EFFECT OF TWO ISOMERIC TETRACHLOROBIPHENYLS ON RATS AND THEIR HEPATIC ENZYMES

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Oral administration of two isomeric tetrachlorobiphenyls (TCBs) (2, 3, 4, 5- and 3, 5, 3', 5'-tetrachlorobiphenyls) to rats at three dosage levels does not cause any adverse effect on their growth. However, several hepatic enzyme systems are affected, even at the lowest level (5 mg/kg). Ethylmorphine *N*-demethylase and aniline hydroxylase activities are slightly induced by 2, 3, 4, 5-TCB but, at a medium dose level (20 mg/kg), the activity of *para*-nitroanisole *O*-demethylase is induced two-fold. Also, 3, 5, 3', 5'-TCB induces these enzyme systems in male rats but decreases the activities of aniline hydroxylase and ethyl morphine *N*-demethylase in female rats at higher doses. An extra polar metabolite occurs in the *in vitro* metabolism of oestradiol-^{3}H when female rats are treated with 2, 3, 4, 5-TCB at a dosage level of 40 mg/kg. UDP-Glucuronyl/transferase and δ-aminolevulinic acid synthetase activities are not affected by the administration of these two TCBs.

Polychlorinated biphenyls (PCBs) are used as industrial chemicals in a wide variety of applications and, as a result, enter the environment by different routes. The presence of PCB residues in the fat samples of various wildlife species and ecosystem has already proven to be a ubiquitous pollution problem (Jensen 1966; Holmes *et al.* 1967, Jensen *et al.* 1969, Duke *et al.* 1970, Risebrough *et al.* 1968). Only limited toxicology work was done in relation to the occupational health, in the early days of the use of PCB. However, recent studies show that these agents can cause liver damage, skin disease, and hepatic porphyria in several species of vertebrates (Vos and Beems 1971, Kuratsune *et al.* 1972, Vos and Koeman 1970). Since PCBs, like most chlorinated hydrocarbon pesticides, are persistent chemicals, their long-term- and immediate toxic effects pose a serious threat to the public health.

Biological effects of PCBs have been the subject of several investigations (Villeneuve *et al.* 1971, Pardini 1971, Keil *et al.* 1971). However, since these studies utilized complex mixtures, it is difficult to ascribe the effects to a specific or even joint action of unidentifiable groups of compounds. Therefore, there is a need to study some of the biological effects of individual isomeric polychlorinated biphenyls.

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Methods and materials

Two tetrachlorobiphenyls (TCBs) (2, 3, 4, 5–tetrachlorobiphenyl and 3, 5, 3′, 5′–tetrachlorobiphenyl) were synthesized in this institute (Oswald *et al.* 1972). The purities of these compounds are greater than 95%, based on the determination by gas–liquid chromatography (GLC). In most of the experiments, male (160 g) and female (190 g) Charles River rats were divided into 4 groups of 4 animals (one control and 3 treated groups) and dosed orally daily for 5 days with corn oil or TCBs (5, 20, 40 mg/kg) in corn oil. Rats were sacrificed after the weight gain was recorded on the 7th day. Liver was weighed and homogenized, in 4 volumes of ice–cold 0.1M tris–HCl buffer (pH 7.4), with a Teflon glass homogenizer. Microsomes were isolated as a pellet from post–mitochondrial supernatant at 100,000 x *g* for 60 min; the microsomal pellet was suspended in the same buffer. (One ml of this suspension is equal to 250 mg of liver.) Ethyl morphine and *para*–nitroanisole demethylase activities were determined by the method of Kato and Gillette (1965). Aniline hydroxylase activity was assayed by the method of Schenkman *et al.* (1967). UDP–Glucuronyltransferase activity was assayed using the method of Lucier *et al.* (1971) with ^{14}C–naphthol as substrate. In the experiment on mitochondrial δ–aminolevulinic acid (ALA) synthetase, female rats (200 g) were dosed orally with each of two TCBs in corn oil for 10 days at the levels of 10 mg/kg and 20 mg/kg. Liver mitochondria were isolated according to the method of Hogeboom (1962). Activity of ALA synthetase was assayed following the method of Urata and Granick (1963). β–Oestradiol–6, 7–^{3}H was purchased from New England Nuclear with a specific activity of 46.6 Ci/mM. The *in vitro* metabolism of β–oestradiol was studied according to the method of Risebrough *et al.* (1968) except the metabolites were chromatographed on TLC using a cyclohexane–ethyl acetate mixture (1:1 by volume) as a solvent and the radioactive spots were monitored by an autographic strip scanner. Protein determination was carried out using the method of Lowry *et al.* (1951).

Results

The effects of short–term feeding of the two TCBs on body weight gain and/or liver growth are shown in Table I. Rats receiving up to 40 mg/kg of 2, 3, 4, 5–TCB or 3, 5, 3′, 5′–TCB maintain normal growth when compared to the control rats. A trend of slight liver enlargement occurs in the male rats treated with 3, 5, 3′, 5′–TCB.

2, 3, 4, 5–TCB induces the activity of ethyl morphine *N*–demethylase in both male and female rats (Table II). The activity reaches maximum when rats receive this compound at 20 mg/kg; it remains at the same level in the male rats when 40 mg/kg of 2, 3, 4, 5–TCB is given to the animals. However, when activity is expressed as mμmoles of formaldehyde formed per gram of liver per hour, the response of this enzyme activity is progressively greater with the increased amount of compound used. A similar effect is observed with male rats treated with 3, 5, 3′, 5′–TCB but the activity is decreased, in both expressions, when female rats are treated with higher doses of this compound (Table II).

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Table I. *Effect of Feeding TCBs on the Body–Weight Gain and Liver Weight of Rats*

Compound	Daily dose[a], mg/kg	Body weight gain, g[b,c]		Liver weight, g[c]	
		Male	Female	Male	Female
2, 3, 4, 5–TCB	0	47.1 ± 7.8	22.0 ± 5.6	10.4 ± 1.7	7.85 ± 0.50
	5	45.2 ± 5.6	24.0 ± 6.1	10.7 ± 0.9	7.70 ± 0.50
	20	47.1 ± 5.6	21.0 ± 5.6	10.4 ± 1.0	8.20 ± 0.45
	40	55.4 ± 5.4	20.0 ± 1.0	10.2 ± 0.5	8.10 ± 0.40
3, 5, 3′, 5′–TCB	0	47.5 ± 4.5	22.4 ± 5.3	11.2 ± 1.3	8.40 ± 0.60
	5	47.0 ± 6.5	19.6 ± 6.5	11.8 ± 0.6	7.80 ± 3.70
	20	44.1 ± 4.1	20.0 ± 5.7	12.8 ± 1.2	9.10 ± 0.70
	40	49.5 ± 9.5	19.0 ± 6.7	13.1 ± 0.5	8.90 ± 0.80

[a] Administered in corn oil by oral intubation for 5 days; mg per kg body weight.

[b] Total body weight gain in 5 days.

[c] All values represent the mean ± S.E.M. of 4 determinations.

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[c] All values represent the mean ± S.E.M. of 4 determinations.

Table II. *Ethyl Morphine N–Demethylase Activity in Liver Microsomes of Rats*

Compound	Daily dose[a], mg/kg	Formaldehyde formed[b]			
		mμmole/mg protein/30 min		mμmole/g liver/hr	
		Male	Female	Male	Female
2, 3, 4, 5–TCB	0	7.06 ± 0.55	2.70 ± 0.42	220 ± 25.6	176 ± 11.9
	5	8.70 ± 1.80	3.80 ± 0.61	284 ± 21.0	216 ± 17.2
	20	10.90 ± 2.00	3.80 ± 1.40	318 ± 29.0	240 ± 21.1
	40	10.80 ± 0.10	2.85 ± 1.00	358 ± 18.0	296 ± 34.0
3, 5, 3′, 5′–TCB	0	10.70 ± 1.40	4.10 ± 0.40	280 ± 26.4	264 ± 10.3
	5	12.30 ± 1.20	3.10 ± 0.14	314 ± 18.4	240 ± 4.60
	20	13.40 ± 1.20	2.90 ± 0.12	352 ± 13.2	218 ± 14.3
	40	14.50 ± 1.80	2.70 ± 0.19	380 ± 15.0	167 ± 10.1

[a] Administered in corn oil by oral intubation for 5 days; mg per kg body weight.

[b] All values represent the mean ± S.E.M. of 4 determinations.

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In general, aniline hydroxylase activity is slightly enhanced, except in the female rats treated with 3, 5, 3′, 5′–TCB (Table III). The medium dose (20 mg/kg) seems to be the most effective level in inducing the enzyme when rats are treated with 2, 3, 4, 5–TCB. Activity of this enzyme in 3, 5, 3′, 5′–TCB–treated male rats is raised at a low dose (5 mg/kg); it drops to the control level at higher doses. However, activity is inhibited when female rats receive high doses of 3, 5, 3′, 5′–TCB.

Administration of each of the TCBs to the rats immediately enhances the activity of *para*–nitroanisole *O*–demethylase (Table IV). The activity is progressively greater with the increase in doses. Enhancement of this activity is considerably more apparent when the activity is expressed in mμmoles of *para*–nitrophenol per gram of liver per hour.

UDP–Glucuronyltransferase, like mixed–function oxidases, also plays an important role in the metabolism and detoxification of xenobiotics. Using ^{14}C–1–naphthol as a substrate, this enzyme activity was monitored in TCB–treated and control rats (Table V). Treatment with either of the two TCBs does not have any significant effect on this conjugating system in the rat.

The treatment of female rats with the two TCBs affects the metabolism of β–oestradiol –6, 7–^{3}H by the liver microsome system prepared from the treated animals, as demonstrated by the TLC chromatograms shown in Fig. 1. The microsomes from the control and treated animals give rise to a common metabolite more polar than oestradiol but those from the 2, 3, 4, 5–TCB–treated rats produce a second, more polar metabolite which is dose dependent. On the basis of the peak height of the common metabolite, there seems to be a change of the steroid metabolism in the female rats treated with 3, 5, 3′, 5′–TCB.

Induction of hepatic mitochondrial ALA synthetase can not be observed in treated animals because the enzyme activities of both control and treated rats are so low that the employed method is unable to measure them (Urata and Granick 1963).

Discussion

Short–term, oral administration of two isomeric tetrachlorobiphenyls to rats does not affect body weight gain significantly. This is in general agreement with earlier investigations in which complex Aroclors were used (Villeneuve *et al.* 1971, Street *et al.* 1969). Significant liver enlargement, described in the cited investigations, was not observed in the experiments reported here, a finding which might be the result of the low dose and short duration of the current treatments. Induction effects of PCBs on the activities of several hepatic mixed–function oxidases were also studied by Villeneuve *et al.* 1971 and Street *et al.* 1969. Either crude homogenate or post–mitochondrial supernatant were used as the enzyme source in the cited investigations. Since these oxidases are exclusively located in the microsomal fraction, a study using microsomes as enzyme source was considered to be more desirable.

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Table III. *Microsomal Aniline Hydroxylase Activity of Rat Liver*

Compound	Daily dose[a], mg/kg	Amount of *para*–aminophenol formed[b]			
		mμmole/mg protein/30 min		mμmole/g liver/hr	
		Male	Female	Male	Female
2, 3, 4, 5–TCB	0	23.5 ± 1.4	21.4 ± 1.6	1786 ± 21.0	1237 ± 23.3
	5	31.9 ± 0.7	23.2 ± 2.0	2068 ± 27.3	1352 ± 28.9
	20	35.4 ± 1.8	28.4 ± 0.7	2410 ± 24.1	1588 ± 22.5
	40	32.5 ± 0.4	29.3 ± 0.9	2117 ± 16.0	1942 ± 18.0
3, 5, 3′, 5′–TCB	0	23.4 ± 2.0	16.5 ± 0.6	1689 ± 22.1	846 ± 10.6
	5	31.6 ± 3.2	22.0 ± 2.6	2106 ± 36.0	1145 ± 19.6
	20	27.1 ± 4.5	12.7 ± 2.8	1978 ± 34.0	712 ± 14.6
	40	25.0 ± 1.6	9.1 ± 0.2	1834 ± 28.0	448 ± 12.8

[a] Administered in corn oil by oral intubation for 5 days; mg per kg body weight.

[b] All values represent the mean ± S.E.M. of 4 determinations.

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Table IV. *Para–Nitroanisole O–Demethylase Activity in the Liver Microsome of Rats*

Compound	Daily dose[a], mg/kg	Amount of *para*–nitrophenol formed[b]			
		mμmole/mg protein/30 min		mμmole/g liver/hr	
		Male	Female	Male	Female
2, 3, 4, 5–TCB	0	1.05 ± 0.3	1.2 ± 0.3	93 ± 6.7	102 ± 5.2
	5	1.85 ± 1.2	1.7 ± 0.4	157 ± 7.2	152 ± 4.6
	20	2.10 ± 0.2	2.0 ± 0.4	172 ± 6.1	164 ± 7.2
	40	2.50 ± 1.1	7.95 ± 0.7	234 ± 4.3	254 ± 5.8
3, 4, 3′, 5′–TCB	0	2.1 ± 0.6	1.6 ± 0.2	108 ± 5.2	98 ± 2.4
	5	3.4 ± 0.25	2.3 ± 0.77	167 ± 7.6	172 ± 6.6
	20	6.1 ± 0.14	5.2 ± 1.2	310 ± 3.3	340 ± 2.1
	40	7.6 ± 1.6	6.4 ± 1.6	549 ± 3.1	550 ± 4.3

[a] Administered in corn oil by oral intubation for 5 days mg per kg body weight.

[b] All values represent the mean ± S.E.M. of 4 determinations.

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[a]All values represent the mean ± S.E.M. of 4 determinations.

Table V. *Activities of UDP–Glucuronyltransferase in Liver Microsomes of Rats*

Compound	Daily dose[a], mg/kg	Amount of 1–naphthyl glucuronide formed[b]			
		mμmole/mg protein/30 min		mμmole/g liver/hr	
		Male	Female	Male	Female
2, 3, 4, 5–TCB	0	127.3 ± 2.6	118.5 ± 3.4	1268 ± 8.6	1224 ± 5.4
	5	145.2 ± 3.1	134.0 ± 3.6	1408 ± 11.2	1328 ± 14.8
	20	148.0 ± 1.2	123.0 ± 2.2	1442 ± 9.6	1264 ± 9.1
	40	138.0 ± 2.1	118.2 ± 2.7	1304 ± 6.6	1232 ± 8.2
3, 5, 3′, 5′–TCB	0	123.0 ± 3.4	112.0 ± 2.6	1242 ± 7.8	1226 ± 8.6
	5	134.0 ± 2.1	128.0 ± 1.7	1406 ± 8.4	1334 ± 9.6
	20	128.0 ± 1.6	131.0 ± 3.1	1264 ± 7.4	1326 ± 4.6
	40	126.0 ± 0.6	121.0 ± 2.2	1486 ± 5.2	1262 ± 6.6

[a]Administered in corn oil by oral intubation for 5 days; mg per kg body weight.

[b]All values represent the mean ± S.E.M. of 4 determinations.

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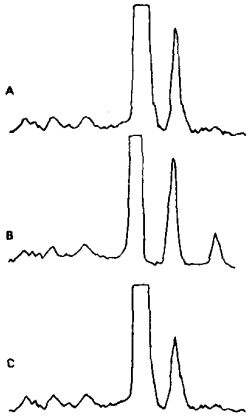

Fig. 1. Thin-layer chromatogram of metabolites formed by *in vitro* metabolism of *beta*-oestradiol-6, 7-^{3}H with female rat liver microsomes; the large (off-scale) peak is oestradiol; A – control rat, B – rat treated with 40 mg/kg of 2, 3, 4, 5-TCB, and C rat treated with 40 mg/kg of 3, 5, 3', 5'-TCB

2, 3, 4, 5-Tetrachlorobiphenyl acts as a mild inducer of *N*-demethylase and aryl hydroxylase activities. But, at medium dose levels (20 mg/kg), the activity of *O*-demethylase is induced 2-fold. With 3, 5, 3', 5'-tetrachlorobiphenyl, the responses of these three oxidases is positive (i.e., induction occurs as expected in treated male animals), *O*-demethylase being the most sensitive enzyme system. There is a 4-fold induction of activity of *O*-demethylase in female rats at 40 mg/kg. However, it is surprising that both activities of ethyl morphine demethylase and aniline hydroxylase are inhibited, at higher doses, in treated female rats. Instances of inhibition of *N*-demethylase and/or aryl hydroxylase by polycyclic hydrocarbons has been reported in the literature (Conney *et al* 1959, Kato and Takayanaghi 1966, Venkatesan *et al.* 1968). Thus, it is apparent that stimulation of drug metabolism is not an exclusive effect exerted by the "microsomal enzyme inducers" but that these agents are also capable of inhibiting some microsomal enzymes. The fact that inhibition only occurs in the female rats suggests that it is sex specific, also.

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A recent study in Japanese quail revealed that dietary DDT decreases the activities of aniline hydroxylase and aminopyrine *N*–demethylase (Sell *et al.* 1972). These authors show that the inhibition of aniline hydroxylation can be duplicated by adding DDT or DDE in the assay medium and they conclude that DDT residues in the microsomes might be sufficient to depress aniline hydroxylase activity. Inhibition of rat microsomal aniline hydroxylase activity by mirex, another chlorinated hydrocarbon pesticide, is known as a result of work in this laboratory (Mehendale *et al* 1972a). Unlike DDT, the presence of mirex in the assay medium does not affect the enzyme activity. Since mirex is not metabolized in rats (Mehendale *et al.* 1972b), inhibition of this enzyme by mirex metabolite(s) is unlikely. Thus, it is clear that 3, 5, 3′, 5′–TCB and other chlorinated hydrocarbons are also capable of inducing some microsomal enzymes while, at the same time, selectively inhibiting other(s).

The enhancement of β–oestradiol metabolism by PCBs in several species of birds is known (Risebrough *et al.* 1968, Nowicki and Norman 1972). Our study shows that the metabolism of oestradiol is, at least, qualitatively altered because a second polar metabolite (Fig. 1) is formed by the microsomal system prepared from 2, 3, 4, 5–TCB treated female rats. The effect of this abnormal oestradiol metabolism on the reproductive system remains to be evaluated thoroughly.

Instances of hepatic porphyria induced by several commercial PCBs have been reported, previously (Vos and Koeman 1970). Vos *et al.* (1971) recently reported that porphyrogenic effect of PCBs is closely associated with an increase of mitochondrial ALA synthetase, which is the limiting enzyme in the biosynthesis of heme, and that excessive porphyrins are excreted in both urine and feces of the treated animals. Failure to observe any elevation of ALA synthetase activity in the experiment made in this laboratory possibly is the result of an inability to measure small increase of the ALA synthetase activity by the method employed or the fact that induction does not occur. Another possibility is that a higher chlorine substitution on biphenyl ring is required for the porphyrogenic action because Vos and Notenboomram (1972) were able to induce porphyria in rabbits with a hexachlorobiphenyl. As a result of the data obtained in this laboratory, it is apparent that the effect of individual isomeric PCBs on rats is quite different from that of commercial Aroclors. Thus, the total effect of Aroclors is probably the result of interactions between each component in them and is not merely the cumulative effect of each individual component.

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