Effects of PCB Administration on Microsomal Enzyme Activity in Pregnant Rabbits

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Polychlorinated biphenyls (PCB's) are industrial chemicals used as plasticizers in paints, resins and plastics, as well as for insulators, heat exchange fluids and a wide variety of other applications (1). PCB residues have been reported in fat samples of various wildlife species, particularly fish and fish-eating birds (1-6)

The limited amount of toxicological data on these compounds has been recently reviewed by Peakall and Lincer (7) and demonstrates the lack of information and effects of PCB's in pregnant animals. This work was designed to study the biochemical and physiological effects of PCB administration on pregnant rabbits and their fetuses.

Methods and Materials

Aroclors* 1221 and 1254 were obtained through the courtesy of Monsanto Company, U.S.A. Twenty-four mature female rabbits, 2500-3000 g body weight were randomized into 6 groups of four animals (2 control and 4 treatment groups) and after mating were dosed daily with either corn oil (c. o.), Aroclor 1221 (1.0 and 10 mg/kg body weight), or Aroclor 1254 (1.0 and 10 mg/kg body weight). Both Aroclors were made up as a 50% (w/v) solution in corn oil.

Twenty-eight days later the does were killed, and their organs removed and weighed. Placentae and fetuses were removed from pregnant does. Tissues were placed on ice for immediate use in enzyme assays and the remainder frozen. Blood samples were taken from the does at the time of death by venapuncture of the abdominal aorta and serum cholesterol determined (8). Liver vitamin A was determined by a colorimetric method using antimony trichloride following saponification in KOH (9). Carboxylesterase activity was determined on liver homogenates using o-nitrophenylbutyrate (ONPB) as substrate (10). Protein was determined according to the method of Gornall et al. (11) and enzyme activity was expressed as uMoles substrate hydrolyzed per hour per mg protein at 25°C. Parathion (O,O-diethyl O-4-

*Registered trade names, Monsanto Co., St. Louis, Missouri, U.S.A.

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nitrophenyl phosphorothioate) degradation by liver microsomes was determined by the method of Nakatsugawa et al. (12). Results are expressed as uMoles paraoxon (O,O-diethyl O-4-nitrophenyl phosphate) or p-nitrophenol formed per hour per mg protein. All analyses were done on paraoxon treated microsomes (13). In vitro drug metabolizing activities were determined on a 20,000 G x 20 min supernatant of freshly homogenized liver tissue (10% w/v in 0.25M sucrose). The activities monitored were the N-demethylation of aminopyrine (14) to 4-aminoantipyrine and the aromatic hydroxylation of aniline to p-aminophenol (15). The rates of reaction were linear with respect to protein concentration and incubation time.

Results

Table I summarizes the data on doe body weight gain, conception rate and number of fetuses in the pregnant rabbits.

TABLE I
Effect of Aroclors on body weight gain, conception rate and number of fetuses in rabbits

Group	Number mated	Number pregnant	Average Body Weight Gain ± S.E.M.	Number of Fetuses per litter
Control	8	4	729±62	8.5
Aroclor 1221 1.0 mg/kg b.w.	4	4	605±100	7.25
Aroclor 1221 10 mg/kg b.w.	4	2	661±104	8.5
Aroclor 1254 1.0 mg/kg b.w.	4	3	596±55	6.0
Aroclor 1254 10 mg/kg b.w.	4	4	603±30	6.75

There was no significant difference between controls and treated groups in body weight gain or the number of fetuses per litter. PCB ingestion had no effect on body weight gain, heart, spleen, kidney or brain weights in the dams. Similarly, there was no significant effect of treatment on the fetal liver or the placentae wet weights (expressed as a percent of the

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fetal body weight) or on the fetal body weights. The only significant changes (expressed as percent body weight) were increased dam liver weights from the 10 mg/kg Aroclor 1254 treatment. The results from the serum cholesterol determinations indicate that there was no difference between treatment groups.

The results of the liver vitamin A stores in pregnant rabbits and fetuses are shown in Table II.

TABLE II
The effects of PCB administration on Vitamin A storage in the livers of pregnant rabbits and fetuses

	Vitamin A ug/g liver	Vitamin A ug/whole liver	Vitamin A ug/g liver	Vitamin A ug/whole liver
	DOES		FETUSES	
Control	99.0[1]±19.7 (4)	9107±1538	13.8[1]±1.9 (8)	33.9±5.2
Aroclor 1221 1.0 mg/kg b.w.	114.2 ± 9.3 (4)	10258±1058	18.1*±1.3 (9)	40.8*±3.3
Aroclor 1221 10 mg/kg b.w.	75.9 ± 6.08 (2)	7263± 768	13.2±1.1 (6)	30.7±5.8
Aroclor 1254 1.0 mg/kg b.w.	52.1*± 8.3 (3)	6216± 885	15.8±0.4 (3)	37.1±5.2
Aroclor 1254 10 mg/kg b.w.	56.0*± 8.5 (4)	8367±1410	11.7*±1.7 (8)	25.9*±7.2

[1]All values represent the mean ±S.E.M.
( )Denotes the number of animals used.
*Denotes significant difference from control at 5% level ("t" test).

The concentration of liver vitamin A was lower in animals receiving Aroclor 1254; however, no significant differences were observed in the total amount of vitamin A stored per liver. Aroclor 1221 was without significant effect on vitamin A storage in the maternal liver. Vitamin A storage was increased in fetal liver by Aroclor 1221 (1.0 mg/kg b.w.) and decreased by Aroclor 1254 (10 mg/kg b.w.). The other levels of Aroclor 1221 and 1254 did not significantly alter the vitamin A content in fetal liver.

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There was no effect of PCB administration on protein levels in maternal, and fetal liver homogenates. The protein concentration of placental homogenates was higher only in animals receiving 10 mg/kg b.w. Aroclor 1254.

Carboxylesterase activity in the doe liver homogenates are shown in Table III.

TABLE III
Carboxylesterase activity[1] in liver of pregnant rabbits

GROUP	umoles ONPB hydrolysed per hour per mg protein	mmoles ONPB hydrolysed per hour per g liver	mmoles ONPB hydrolysed per hour per whole liver
Control	345 ± 43 (4)	50.1 ± 8.9	4957 ±1093
Aroclor 1221 1.0 mg/kg b.w.	384 ± 77 (4)	63.6 ±15	5696 ±1436
Aroclor 1221 10 mg/kg b.w.	278 ± 55 (2)	45.7 ± 6.4	4378 ± 745
Aroclor 1254 1.0 mg/kg b.w.	506 ±162 (3)	71.9 ±21	8200 ±1782
Aroclor 1254 10 mg/kg b.w.	580*± 82 (4)	95.6*±15	14038**±1913

[1]All values represent the mean ±S.E.M.
*Indicates significant difference from controls at 5% level ("t" test).
**Indicates significant difference from controls at 1% level ("t" test)
( )Indicates the number of animals.

Aroclor 1254 (10 mg/kg b.w.) administration increased the carboxylesterase activity of maternal liver homogenates There was no change in the carboxylesterase activities in the fetal livers or in the placentae from the treated does as compared with the controls.

The parathion degradation activities of livers from female rabbits are shown in Table IV.

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TABLE IV
Parathion degradation[1] in pregnant rabbits

GROUP	Paraoxon formed per ml homogenate[2]	Paraoxon formed per mg protein[3]	PNP formed per ml homogenate	PNP formed per mg protein
Control	0.031± 0.005 (4)	0.0094± 0.0022	0.026± 0.004	0.0084± 0.0025
Aroclor 1221 1.0 mg/kg b.w.	0.034± 0.005 (4)	0.0091± 0.0016	0.024± 0.006	0.0061± 0.0008
Aroclor 1221 10 mg/kg b.w.	0.028± 0.001 (2)	0.0077± 0.0016	0.018± 0.004	0.0050± 0.0002
Aroclor 1254 1.0 mg/kg b.w.	0.035± 0.004 (3)	0.0077± 0.0013	0.034± 0.004	0.0079 0.0010
Aroclor 1254 10 mg/kg b.w.	0.043± 0.006 (4)	0.0078± 0.0018	0.052*± 0.008	0.0089± 0.0017

[1]Rates are expressed in terms of umoles paraoxon or PNP formed per hour at 25°C. Values represent the mean ±S.E.M.
[2]Microsomal homogenate.
[3]Microsomal protein.
( )Indicates the number of animals.
*Indicates significant difference at 5% level ("t" test).

Control and treated groups were comparable in their ability to degrade parathion to paraoxon. However, the Aroclor 1254 group (10 mg/kg body weight) showed an increased p-nitrophenol formation when considered per ml homogenate but not per mg protein. There was some parathion degradation activity in the microsomes of the placenta and from the fetal livers but it was too low to be determined accurately.

The results for liver aniline hydroxylase and aminopyrine n-demethylase enzyme activities are shown in Table V.

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TABLE V
Liver aniline hydroxylase and aminopyrine n-demethylase activity in pregnant rabbits

GROUP	umolcs p-AP[1] per hour per 100 mg protein	umoles p-AP per hour per gram tissue (wet wt.)	umoles 4-AAP[2] per hour per 100 mg protein	umoles 4-AAP per hour per gram tissue (wet wt.)
Control	0.59±0.11 (4)	0.47±0.07 (4)	0.17±0.04 (4)	0.15±0.04 (4)
Aroclor 1221 1.0 mg/kg b.w.	0.40±0.05 (4)	0.36±0.05 (4)	0.15±0.04 (4)	0.14±0.04 (4)
Aroclor 1221 10 mg/kg b.w.	0.59±0.18 (2)	0.31±0.0 (2)	0.192±0.05 (2)	0.092±0.04 (2)
Aroclor 1254 1.0 mg/kg b.w.	0.61±0.10 (3)	0.45±0.04 (3)	0.13±0.01 (3)	0.10±0.01 (3)
Aroclor 1254 10 mg/kg b.w.	0.83±0.18 (4)	0.92*±0.16 (4)	0.52*±0.09 (4)	0.61*±0.15 (4)

[1]p-aminophenol.
[2]4-aminoantipyrine.
*Indicates significant difference at 5% level ("t" test).
( )Indicates number of animals.

There was no difference between control and treated groups when the aniline hydroxylase enzyme activity was expressed in terms of mg protein. However, 10 mg/kg b.w. Aroclor 1254 significantly increased aniline hydroxylase activity when expressed per gram of tissue and increased aminopyrine n-demethylase activity both in terms of activity per 100 mg protein and per weight of tissue.

Discussion

Many pesticides are known to influence reproduction in mammalian species (16, 17). Because of the similarity of PCB's to some of the organochlorine insecticides and the finding of PCB residues in animal species, including man (18), it was of interest to determine the effect of sub-acute administration of two PCB's on reproductive parameters in the rabbit. The results indicate that the administration of Aroclors 1221 and

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1254 for the first 28 days of gestation did not affect implantation, fetal development, fetal growth or litter size at dose levels of 1.0 and 10 mg/kg b.w.[1] The only organ weights observed to be affected were liver weights in the does receiving 10 mg/kg Aroclor 1254. Fetal body weights, liver weights and placenta wet weights were unaffected by PCB administration to pregnant does. Placental transfer of both compounds occurred as evidenced by the detection of residues of the PCB's in the fetuses. The details of these results will be reported in another communication. It has been reported that PCB's fed to rats at 100 mg/kg b.w. per day for 28 days caused elevated serum cholesterol levels (20). However, in our case, PCB administration did not cause any alterations in serum cholesterol levels in pregnant does.

Multigeneration studies in rats fed a dietary regimen of 20 p.p.m. DDT have shown that the liver vitamin A of the pups (21 days of age) was decreased (21). In the present experiment vitamin A storage in the fetal liver was decreased only by the highest dose level of Aroclor 1254. This observation will require further study to assess its significance.

One of the parameters of enzyme induction is increased protein synthesis. The only significant increase in protein levels of homogenates was observed with placental homogenates from rabbits receiving 10 mg/kg b.w. Aroclor 1254; however, no change was observed in the carboxylesterase activity of the placenta. Carboxylesterase activity, used as a indicator of microsomal enzyme activity, was elevated in liver homogenates of does receiving 10 mg/kg b.w. Aroclor 1254. However no change was observed in the carboxylesterase activity of fetal liver homogenates. In vitro, parathion is metabolized to either paraoxon or to p-nitrophenol. Neal (22) has demonstrated that pretreatment of rats with the enzyme inducer 3,4 benzpyrene stimulated the formation of paraoxon to a greater degree than the formation of p-nitrophenol. With phenobarbital pretreatment, about equal stimulation of the two pathways of metabolism occured. In this study the ability of rabbit liver microsomes from does exposed to Aroclor 1254 (10 mg/kg b.w.) to degrade parathion to p-nitrophenol was enhanced while paraoxon formation was not affected.

The drug metabolizing enzymes aniline hydroxylase and aminopyrine n-demethylase were both induced by the administration of 10 mg/kg b.w. Aroclor 1254. This suggests that Aroclor 1254 may increase hydroxylation of steroids in vivo and possibly

[1] In a subsequent study it was found that the administration of Aroclor 1254 at levels of 12.5, 25.0 and 50.0 mg/kg b.w. during the first 28 days of gestation had embryotoxic effects in the rabbit. The details of this study will be published elsewhere (19).

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interfere with reproductive processes. However, in this case, no effects on reproduction were noted.

From these results, it appears that the no effect level of Aroclor 1254 for enzyme induction in the pregnant rabbit is between 1.0 and 10 mg/kg body weight when administered for 28 days during gestation.

Aroclor 1221 did not induce any enzyme activity in the does, foetus or placenta so its no-effect level must be considered higher than that for Aroclor 1254. The important point for both Aroclor 1254 and 1221 is that placental transfer does occur, but does not cause any changes in the biochemical or physiological parameters measured.

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References

(1) Lee, E., Chirnside, R.C., Lewis, D.T., Solomons, S.E.B., West, T.S., and Fox, L.L. Report of the Government Chemist 1967. London, 1967.

(2) Jensen, S. New Scientist 32, 612, 1966.

(3) Koeman, J.H., Oskamp, A.A.G., Veen, J., Brouwer, E., Rooth, J., Zwart, P., Brock E.V.D., and van Genderen, H. Meded. Rijksfac. Landbouwwetensch. Gent 32, 841, 1967.

(4) Holmes, D.C., Simmons, J.H., and Tatton, J.O.G. Nature 216, 227, 1967.

(5) Koeman, J.H., ten Noever de Brau, M.C., and de Vos, R.H. Nature 221, 1126, 1969.

(6) Duke, T.W., Lowe, J.I., and Wilson, A.J. Bull. Environ. Contamin. Toxicol. 5, 171, 1970.

(7) Peakall, D.B., and Lincer, J.L. Bioscience 20, 958-964, 1970.

(8) Technicon Auto Analyzer Method File, 1965.

(9) Phillips, W.E.J. Can. J. Biochem. Physiol. 40, 491, 1962.

MONS 084480

(10) Villeneuve, D.C. Ph.D. thesis, McGill University, 1969.

(11) Gornall, A.G., Bardawill, C.J., and David, M.M. J. Biol. Chem. 177, 751, 1948.

(12) Nakatsugawa, T., Tolmam, N.M., and Dahm, P.A. Biochem. Pharmacol. 17, 1517-28, 1968.

(13) Nakatsugawa, T., and Dahm, P.A. Biochem. Pharmacol. 16, 25, 1967.

(14) Brodie, B.B., and Axelrod, J. J. Pharm. Exptl. Therap. 99, 171, 1950.

(15) Schenkman, J.B., Remmer, H., and Estabrook, R.W. Mol. Pharmacol 3, 113, 1967.

(16) Ottoboni, A. Toxicol. Appl. Pharmacol. 14, 74, 1969.

(17) Good, E.E., and Ware, G.W. Toxicol. Appl. Pharmacol. 14, 201, 1969.

(18) Biros, F.J., Walker, A.C., and Medberry, A. Bull. Environ. Contamin. Toxicol. 5, 317, 1970.

(19) Villeneuve, D.C., Grant, D.L., Phillips, W.E.J. and Clegg, D.J. Submitted for publication, 1970.

(20) Tanaka, K., Fujita, S., Komatsu, F., and Tamura, N. Fukuoka Igaku Zasshi 60, 544, 1969.

(21) Phillips, W.E.J., Hatina, G., Villeneuve, D.C., and Grant, D.L. In preparation, 1970.

(22) Neal, R.A. Biochem. J. 103, 183, 1967.

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