The Effect of a PCB (2, 4, 2′, 4′-tetrachlorobiphenyl) on Lipid-Synthesizing Enzymes in Rat Liver Microsomes

by BRUCE J. HOLUB, J. PIEKARSKI, and K. NILSSON
Department of Nutrition, University of Guelph
Guelph, Ontario, Canada

Polychlorinated biphenyls (PCBs) are well known as industrial chemicals of considerable commercial importance. The detection of these compounds in natural food chains and in human diets (FISHBEIN 1974) has raised concern about their possible impact on fish and wildlife in the environment and the health of human beings. Some of the biological effects of the PCBs have been documented (KAY 1973) and include the induction of various microsomal enzymes as well as cytochrome P_{450} (LITTERST and VAN LOON 1974, CHEN and DUBOIS 1973, JOHNSTONE *et al.* 1974). *In vitro* studies have revealed an inhibitory effect of PCBs on oxidative phosphorylation in isolated mitochondria (SIVALINGAN *et al.* 1973) and on certain enzymes such as ATPase (YAP *et al.* 1971).

PCBs and polychlorinated terphenyls (PCTs) have been shown to enhance the formation of lipid droplets (HANSELL and ECOBICHON 1974) and to alter the level of triglycerides and phospholipids in rat liver (LITTERST *et al.* 1972, SOSA-LUCERO *et al.* 1973) and of total lipid in bacteria (GREER *et al.* 1974). Furthermore, various membrane-bound enzymes and cytochrome P_{450} are known to be affected by PCBs and are also dependent upon membrane phospholipid for normal activity (TANAKA and STRICKLAND 1965, STROBEL *et al.* 1970). Therefore, the possible effect of 2, 4,2',4'-tetrachlorobiphenyl on key enzymes responsible for the biosynthesis of phospholipid and triglyceride in rat liver microsomes was investigated.

MATERIALS AND METHODS

Animals and treatments

For the *in vivo* treatments, male rats of the Wistar strain were obtained from Woodlyn Farms (Guelph, Ontario). They were housed individually in screen-bottomed cages and fed laboratory chow and water *ad libitum*. In the first study, rats weighing 105.2 ± 1.0 g (mean ± SE, n=12) were divided at random into two groups; in the second study, rats weighing 164.4 ± 3.3 g (n=12) were similarly divided. In each study, a dosing scheme similar to that reported (SCHWARK and ECOBICHON 1967) was employed. One group was administered the PCB (2,4,2',4'-tetrachlorobiphenyl) intraperitoneally at a dosage of 50 mg/kg

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body weight as a peanut oil solution while the control animals received an equivalent volume of peanut oil alone. The animals were dosed for three days in succession and were sacrificed 96 hr after the final injection. Body weights and food consumption were measured on a daily basis throughout the experimental period. Animals were killed by cervical fracture and the livers were quickly excised, rinsed in 0.25 M sucrose, and weighed.

Preparation of microsomes

Rat livers were homogenized with a cold 0.25 M sucrose solution containing 20 mM Tris-HCl buffer (pH 7.4) and 0.6 mM EDTA and liver microsomes were then isolated as described (HOLUB 1974). The microsomal protein concentration was determined by the method of LOWRY et al. (1951) using bovine serum albumin as the standard.

Enzyme assays and PCB studies in vitro

The enzymatic assay of acyl-CoA :sn-glycero-3-phosphate acyltransferase was conducted under conditions modified from those described elsewhere (HOLUB et al. 1975). The incubation mixture contained 50 mM Tris-HCl buffer (pH 7.4), 40 mM [^{14}C]glycerol-3-phosphate, 17.5 µM palmitoyl-CoA, and 150 µg of microsomal protein in a total volume of 1.0 ml. The formation of product was calculated as µmoles of sn-[^{14}C]glycerol-3-P incorporated into lipid/mg protein/min. All reactions were conducted under conditions in which the rate of product formation was linear with respect to enzyme concentration and time.

CDP-choline:1,2-diacyl-sn-glycerol cholinephosphotransferase was assayed under conditions modified from those described (MCCAMAN and COOK 1966). The standard assay mixture contained 50 mM Tris-HCl buffer (pH 7.4), 100 µl of a 50 mM Tris-HCl solution (containing 4.6 µmoles 1,2-diolein plus 1.5 mg of Tween-20 per ml), 8 mM $MgCl_2$, 24.4 µM CDP-choline [^{14}C], and 100 µg of microsomal protein in a final volume of 0.5 ml. Incubations were conducted at 37 °C for 7 min. The assay for the acyl-CoA:1, 2-diacyl-sn-glycerol acyltransferase was similar to that for the cholinephosphotransferase except that 12 mM $MgCl_2$, twice the diglyceride concentration, 60 µg microsomal protein, and 34.8 µM [^{14}C]palmitoyl CoA rather than CDP-choline were employed.

The acyl-CoA:sn-glycero-3-phosphorylcholine acyltransferase was measured in a medium containing 50 mM Tris-HCl buffer (pH 7.4), 0.18 mM 1-acyl-sn-glycero-3-phosphorylcholine, 34.8 µM [^{14}C]palmitoyl-CoA, and 200 µg of microsomal protein in a total volume of 0.5 ml. The reaction was stopped after 2 min, the lipids extracted (FOLCH et al. 1957), and the rate of [^{14}C]palmitate entry into lecithin was calculated following thin-layer chromatography (WITTELS 1973).

For the in vitro studies on the possible effects of 2,4,2',4'-tetrachlorobiphenyl on lipid-synthesizing enzymes, the PCB was introduced to the incubation medium as a solution in dimethyl sulfoxide either before or 12 sec after the addition of enzyme (SHARP et al. 1974). Dimethyl sulfoxide was added at a concentration of 1% by volume to the medium. Control incubations contained dimethyl sulfoxide alone.

RESULTS

Table 1 gives the results from experiments on the effect of 2,4,2',4'-tetrachlorobiphenyl (PCB) administration to rats on food intake, growth rate, liver weight, and the concentration of microsomal protein in liver.

TABLE 1

The effect of intraperitoneal administration of PCB on food intake, growth, liver weights, and microsomal protein.

	Experiment 1		Experiment 2	
	Control	Treated	Control	Treated
Daily food intake, g	16.8±0.7	16.9±0.4	18.3±0.8	18.0±0.9
Daily weight gain, g	6.6±0.7	6.9±0.4	5.5±0.3	6.0±0.6
Final body weight, g	144.8±5.7	146.3±3.8	198.8±7.5	198.8±5.2
Final liver weight, g	7.4±0.3	7.5±0.3	9.3±0.3	9.9±0.5
Microsomal protein, mg/g liver	12.3±0.8	15.5±0.9*	15.7±1.0	19.5±1.1*

Results are given as means ± SE with 6 rats per group.

* Results are significantly different from the corresponding controls, $p<0.05$.

The initial body weight of the animals just prior to treatment was 105.2 ± 1.0 g (mean ± SE, n=12) and 164.4 ± 3.3 g in experiments 1 and 2, respectively.

In both experiments, there was no significant difference ($P>0.05$) in daily food intake, daily weight gain, final body weight, or final liver weight between the control and PCB-treated animals. There was, however, a significant difference ($P<.05$) in the level of microsomal protein with the treated animals showing concentrations which were 25% greater than controls. Increases in microsomal protein due to dietary (LITTERST and VAN LOON 1974) or intraperitoneal administration (JOHNSTONE et al. 1974) of PCBs have been reported.

The results on the effect of *in vivo* dosing with PCB on the activity of four key microsomal enzymes responsible for lipid biosynthesis are shown in Table 2. None of the small differences in enzyme reaction rates between the microsomes from control and treated groups were statistically significant ($P>0.05$).

TABLE 2

The effect of intraperitoneal administration of PCB on activity of lipid-synthesizing enzymes in rat liver microsomes.

Enzyme	Activity (nmoles/mg protein/min) Control	Treated
Acyl-CoA:*sn*-glycero-3-phosphate acyltransferase	50.4 ± 2.7	46.7 ± 3.0
CDP-choline:1,2-diacyl-*sn*-glycerol cholinephosphotransferase	1.8 ± 0.2	1.8 ± 0.1
Acyl-CoA:1-acyl-*sn*-glycero-3-phosphorylcholine acyltransferase	9.4 ± 0.6	9.5 ± 0.2
Acyl-CoA:1,2-diacyl-*sn*-glycerol acyltransferase	6.1 ± 0.6	5.6 ± 0.5

Results are given as means ± SE of 6 rats per group.

The effects of adding the PCB directly to the enzyme assay medium as solutions in dimethyl sulfoxide are summarized in Table 3.

TABLE 3

The effect of adding PCB to the incubation medium on the activity of lipid-synthesizing enzymes in rat liver microsomes

Enzyme	PCB in medium (ppm)	Order of PCB addition to medium: PCB before enzyme	Order of PCB addition to medium: PCB after enzyme
		(% of control activity*)	
Acyl-CoA:*sn*-glycero-3-phosphate acyltransferase	40	57.5±6.7***	66.6±9.5**
	10	78.0±9.9	72.4±9.9**
CDP-choline:1,2-diacyl-*sn*-glycerol cholinephosphotransferase	40	144.3±8.0***	103.3±4.0
	10	108.3±15.7	101.3±3.6
Acyl-CoA:1-acyl-*sn*-glycero-3-phosphorylcholine acyltransferase	40	56.7±2.7***	61.5±1.4***
	10	76.0±3.7***	60.1±3.8***

Results are given as means ± SE for 3-5 determinations.

* The reaction rates in the controls were not significantly different when dimethyl sulfoxide was added before or after the microsomes.

** Significantly different from control at the $p<0.05$ level.

*** Significantly different from control at the $p<0.005$ level.

A significant decrease in acyl-CoA:sn-glycero-3-phosphate acyltransferase activity was observed due to the addition of PCB at 40 ppm before or after the microsomal preparations. Lowering the PCB concentration further to 10 ppm reduced the degree of inhibition. In contrast, the addition of PCB at 40 ppm before the enzyme actually caused a significant increase in CDP-choline:1,2-diacyl-*sn*-glycerol cholinephosphotransferase activity ($p<0.005$). Acyl-CoA:1-acyl-sn-glycero-3-phosphorylcholine acyltransferase activity was markedly reduced ($p<0.005$) by 43-24% due to the addition of PCB at 40 and 10 ppm, respectively.

DISCUSSION

The administration of PCBs is known to increase the number of lipid droplets in hepatocytes of rats (HANSELL and ECOBICHON 1974) and to alter lipid levels in liver (LITTERST et al. 1972). PCBs also cause the induction of microsomal drug-metabolizing enzymes (JOHNSTONE et al. 1974) and increase the level of cytochrome P_{450} (LITTERST and VAN LOON 1974), which has a requirement for microsomal phospholipid. The present results demonstrate no significant effect of PCB treatment *in vivo* on the activity of four key microsomal enzymes responsible for lipid biosynthesis. This suggests that induction of these enzymes may not be the primary cause of the changes in lipid concentration due to PCB administration mentioned above. It is possible, however, that an altered catabolism or transport of hepatic lipid could be more directly responsible for the reported changes in lipid composition.

The *in vitro* studies reported herein do indicate a marked effect of 2,4,2',4'-tetrachlorobiphenyl on the activities of three microsomal enzymes when this compound is added directly to the incubation medium at a concentration of 40 or 10 ppm. These latter effects may result from the interaction of the microsomal enzymes with an oil-in-water dispersion of the PCB (SHARP et al. 1974). The significance of these *in vitro* results in explaining the shifts in tissue lipid composition due to PCB exposure under physiological conditions awaits further investigation. It should be pointed out that levels of PCBs averaging 2.1 ppm have been reported in salmon from Lake Erie (CARR et al. 1972) and even higher in certain species of wildlife (RISEBROUGH et al. 1968). Furthermore, liver is known to accumulate the highest concentration of PCBs next to adipose tissue (GRANT et al. 1971, HANSEN et al. 1971) in some animals and fish.

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REFERENCES

CARR, R.L., C.E. FINSTERWALDER and M.J. SCHIBI: Pestic. Monit. J. 6, 23 (1972).

CHEN, T.S. and K.P. DUBOIS: Toxicol. Appl. Pharmacol. 26, 504 (1973).

FISHBEIN, L.: Ann. Rev. Pharmacol. 14, 139 (1974).

FOLCH, J., M. LEES and G.H. SLOANE STANLEY: J. Biol. Chem. 226, 496 (1957).

GRANT, D.L., W.E.J. PHILLIPS and D.C. VILLENEUVE: Bull. Environ. Contam. Toxicol. 6, 102 (1971).

GREER, D.E., J.E. KEIL, L.W. STILLWAY and S.H. SANDIFER: Bull. Environ. Contam. Toxicol. 12, 295 (1974).

HANSELL, M.M. and D.J. ECOBICHON: Toxicol. Appl. Pharmacol. 28, 418 (1974).

HANSEN, D.J., P.R. PARRISH, J.I. LOWE, A.J. WILSON and P.D. WILSON: Bull. Environ. Contam. Toxicol. 6, 113 (1971).

HOLUB, B.J.: Biochim. Biophys. Acta 369, 111 (1974).

HOLUB, B.J., J.T.H. CONNOR and S.J. SLINGER: J. Fish. Res. Bd. Can. 32, 61 (1975).

JOHNSTONE, G.J., D.J. ECOBICHON and O. HUTZINGER: Toxicol. Appl. Pharmacol. 28, 66 (1974).

KAY, K.: Environ. Res. 6, 202 (1973).

LITTERST, C.L., T.M. FARBER, A.M. BAKER and E.J. VAN LOON: Toxicol. Appl. Pharmacol. 23, 112 (1972).

LITTERST, C.L. and E.J. VAN LOON: Bull. Environ. Contam. Toxicol. 11, 206 (1974).

LOWRY, O.H., N.J. ROSENBROUGH, A.L. TARR and R.J. RANDALL: J. Biol. Chem. 193, 265 (1951).

MCCAMAN, R.E. and K. COOK: J. Biol. Chem. 241, 3390 (1966).

RISEBROUGH, R.W., P. RIECHE, S.G. HERMAN, D.B. PEAKALL and M.N. KIRVEN: Nature 220, 1098 (1968).

SCHWARK, W.S. and D.J. ECOBICHON: Can. J. Physiol. Pharmacol. 46, 207 (1967).

SHARP, C.W., D.G. HUNT, S.T. CLEMENTS and W.E. WILSON: Mol. Pharmacol. 10, 119 (1974).

SIVALINGAN, P.M., T. YOSHIDA and Y. INADA: Bull. Environ. Contam. Toxicol. 10, 242 (1973).

SOSA-LUCERO, J.C., F.A. DE LA IGLESIA and G.H. THOMAS: Bull. Environ. Contam. Toxicol. 10, 248 (1973).

STROBEL, H.W., A.Y. LU, J. HEIDEMA and M.J. COON: J. Biol. Chem. 245, 4851 (1970).

TANAKA, R. and K.P. STRICKLAND: Arch. Biochem. Biophys. 111, 583 (1965).

WITTELS, B.: J. Biol. Chem. 248, 2906 (1973).

YAP, H.H., D. DESAIAH and L.K. CUTKOMP: Nature 223, 61 (1971).