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Effects of Dietary Phenochlor DP5 on Microsomal Enzymes, Liver, and Blood Lipids in Adult Male and Female Rats After Subchronic and Perinatal Exposures

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Polychlorinated biphenyls (PCBs) are widely spread in the environment, and their residues have been detected in the tissues of fish, wild and domestic animals and in humans, especially in breast milk of mothers occupationnally exposed. PCBs have numerous toxic effects on laboratory animals, namely hepatotoxicity, immunotoxicity, reproductive and hormonal effects, mutagenic and carcinogenic potency (Safe 1984). They have been recognized as potent inducers of many microsomal drug metabolizing enzymes in several species (Litterst et al. 1972). Moreover, treatment of rats with PCBs gave rise to altered lipid metabolism with accumulation of lipids in the liver (Hinton et al. 1978 ; Sandberg and Glaumann 1980 ; Kholi et al. 1983 ; Carter 1985 ; Kato et al. 1982). In most of these studies male rats have been used. However, sex differences in the effects of xenobiotics on microsomal drug metabolizing enzymes have been shown (Skett and Paterson 1985) particularly with PCBs (Narbonne 1978) and little was known about differences in the effects of PCBs on lipid metabolism.

This study was designed to investigate the effects of a subchronic treatment with Phenochlor DP5 on some microsomal drug metabolizing enzyme activities and on liver and blood lipids of male and female rats. The long-term effects of DP5 administration during pre and postnatal period on adult microsomal enzyme activities and liver and blood lipids of both sexes have also been studied. Indeed, a possible xenobiotic imprinting of the hepatic monooxygenase system during neonatal period has been shown recently (Bagley and Hayes 1985) and it has been recognized that some functional defects, for example in organ metabolism, which often manifest themselves in adult period may be induced prenatally or before weaning (Neubert et al. 1985).

MATERIALS AND METHODS

Male (320-350g) and female (220-260g) Wistar rats (Iffa-Credo, France) were housed in an air conditioned room and allowed free access to a semi-synthetic diet (18% casein, 40% potato starch, 31,5% sugar, 5% peanut oil, 4% salt mixture and 1% vitamin

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mixture) and water ad libitum. Phenochlor DP5 (Prodelec France) was dissolved in peanut oil and incorporated into the diet at a final dose of 250 mg/kg during 8 days. After a 12 hours starvation period, rats were lightly anesthetized with ether, and blood, withdrawn from aorta, was frozen at -20°C. Liver lipid extraction procedure, triacylglycerol, cholesterol and phospholipid estimations in blood and liver extracts, liver microsome preparation and microsomal proteins, cytochrome P450, aminopyrine demethylase, aniline hydroxylase, UDPG transferase activity dosage methods have been described elsewhere (Poul 1983). For time course effect study of PCBs on blood lipids, male rats were allowed the diet supplemented with 250 mg/kg of DP5 during 16 days. Blood was collected from rats starved for 6 hours, by tail section, and cholesterol and triacyglycerol were measured as described.

Virgin female rats (220-240g) were mated overnight with males. Vaginal smears were taken in the morning and the presence of sperm was considered as day 0 of gestation. On day 2 of gestation, treated females were fed with a semi-synthetic diet (22% casein, 36,5% potato starch, 30% sugar, 5% peanut oil, 4% salt mixture supplemented with Co++, Cu++, Mn++, Zn++, and 1,5% vitamine mixture) supplemented with 50 mg/kg of Phenochlor DP5. This treatment was continuously administred throughout gestation and lactation. One day after parturition, litters were reduced to 8 offsprings (4 males and 4 females when possible). After weaning, all pups received the control diet until 110-125 days of age, when the rats were randomized according to sex as follows : 1) control group (C/C) and pre and postnatally DP5 treated group (DP5/C), these animals were sacrificed and measurements of cytochrome P450, aminopyrine demethylase, microsomal proteins, liver and blood lipids were performed, 2) control group (C/DP5) and pre and postnatally DP5 treated group (DP5/DP5) were retreated with DP5 (50 mg/kg) via the diet during 8 days. Animals were then sacrificed and dosages of cytochrome P450, aminopyrine demethylase and microsomal proteins were performed.

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

RESULTS AND DISCUSSION

The data representing the effects of DP5 on activities of microsomal drug metabolizing enzymes are summarized in table 1. All parameters measured were enhanced by the treatment in male and female rats. Basal activities of aminopyrine demethylase, aniline hydroxylase and UDPG transferase were smaller in females when concentrations in microsomal proteins were similar in both sexes. Inducing effects of DP5 were generally more important in the males except for the induction of UDPG transferase activity which was threefold higher in female and 1,6 in male rats.

Table 1. Microsomal drug metabolism parameters in male and female rats in basal conditions and after DP5 (250 mg/kg for 8 days) exposure.

	Control males	DP5 males	Control females	DP5 females
Liver weight (g/100 g)	2.93±0.03	4.78±0.22*	2.54±0.04	3.10±0.15*
Microsomal proteins (mg/g)	21.2±0.5	34.4±1.1*	20.0±0.7	24.6±0.7*
Cytochrome P450 (nmole/mg)	0.91±0.03	2.87±0.15*	0.67±0.03	1.51±0.09*
Aminopyrine demethylase (nmole/mn/mg)	3.08±0.18	6.62±0.44*	1.79±0.08	3.02±0.23*
Aniline hydroxylase (nmole/mn/mg)	0.91±0.03	1.55±0.05*	0.53±0.02	0.73±0.04*
UDPGtransferase (nmole/mn/mg)	16.7±1.2	26.5±1.0*	7.7 ±0.9	23.2±1.5*

6 rats per group. * : significantly different from respective control ($P < 0.05$)

In male liver, DP5 increased all class of lipids significantly. Th increase in total cholesterol was mainly reflected in esterified fraction (results not shown). In the female liver, phospholipid concentration was enhanced, total cholesterol was unaffected and triacylglycerol slightly decreased (table 2).

Table 2. Blood and liver lipids in male and female rats in basal conditions and after DP5 exposure.

	Control males	DP5 males	Control females	DP5 females
Liver lipids (mg/g)				
Triacylglycerol	6.6±0.4	12.2±1.4*	11.4±0.9	8.1±0.4*
Total cholesterol	3.4±0.2	5.9±1.1*	3.0±0.1	3.1±0.1
Phospholipids	38.7±0.6	51.4±0.9*	38.5±0.4	41.8±0.4*
Blood lipids (mg/100 ml)				
Triacylglycerol	52.8±5.0	113.2±9.6*	76.6±5.3	92.6±21.5
Total cholesterol	67.4±7.1	117.0±14.7*	96.9±8.3	70.2± 7.5

6 rats per group. * : significantly different from respective control ($P < 0.05$)

These sex differences in DP5 effects on lipid metabolism were confirmed by measurements of blood lipids (table 2). In males, DP5 treatment increased the concentration of both triacylglycerol and cholesterol and in females, blood lipid concentration was not statistically different from that of controls.

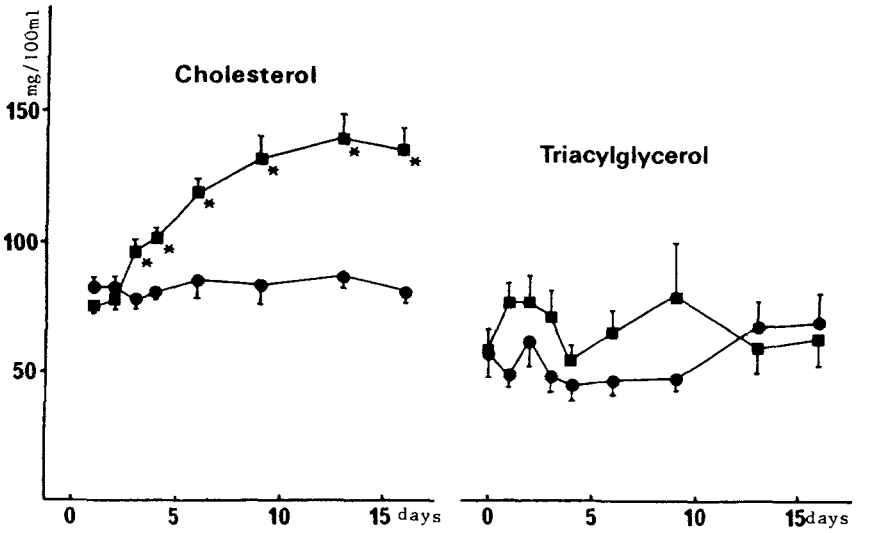

Figure 1. Time course of blood lipids in male rats fed dietary DP5 (250mg/kg). ● : control, ■ : DP5. Asteriks indicate statistically different mean at $P < 0.05$.

Time course effect of DP5 (250 mg/kg diet) on blood lipids was examined in male rats starved for 6 hours (each animal being its own control throughout the 16 days of experiment). Figure 1 shows that the rise in blood total cholesterol began after a few days' treatment and seemed to be maximum after 12 days. Blood triacylglycerol was slightly elevated in rats treated with DP5 during the most part of the exposure period but the differences with control were never statistically significant. From comparison with the above-mentioned results on animals starved for 16 hours, it appeared that the nutritional status of rats may be taken into consideration for the interpretation of PCBs effects on blood triacylglycerol.

In male and female rats treated in utero and during lactation, via the diet of mothers, by Phenochlor DP5 (50 mg/kg), basal liver weight, aminopyrine demethylase activity and concentration of cytochrome P450 and microsomal proteins have been investigated in adulthood (110-125 days old rats) (table 3).

In male rats, specific activity of aminopyrine demethylase and cytochrome P450 were at the control level, but when expressed by 100 g body weight, these two parameters were slightly above control and activity of aminopyrine demethylase was increased by 20% ($P<0.05$). In females, no difference was found between DP5 treated rats and controls. In these experimental conditions, a pre and postnatal exposure to 50 mg/kg of DP5 through the dams weakly only affected some parameters of microsomal drug metabolism in the adult. However, males seemed more sensitive than females to the treatment.

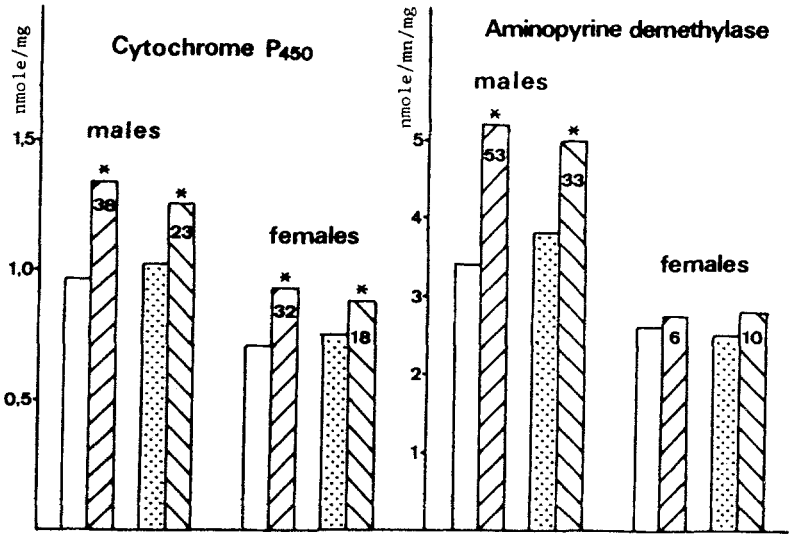

Figure 2. Microsomal enzyme activities in male and female rats with respect to pre and postnatal and further adult treatment with DP5. ☐ C/C, ▨ C/DP5, ⁘ DP5/C, ▧ DP5/DP5. (see details in materials and methods). The numbers in bars indicate the percentage of increase in activities compared to corresponding control. Asteriks indicate statistically different mean at $P < 0.05$.

Table 3. Long-term effect of DP5 exposure during pre and postnatal period on adult (110-125 days old) male and female microsomal drug metabolism parameters.

	Control males	DP5 males	Control females	DP5 females
Liver weight (g/100 gBW)	2.73±0.04	2.80±0.07	2.70±0.09	2.75±0.05
Microsomal proteins (mg/100 gBW)	52.0±2.3	55.9±2.3	50.1±1.8	51.9±1.7
Cytochrome P450				
(nmole/mg)	0.96±0.03	1.01±0.03	0.70±0.04	0.74±0.02
(nmole/100 gBW)	49.6±2.2	56.5±2.8**	34.9±1.8	38.5±2.1
Aminopyrine demethylase				
(nmole/mn/mg)	3.41±0.18	3.79±0.17	2.58±0.10	2.52±0.14
(nmole/mn/100 gBW)	176.1±9.1	211.9±13.5*	128.2±3.7	131.5±10.7

8 rats per group. * : significantly different from respective control ($P < 0.05$). (**: $0.05 < P < 0.10$). BW : body weight.

Inducing effects of a further subacute dose of DP5 (50 mg/kg of diet) administered in adult period to rats treated pre and postnatally and comparison with corresponding controls are shown in figure 2. In males, a secondary adult DP5 exposure caused

significant increases in aminopyrine demethylase and cytochrome P450 irrespective of the perinatal treatment, but the percentage of increase seemed to be slightly smaller in rats pretreated in utero and during lactation with DP5.
In female treated pre and postnatally there was no difference between groups except for cytochrome P450 (figure 2) and relative liver weight (results not shown). Here too, differences between males and females in basal and induced activities of microsomal drug metabolism parameters could be noted.

Liver lipids of control and pre and postnatally DP5 treated rats assayed at 110-125 days were not significantly different in both sexes (results not shown). However, blood triacylglycerol in males and blood cholesterol in females are decreased by the pre and postnatally exposure to PCBs (table 4).

Table 4. Long-term effects of DP5 exposure during pre and postnatal period on adult male and female blood lipids.

	Control males	DP5 males	Control females	DP5 females
Triacylglycerol (mg/100 ml)	138.3±9.5	103.2±13.0*	71.1±5.6	59.7± 6.4
Total cholesterol (mg/100 ml)	98.6±5.5	86.4± 6.0	76.8±5.4	57.3± 4.9*

8 rats per group. * : significantly different from respective control ($P < 0.05$).

The results of this study would indicate that basal activities of aminopyrine demethylase, aniline hydroxylase, UDPG transferase and concentration of cytochrome P450 were higher in male than in female adult rats. These sex differences in the activities of microsomal drug metabolizing enzymes have been previously shown (Kato 1974). A possible explanation of these findings was an imprinting of drug metabolizing enzymes activities by androgens via the hypotalamus-hypophysis-liver axis during the neonatal period (Skett and Gustafson 1979). Treatment of male and female rats by Phenochlor DP5 (250 mg/kg in the diet during 8 days) gave rise to relative liver weight, concentrations of microsomal proteins and cytochrome P450 and activities of aminopyrine demethylase, aniline hydroxylase and UDPG transferase in both sexes. However, male rat response to the inducing effect was stronger than female except for UDPG transferase activity which was triplicated in females, reaching the level of males after induction. These sex differences were depending on the xenobiotics administered and the microsomal enzymes studied (Skett and Paterson 1985).

Subchronic treatment with DP5 altered the lipid metabolism in male rats. Liver triacylglycerol, total cholesterol and phospholipid fractions, blood triacylglycerol and cholesterol concentrations were enhanced in animals fasting for 16 hours. Study of time course effect of DP5 on blood lipids in male rats starved for 6

hours indicated a rapid increase of cholesterol concentration as early as the third day of exposure. The concentration of triacylglycerol remained at the control level throughout the duration of treatment. The rise of blood cholesterol after PCBs ingestion has been observed in different studies (Kato and Yoshida 1981 ; Yagi and Itokawa 1980 ; Kling and Gamble 1982) and among lipoprotein fractions, HDL-cholesterol seemed selectively enhanced in rat (Carter 1985) and in man after drug induced liver induction (Luoma et al. 1983). The effects of PCBs on blood triacylglycerol were more controverted with indications of enhancement (Baumann et al. 1983 ; Quazi et al. 1983a), decrease (Sandberg and Glaumann 1980) or no change (Yagi and Itokawa 1980) in blood after PCBs exposure. These results might be explained by various experimental conditions, particularly length of exposure, nutritional factors, inducers or species used.

After PCB administration in rats, fatty liver was generally observed (Litterst et al. 1972 ; Hinton et al. 1978) but it seemed that there was no close relationship between induction of microsomal enzymes and steatosis because some isomers of PCBs (Kholi et al. 1979) and inducers like methylcholanthrene (Goldberg et al. 1981) had no effect on the liver lipid concentrations. The hypothesis of a partial block of intracellular migration of VLDL particles in liver after PCBs resulting in accumulation of triacylglycerol and cholesterol in the liver has been proposed (Sandberg and Glaumann 1980).

In female rats treated with DP5, liver phospholipid concentration was enhanced indicating some proliferation of endoplasmic reticulum, but liver cholesterol was unchanged and triacylglycerol was significantly decreased compared with control. In blood, total cholesterol concentration decreased and triacylglycerol concentration increased but the differences from controls was under the level of signification. Few studies have investigated the effects of PCBs on blood and liver lipids in female rats. Quazi et al. (1983b) have indicated an increase of blood and liver cholesterol in female rats after Arochlor 1245 exposure. Discrepancy with the results of our study comes perhaps from differences in age of animals and diet composition. However it may be, more detailed works on sex responses in lipid metabolism after PCB administration are needed.

Long-term effects of fetal and neonatal exposure of rats to DP5 (50 mg/kg of mother's diet) on adult microsomal enzymes and liver and blood lipids have been investigated. In these conditions, PCBs were transferred from mothers to fetus and pups via the placenta and the milk (Bresner et al. 1984). In adult male rats (110-125 days old) a weak increase in cytochrome P450 (14%) and aminopyrine demethylase activity (20%), expressed by 100 g body weight, was noted. In females, none of the parameters related to the microsomal enzyme induction were affected by the pre and postnatal exposure to DP5. Moreover, a secondary treatment of adult with DP5 (50 mg/kg diet during 8 days) in rats of both sexes induced a similar rise in level of enzyme activities in pre and

postnatal controls and DP5-treated rats, and so, the percentage of increased activity from respective controls seemed to be slightly lower in rats treated in utero and during lactation with PCBs.

Some experiments have related the possible imprinting of the hepatic monooxygenase enzymes by xenobiotics (particularly phenobarbital) administered prenatally (Faris and Campbell 1983 ; Simpson and Chung 1982). This effect could be mediated by interaction between the inducers and sexual hormones and perhaps by synthesis in neonatal period of novel forms of cytochrome P450 different from adult ones (Bagley and Hayes 1985). Our results with Phenochlor DP5 show a weak effect of a pre and postnatal exposure on long-term adult basal activities of some microsomal enzymes only in male rats, and a similar inducing effect of a further treatment with a low dose of DP5 in adult period. Complementary experiments in that field are obviously required, particularly with larger doses of DP5 during the lactation period, measurement of other microsomal enzyme activities and time course study of the effect of the perinatal treatment on microsomal drug metabolism paramaters during the life span of rats.

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