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Studies on the Enzyme Inducing Effect of Polychlorinated Biphenyls[1]

THERESA S. CHEN[2,3] AND KENNETH P. DUBOIS

Toxicity Laboratory, Department of Pharmacology, University of Chicago, Chicago, Illinois 60637

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Studies on the Enzyme Inducing Effect of Polychlorinated Biphenyls. CHEN, T. S. and DUBOIS, K. P. (1973). *Toxicol. Appl. Pharmacol.* 26, 504–512. Aroclor 1260 was fed to weanling male and female rats in the diet at levels of 1, 5, 25 and 50 ppm to determine its enzyme inducing capacity over various feeding periods. Dose-dependent increases in the activity of 3 hepatic microsomal enzymes were observed; maximal induction occurred in the first 3 weeks of the feeding period. Among the 3 enzyme systems studied, *N*-demethylase was the most sensitive to the inducing effect of Aroclor 1260. The enzyme induction was more pronounced in male rats than in females. An age difference in susceptibility of male rats to the enzyme inducing effect was also noted, young rats being more susceptible than adults. Data obtained by simultaneously feeding PCB and DDT to rats showed that these compounds have additive inducing effects on *N*-demethylase activity. However, PCB and DDT were not, when combined, additive on the *O*-demethylase and EPN-detoxification systems under the test conditions employed. Comparison of enzyme inducing effects of three Aroclor compounds, Aroclor 1221, 1254 and 1260, clearly indicates that both microsomal *N*-demethylase and EPN detoxification activity were increased in proportion to the chlorine content of the Aroclor compounds. Maximal induction of *O*-demethylase occurred at 54% chlorination.

Polychlorinated biphenyls (PCB) are widespread industrial pollutants and have been extensively used as plasticizers and lubricants, heat transfer fluids and insulators. The presence of PCB residues has been demonstrated in fish, birds (Holmes *et al.*, 1967) and human adipose tissue (Biros *et al.*, 1970). The hepatic microsomal enzyme inducing capacity of PCB is well documented. These compounds have been shown to induce hepatic hydroxylating enzymes in the pigeon (Risebrough *et al.*, 1968), rat (Street *et al.*, 1969) and American kestrel (Lincer and Peakall, 1970). In addition Litterst *et al.*, (1972) have recently demonstrated a stimulating effect on microsomal nitroreduction, demethylation and hydroxylation in the rat. However, little information is available on the quantitative aspects of enzyme induction during prolonged administration of PCBs. The present investigation was thus undertaken to ascertain the rate and extent of induction of microsomal enzyme activity by PCBs administered at various dietary

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[2] University of Chicago Postdoctoral Fellow in Toxicology.

[3] Present address: Department of Pharmacology, University of Louisville, Louisville, Kentucky.

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levels over a 13-week period. Enzyme inducing effects were evaluated in terms of hepatic *N*-demethylase, *O*-demethylase and *O*-ethyl *O*-(4-nitrophenyl)phenyl phosphonothioate (EPN) detoxification activity in the rat. A comparison was also made between the effects of PCBs of different chlorine content.

It has been shown that DDT can cause induction of hepatic microsomal enzymes (Hart and Fouts, 1963). In view of the structural similarity of PCB to DDT and the reported synergistic toxic effects of PCB, DDT and other insecticides (Lichtenstein *et al.*, 1969), the combined effects of PCB and DDT on microsomal enzyme activity were also determined.

METHODS

Animals and diets. Weanling Holtzman rats of both sexes were used. They were obtained at 22 days of age and housed in an air-conditioned room maintained at 68–75 F. After feeding the Rockland Rat Diet pellets and water ad libitum for 1 week, they were placed on a grain form of the diet containing various levels of PCBs. The PCBs used in these studies were Aroclor 1221, Aroclor 1254 and Aroclor 1260, which contain 21, 54 and 60% chlorine by weight, respectively; they were kindly supplied by the Monsanto Company, St. Louis, Missouri.

The PCBs were dissolved in corn oil in appropriate concentrations to permit the addition of 5 ml/kg of diet and mixed with the ground diet using a mechanical mixer. Groups of 15 male and 15 female weanling rats each were fed diets containing Aroclor 1260 at 1, 5, 10, 25 and 50 ppm. Control rats of the same age were fed the diet without added PCBs. After feeding periods of 1, 3, 6 and 13 weeks, 3 male and 3 female rats from each treated and untreated group were sacrificed for enzyme assays.

Aroclor 1221 and Aroclor 1254 were also fed to weanling male rats at dietary levels of 10, 25 and 50 ppm. At the end of 1 and 3 weeks, groups of 3 rats were sacrificed.

In studies on the combined effects of PCB and DDT on enzyme induction, 5 ml of solutions containing the appropriate concentrations of PCB and DDT were added to each kilogram of feed. Groups of 6 males were fed a diet containing 5 ppm of DDT and 5 ppm of PCB or 5 ppm of DDT and 1 ppm of PCB for 1 or 3 weeks. Animals were sacrificed at the end of the feeding periods, and the liver was removed for measurement of enzyme activity.

Enzyme assays. Animals were sacrificed by decapitation, and whole liver homogenates were used for assays of hepatic microsomal enzymes. Phosphorothioate detoxification was measured by the method of Neal and DuBois (1965) using EPN as the substrate, and *O*-demethylase activity was measured by the procedure of Netter and Seidel (1964) using *p*-nitroanisole as the substrate. The *N*-demethylation of aminopyrine was measured by the method of La Du *et al.* (1955). The details of each of these methods as applied to quantitative measurements of enzyme induction have been described previously by Kinoshita *et al.* (1966). N-Demethylase activity was expressed as micrograms of 4-aminoantipyrine formed per 100 mg of liver in 30 min. The activities of the phosphorothioate detoxification system and *O*-demethylase were expressed as micrograms of *p*-nitrophenol formed by 50 mg of liver in 60 min from EPN and *p*-nitroanisol, respectively. The data were analysed throughout by the students *t* test.

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RESULTS

Induction of N-Demethylase by Dietary Intake of PCB

The effects of Aroclor 1260 at various dietary levels on hepatic *N*-demethylase activity in male and female rats are shown in Fig. 1. The values are expressed as per cent of control activity. Dose-related increases in enzyme activity were observed with a maximal response occurring in about 3 weeks in both male and female rats. However, there was a difference between sexes in susceptibility to the *N*-demethylase inducing effect of Aroclor 1260. Maximal enzyme activities reached at the highest dietary level,

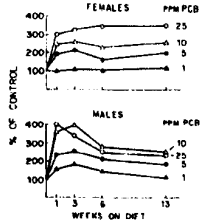


FIG. 1. Rate and extent of induction of *N*-demethylase in the livers of male and female rats by various dietary levels of Aroclor 1260. The activities (Means ± SE) of *N*-demethylase in control males at 1, 3, 6 and 13 weeks were 5.69 ± 0.25, 8.96 ± 0.63, 10.63 ± 0.20 and 9.79 ± 0.38 μg of 4-aminoantipyrine/100 mg of liver/30 min, respectively. In females, the corresponding values were 3.89 ± 0.24, 3.97 ± 0.21, 3.32 ± 0.16 and 2.93 ± 0.09, respectively.

25 ppm were about 300% of control values in females and 400% in males. At a dietary level of 1 ppm there was no significant change ($p > 0.05$) in enzyme activity in female rats in contrast to the 61% ($p < 0.05$) increase observed in males. At higher dietary levels the degree of enzyme induction was also greater in male than in female rats, indicating that the female is less susceptible to the enzyme inducing effects of Aroclor 1260. Sex differences were also observed in the pattern of enzyme induction. In female rats the enzyme activity remained relatively constant at the elevated level throughout the remainder of the 13-week period whereas in male rats the initial increase was followed by a gradual decline in activity.

Induction of EPN Detoxification by PCB

Figure 2 shows the rate and extent of induction of the hepatic EPN detoxification system by Aroclor 1260. The lowest dose of Aroclor 1260, 1 ppm, caused no significant change ($p > 0.05$) in the activity of this enzyme system. Over the range of dietary levels from 1 to 25 ppm, there was a dose-dependent increase in enzyme activity, the extent of induction was about the same in male and in female rats. Data obtained from male rats showed maximum induction during week 3 of feeding except at the 25 ppm level, where the greatest degree of induction was observed after 1 week. These increases in activity were maintained at a nearly constant level over the entire feeding period. In

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females the greatest increases were observed at the end of the 13-week feeding period in all of the dietary levels studied, except at 25 ppm, where the greatest induction occurred after 3 weeks. EPN detoxification activity remained at elevated levels throughout the remainder of the 13-week study.

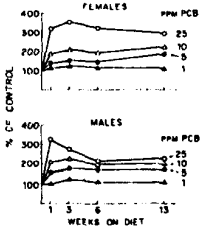


FIG. 2. Rate and extent of induction of EPN detoxification in the livers of male and female rats by various dietary levels of Aroclor 1260. The activities (Means ± SE) of the EPN detoxification system in control males at 1, 3, 6 and 13 weeks were 5.25 ± 0.11, 6.67 ± 0.19, 6.98 ± 0.12 and 7.25 ± 0.21 μg of *p*-nitrophenol/30 mg of liver/60 min, respectively. In females, the corresponding values were 3.17 ± 0.07, 2.78 ± 0.11, 2.72 ± 0.12 and 3.5 ± 0.18, respectively.

Induction of O-Demethylase by PCB

The results of measurements of hepatic *O*-demethylase activity in rats fed Aroclor 1260 are presented in Fig. 3. Similar patterns of response were observed in male and female rats. At each dietary level above 1 ppm, Aroclor 1260 produced a dose-dependent increase in *O*-demethylase activity. In male and female rats, the greatest extent of enzyme induction was seen at the 25-ppm feeding level, which caused an increase to

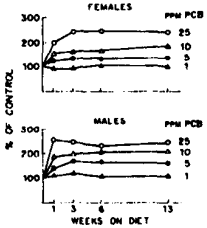


FIG. 3. Rate and extent of induction of *O*-demethylase in the livers of male and female rats by various dietary levels of Aroclor 1260. The control activities (Means ± SE) of *O*-demethylase in males at 1, 3, 6 and 13 weeks were 7.84 ± 0.21, 7.71 ± 0.35, 7.41 ± 0.30 and 7.41 ± 0.09 μg of *p*-nitrophenol/50 mg of liver/60 min, respectively. In females, the corresponding values were 6.61 ± 0.17, 6.07 ± 0.18, 6.00 ± 0.24 and 6.37 ± 0.19, respectively.

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250% of control activity within the first 3 weeks of feeding and the increased activity was maintained at a constant level over the remainder of the 13-week treatment period. Thus, no sex difference in extent of enzyme induction was observed in the *O*-demethylase activity.

From the results of assays for the 3 enzymes it is clear that Aroclor 1260 affects each of the enzyme systems studied with *N*-demethylase being the most sensitive to the enzyme inducing activity of this compound.

Enzyme Induction by Aroclor 1260 in Weanling and Adult Male Rats

In another series of experiments, adult (60-day-old) and weanling (30-day old) rats were fed Aroclor 1260 at dietary levels of 10 ppm and 50 ppm for 1 week. The activity of 3 hepatic microsomal enzyme systems was measured at the end of 1 week of feeding.

The results in Table 1 clearly indicate that young rats are more susceptible to the inducing effects of Aroclor 1260. These age differences in susceptibility to the enzyme inducing effect were observed only with *N*-demethylase and the EPN detoxification system.

TABLE 1

EFFECT OF AROCLOR 1260 ON HEPATIC MICROSOMAL ENZYME ACTIVITY IN WEANLING AND ADULT RATS

		Enzyme activity					
		N-demethylase[a]		EPN detoxification[b]		*O*-demethylase[b]	
Rats	Dietary level (ppm)	Mean ± SE	% of control	Mean ± SE	% of control	Mean ± SE	% of control
Weanling	10	20.08 ± 1.60[c]	353	11.40 ± 0.37[c]	217	14.42 ± 0.20[c]	184
	50	24.04 ± 0.36[c]	423	17.24 ± 0.13[c]	328	20.50 ± 0.44[c]	262
Adult	10	21.67 ± 1.42[c]	242	9.79 ± 0.83[c]	151	11.29 ± 0.26[c]	168
	50	25.17 ± 0.44[c]	281	17.63 ± 0.69[c]	272	18.99 ± 0.48[c]	282

[a] Expressed as μg of 4-amino antipyrine/100 mg liver/30 min.
[b] Expressed as μg of *p*-nitrophenol/50 mg liver/ 60 min.
[c] Significantly different from controls ($p < 0.05$).

Effect of Simultaneous Feeding of PCB and DDT on Enzyme Activity.

PCB and DDT each at dietary levels of 5 ppm or 1 ppm PCB and 5 ppm DDT were fed to weanling male rats, and the 3 hepatic enzyme activities were measured at the end of 1- and 3-week feeding periods. The results (Table 2) indicate that PCB and DDT have an additive inducing effect of *N*-demethylase activity. Each compound alone, administered at 5 ppm, caused only a doubling of activity, whereas a combination of DDT 5 ppm and PCB 5 ppm increased the activity to about 450% of control values. A similar additive inducing effect on *N*-demethylase activity was observed when rats were fed the diet containing PCB 1 ppm and DDT 5 ppm. In contrast the effects of PCB and DDT on *O*-demethylase and EPN detoxification activity were not additive.

Comparison of Enzyme Inducing Effects of Aroclor 1221, 1254 and 1260

Aroclor 1221, 1254 and 1260 were fed to weanling male rats at dietary levels of 10, 25 and 50 ppm for 1 and 3 weeks. The effects of these compounds on 3 hepatic microsomal enzyme activities are summarized in Table 3. In general, dose-dependent

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TABLE 2

INFLUENCE OF VARIOUS DIETARY LEVELS OF PCB AND DDT ON HEPATIC MICROSOMAL ENZYME ACTIVITY IN MALE RATS

Compound	Dietary level (ppm)	Length of time of feeding (weeks)	Enzyme activity[a]					
			N-Demethylase		EPN detoxification		*O*-Demethylase	
			Mean ± SE	% of control	Mean ± SE	% of control	Mean ± SE	% of control
PCB	1	1	9.14 ± 0.52[b]	161	5.47 ± 0.13	104	8.84 ± 0.33	113
		3	16.37 ± 1.03[b]	183	8.12 ± 0.20[b]	122	9.41 ± 0.22[b]	122
PCB	5	1	13.60 ± 1.10[b]	239	8.38 ± 0.67[b]	160	11.33 ± 0.37[b]	145
		3	23.30 ± 0.62[b]	260	12.10 ± 0.04[b]	181	13.28 ± 0.13[b]	172
DDT	5	1	11.97 ± 0.62[b]	210	7.94 ± 0.17[b]	151	11.43 ± 0.31[b]	146
		3	23.38 ± 0.95[b]	260	7.88 ± 0.24[b]	118	9.42 ± 0.18[b]	122
PCB + DDT	1	1	16.95 ± 1.35[b]	298	7.85 ± 0.34[b]	150	10.87 ± 0.43[b]	139
	5	3	26.97 ± 0.47[b]	301	9.51 ± 0.45[b]	143	11.56 ± 0.43[b]	150
PCB + DDT	5	1	25.18 ± 1.43[b]	443	10.91 ± 0.66[b]	208	13.11 ± 0.52[b]	167
	5	3	30.77 ± 0.45[b]	343	12.60 ± 0.41[b]	189	13.30 ± 0.21[b]	173

[a] For units, see Table 1.
[b] Significantly different from controls ($p < 0.05$).

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TABLE 3

INFLUENCE OF VARIOUS DIETARY LEVELS OF THREE AROCLOR COMPOUNDS ON HEPATIC MICROSOMAL ENZYME ACTIVITY IN MALE RATS

Compound	Dietary level (ppm)	Length of time of feeding (weeks)	Enzyme activity[a]					
			N-Demethylase		EPN detoxification		*O*-Demethylase	
			Mean ± SE	% of control	Mean ± SE	% of control	Mean ± SE	% of control
Aroclor 1260	10	1	20.08 ± 1.60[b]	353	11.40 ± 0.37[b]	217	14.42 ± 0.20[b]	184
		3	34.85 ± 2.10[b]	389	15.60 ± 0.54[b]	226	14.90 ± 0.29[b]	193
	25	1	23.01 ± 1.40[b]	404	17.64 ± 0.66[b]	336	19.71 ± 0.57[b]	251
		3	31.09 ± 1.20[b]	347	18.56 ± 0.72[b]	278	18.91 ± 0.51[b]	245
	50	1	24.04 ± 0.36[b]	423	17.24 ± 0.13[b]	328	20.50 ± 0.44[b]	262
		3	28.58 ± 1.90[b]	319	18.90 ± 0.95[b]	283	21.65 ± 0.33[b]	281
Aroclor 1254	10	1	10.41 ± 0.73[b]	183	9.50 ± 0.65[b]	181	17.79 ± 0.50[b]	188
		3	12.68 ± 1.05[b]	142	10.45 ± 0.55[b]	157	20.96 ± 1.99[b]	217
	25	1	13.32 ± 0.74[b]	234	12.32 ± 0.22[b]	235	31.00 ± 0.87[b]	327
		3	17.00 ± 0.88[b]	190	13.36 ± 0.53[b]	200	33.87 ± 0.71[b]	350
	50	1	14.72 ± 0.66[b]	259	15.60 ± 0.64[b]	297	38.73 ± 0.63[b]	408
		3	19.21 ± 1.08[b]	214	16.42 ± 0.34[b]	246	44.77 ± 1.38[b]	463
Aroclor 1221	10	1	5.68 ± 0.04	100	6.65 ± 0.28	126	10.01 ± 0.51	106
		3	9.18 ± 0.40	102	7.58 ± 0.27	114	9.28 ± 0.52	100
	25	1	9.08 ± 0.10[b]	159	6.88 ± 0.12[b]	131	11.38 ± 0.06[b]	120
		3	13.68 ± 0.28	152	7.06 ± 0.09	106	10.04 ± 0.29	104
	50	1	5.41 ± 0.02	100	6.46 ± 0.35	123	10.06 ± 0.38	106
		3	9.64 ± 0.45	108	8.54 ± 0.36	128	10.81 ± 0.58	112

[a] For units, see Table 1.
[b] Significantly different from controls ($p < 0.05$).

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increases in enzyme activity were observed with Aroclor 1254 and 1260 at each dietary level studied. In animals receiving Aroclor 1221, the enzyme activities were not significantly different from those of controls except at 25 ppm.

With *N*-demethylase and the EPN detoxification system, the extent of induction by the Aroclors was of the order 1260 > 1254 > 1221. Thus, there is a direct relationship between the chlorine content of the Aroclors and the ability to induce these two enzyme systems. The data in Table 3 demonstrate a different type of relation between chlorine content and *O*-demethylase activity. No significant increase in *O*-demethylase activity was observed with Aroclor 1221. Aroclor 1254 and 1260 produced dose-related increases to 408% and 262%, respectively, of control activities at the dietary level of 50 ppm.

It appears that there is a direct relationship between chlorine content of the biphenyl molecule and magnitude of induction of the *N*-demethylase and EPN detoxification systems, whereas maximal induction of *O*-demethylase occurs at 54% chlorination, then decreases at the higher level of chlorination.

DISCUSSION

Results of the present investigation demonstrate the inducing effects of various dietary levels of PCB on three hepatic microsomal enzymes in the rat during a 13-week feeding period. The PCBs resembled the chlorinated hydrocarbon insecticides, DDT and toxaphene (Kinoshita *et al.*, 1966) and the substituted urea herbicides, Herban and diuron (Kinoshita and DuBois, 1970) in the rate and persistence of enzyme induction when compared under the same experimental conditions used in our previous studies.

Among the three hepatic microsomal enzymes studied, *N*-demethylase was most sensitive to the inducing effect of Aroclor 1260. Significant but less profound increases in the EPN detoxification system and *O*-demethylase activity were also observed. Dose-dependent increases in the enzyme activity were observed in nearly all cases with maximum induction occurring during the first 3 weeks of feeding. There was a gradual decline to lower but still elevated activities throughout the remainder of the 13 weeks of treatment. Thus, prolonged exposure to the Aroclor compounds would be expected to result in persistent enzyme induction.

Sex differences in the pattern of enzyme induction and in susceptibility to the enzyme inducing effect of Aroclor 1260 were noted in *N*-demethylation with males being more susceptible than females. The patterns of response and the extent of induction of *O*-demethylase and EPN detoxification were about the same in both sexes. A marked sex difference in the amount of enzyme induction was observed in previous studies on DDT and toxaphene (Kinoshita *et al.*, 1966).

The results obtained from feeding Aroclor 1260 to weanling and adult male rats demonstrate that young rats are more susceptible to the enzyme inducing effects of Aroclor 1260, particularly with respect to the *N*-demethylase and EPN detoxification systems.

In studies on the combined effects of PCB and DDT on enzyme activity, it was found that these compounds have additive inducing effects on the *N*-demethylase system. Such as additive effect on the *O*-demethylase and EPN detoxification systems was not observed at the dose levels used. Since PCBs are one of the most abundant among the

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