45	27.3 ± 1.6	1415 ± 98	0.48 ±0.03
DP5GL (13)	2.53 ±0.06	20.3 ± 0.4	0.76 ±0.02	2.73 ±0.09	0.66 ±0.02	13.5* ± 0.9	697* ± 57	31.7* ± 1.6	1633* ± 95	0.52 ±0.03

( ):number of rats per group; * :significantly different from respective control (P<0.05)
Enzyme specific activity is expressed as nM/minute/mg of microsomal proteins
Total enzyme activity is expressed as nM/mn/100 g body weight
APDM :aminopyrine-demethylase; AH :aniline-hydroxylase; UDPGt :UDPglucuronyl-transferase
BPH :benzo(a)pyrene-hydroxylase; AE :aldrin-epoxidase

activities and cytochrome P450 contents were at the control level.

Activation of aminofluorene into genotoxic metabolites by S9 homogenates of control and DP5L rats, at PND300, have been investigated, in vitro, with the Ames test (Figure 2). No significant differences could be seen between the groups, with however a trend towards an increase of mutagenicity of aminofluorene by the DP5 treated rat S9 homogenates.

The results of this study indicate that PCB administration to dams, during the end of gestation and the lactation, led to long term (at least until PND300) modifications of some basal microsomal enzyme activities and of blood triacylglycerols. Exposure during lactation induces only temporary changes which disappeared at PND300. PCB tissue concentrations related to both exposures are the same at PND300 (10 to 20ppm).

PCBs are stronger inducers of mixed function oxidases but their long lasting effects seem less marked than those of phenobarbital. However, their effects on activation of genotoxic metabolism of drugs need further investigations, particularly after exposure during gestation or perinatal period. The rise of blood triacylglycerols in adults might also have some importance in the increase of plasma lipids with age.

Production of PCBs is stopped or limited in most countries, but these substances still persist in the environment. Evaluation of PCBs effects on foetus and newborns has been recently recommended by the World Health Organization (1990). The present data and other works (Lilienthal et al; 1990; Jacobson et al. 1990) indicate that, although PCB transfer from dams to offspring is much less important during gestation than lactation (Takagi et al. 1986), the greatest risk to development seems to be associated with the exposure during the perinatal period.

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