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Effect of Polychlorinated Biphenyls on Hepatic Microsomal Enzymes in the Rat[1]

C. L. LITTERST,[2] T. M. FARBER, A. M. BAKER AND E. J. VAN LOON

Special Pharmacological Animal Laboratory, Bureau of Foods, Food and Drug Administration, Department of Health, Education, and Welfare Washington, D.C. 20204

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Effect of Polychlorinated Biphenyls on Hepatic Microsomal Enzymes in the Rat. LITTERST, C. L., FARBER, T. M., BAKER, A. M. and VAN LOON, E. J. (1972). *Toxicol. Appl. Pharmacol.* 23, 112–122. Polychlorinated biphenyls (PCB) are widely used industrial synergists and stabilizers that have been detected in various animal systems throughout the world. Although these chemicals possess a low acute toxicity, recent indications are that they may possess a high chronic toxicity. To evaluate further the toxicity of PCB mixtures, rats were fed diets containing 4 different Aroclors varying in chlorine content from 42 to 60% for 4 wk at levels of 0.5, 5, 50, and 500 ppm. The influence of PCB was studied by determining liver weights, hepatic triglyceride concentration and glucose-6-phosphatase activity, microsomal enzyme activities (nitroreduction, demethylation and hydroxylation) and microsomal cytochrome P-450 content. Liver-to-body weight ratios and cytochrome P-450 content were significantly increased at 50 and 500 ppm for all PCB mixtures; the increase in cytochrome P-450 was somewhat greater with increasing chlorine content. Significant increases in liver triglycerides were seen at 500 ppm, with a maximum effect occurring at approximately 50% chlorine content. A dose-related increase in demethylase activity was observed, with a 2.5- to 3-fold increase over control values at 500 ppm for all mixtures. Effects were maximum at approximately 50% chlorine content. A dose-related increase in pentobarbital hydroxylation was seen, and the induction of hydroxylation increased with increasing chlorine content. Nitroreductase activity was markedly stimulated with increasing doses of PCB, and induction was seen at levels as low as 0.5 ppm for all mixtures. The maximal effect appears to be at levels higher than 500 ppm. The highest levels of activity were seen with 60% chlorination.

In the mid-1960s, analytical chemists first demonstrated in various ecosystems the presence of a class of industrial chemicals known as polychlorinated biphenyls (PCB). These materials are widely used industrial stabilizers and synergists and are found in such diverse products as paint, rubber, asphalt, printer's ink and pesticide formulations. Soon after the occurrence of these substances in the environment was reported, accounts of their toxicity to environmental components began to appear. In one of the earliest of these toxicity studies, Flick *et al.* (1965) noted that the toxicity seen after feeding PCB

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to cockerels was similar to the signs of chick edema disease in poultry. Duke *et al.* (1970) reported the effect of PCB on various marine fauna, and Zitko (1970) studied PCB for their toxicity to salmon. Bitman and Cecil (1970) determined that some of these chemicals with low chlorine content had an estrogenic action on immature female rats, while Lincer and Peakall (1970) showed an effect on estrogen metabolism in kestrels.

The possible health hazard to humans presented by PCB is as yet obscure, but recent disclosures of the presence of PCB in human adipose tissues (Biros *et al.*, 1970) and in human milk (Acker and Schulte, 1970) raise questions as to the effect of these tissue contaminants on human health. Reports have been made of the ability of PCB to increase host susceptibility to viral infection (Friend and Trainer, 1970) and to synergize the toxicity of pesticide residues (Lichtenstein *et al.*, 1969). The toxicity of these chemicals to human cells has been documented (Litterst and Lichtenstein, 1971). The ability of PCB to induce enzymes was first suggested by Risebrough *et al.* (1968) and was further defined for liver microsomal enzymes by Street *et al.* (1969). An increase in hepatic microsomal enzyme activity in rats following dietary exposure to PCB has been correlated with smooth endoplasmic reticulum proliferation, shown by electron microscopy (Norback and Allen, 1970). The lowest dose of PCB used by Street *et al.* (1969) was 25 ppm in the diet; this level was found effective for enzyme induction. Their results suggested to us that minute doses of these materials might be capable of producing significant disruptions in the activity of drug-metabolizing enzymes located in hepatic microsomes. The present study was designed to define a minimum inductive dose of PCB in rats and to extend the knowledge about the range of enzymes affected by these chemicals.

METHODS

Male Osborne-Mendel rats, weighing between 180 and 220 g, were allotted to groups of 6 rats each and were fed diets containing 0, 0.5, 5.0, 50 or 500 ppm of 1 of 4 commercial PCB plasticizers incorporated into ground Purina rat chow. The PCB was added undiluted to dry feed while mixing; serial dilutions were then made until the desired concentrations were obtained. The PCB mixtures used were Aroclors®[3] 1242, 1248, 1254 and 1260, which contain 42, 48, 54 and 60% chlorine by weight, respectively. Body weights were determined each week throughout the course of the experiment and again immediately before death. After the rats had been fed the experimental diets for 4 wk, they were fasted for 16 hr but given water ad libitum and then killed by cervical dislocation. Livers were excised and weighed, then were placed immediately into ice-cold buffer and minced into small pieces with scissors. All subsequent manipulations were done at 4°C. Livers from 6 replicate animals per treatment dose were pooled for enzyme analyses and were homogenized in buffer by utilizing a Potter-Elvehjem homogenizer with a Teflon® pestle and a constant torque motor. Homogenates were appropriately diluted and then, depending on the assay to be conducted, either used directly or centrifuged for 30 min at 9000 *g* in a refrigerated International PR-2 clinical centrifuge. All determinations were made in triplicate.

For analyses of liver triglyceride content and glucose-6-phosphatase (G-6-Pase) activity, 1 g liver samples were homogenized in 9 volumes of 0.1 M citrate buffer (pH 6.5)

[3] Supplied by Monsanto Chemical Company, St. Louis, Missouri.

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All other enzymes assays were conducted with a 9000 *g* supernatant from livers homogenized in 4 volumes of a pH 7.4 buffer containing mannitol (0.25 M), sucrose (0.07 M), EDTA (0.001 M) and Tris (0.01 M). The supernatant was adjusted after centrifugation so that 1 ml of the final preparation contained 200 mg of liver tissue.

The triglyceride content of 1:10 homogenates of liver tissue in citrate buffer was determined by a modification of the Turner fluorometric procedure for serum triglycerides (Phillips, 1969). The G-6-Pase activity was assayed by determining the amount of inorganic phosphate liberated when 16 μmoles of glucose 6-phosphate was incubated with 5 mg of liver tissue according to the method of Harper (1963).

The 9000 *g* supernatant fraction from a 1:5 dilution of liver in the adjusted mannitol-sucrose buffer was used to study the effect of PCB on the following microsomal enzyme reactions, with the substrates and respective quantities specified: the demethylation of aminopyrine (10 μmoles), codeine (10 μmoles) and ethyl morphine (10 μmoles) to formaldehyde by the method of Cochin and Axelrod (1959); the side-chain hydroxylation of ^{14}C-pentobarbital (1 μmole) by the method of Kuntzman *et al.* (1967); and the nitroreduction of *p*-nitrobenzoic acid (10 μmoles) to *p*-aminobenzoic acid by the method of Smith and Van Loon (1969). Cytochrome P-450 content was determined by the method of Omura and Sato (1964).

For demethylation, hydroxylation and nitroreduction assays, each incubation flask contained 50 μmoles each of nicotinamide and $MgCl_2$, 25 μmoles of glucose 6-phosphate and 0.6 μmoles of NADP. To the cofactors in the incubation flasks, 1 ml of substrate was added and the volume was adjusted to 5 ml with 0.5 M Na-K phosphate buffer, pH 7.4. In addition, all demethylation flasks contained 0.1 ml of a 5% semicarbazide solution. Flasks were incubated in a metabolic shaker at 37°C for 30 min in an oxygen atmosphere. Nitroreductase activity was determined under nitrogen.

RESULTS

The effect of dietary administration of Aroclor 1260 on the growth rate of rats throughout the 4 wk treatment period is presented in Fig. 1. The results represent in general the effect on growth rate for all four PCB mixtures tested; as can be seen, no significant effect was observed for any dose tested.

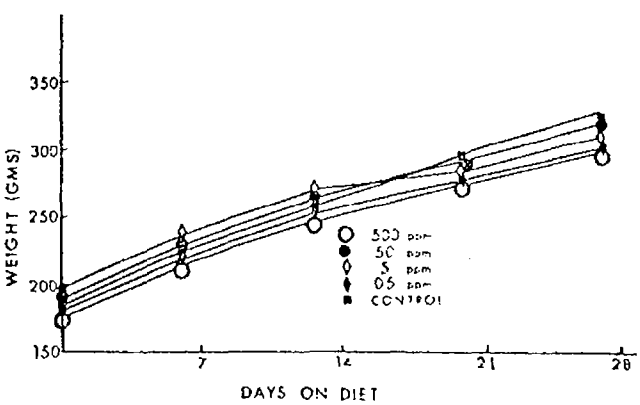


FIG. 1. Growth rate of rats fed varying concentrations of Aroclor 1260 for 4 wk. Each point is the mean of 6 animals.

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Table 1 presents the effects of PCB on liver weights and on liver:body weight ratios. Increases in liver weights and corresponding significant increases in liver:body weight ratios were seen for the higher dose levels of all PCB mixtures studied; no effect was seen at 0.5 or 5 ppm. An increase in liver:body weight ratio was observed for all 4 Aroclors at 500 ppm and for Aroclors containing more than 48% chlorine at the 50-ppm dose level.

TABLE 1

EFFECT OF PCB TREATMENT ON FINAL LIVER WEIGHTS AND ON LIVER:BODY WEIGHT RATIO IN MALE OSBORNE-MENDEL RATS[a]

Aroclor No.[b]	Dose (ppm)	Final body weight (g)	Liver weight (g)	Liver:body weight ratio
1242	500	273 ± 20	12.98 ± 1.03	0.047 ± 0.002[c]
	50	297 ± 31	9.93 ± 1.40	0.033 ± 0.002
	5	295 ± 33	9.19 ± 1.16	0.031 ± 0.001
	0.5	290 ± 7	8.71 ± 0.67	0.030 ± 0.001
	0	287 ± 18	8.69 ± 0.69	0.030 ± 0.001
1248	500	307 ± 25	16.90 ± 2.31	0.055 ± 0.005[c]
	50	329 ± 16	11.59 ± 1.16	0.035 ± 0.003[d]
	5	290 ± 30	8.70 ± 1.64	0.030 ± 0.003
	0.5	274 ± 13	8.53 ± 0.95	0.031 ± 0.002
	0	318 ± 26	9.93 ± 1.24	0.031 ± 0.002
1254	500	240 ± 33	15.14 ± 2.53	0.063 ± 0.003[c]
	50	255 ± 24	9.43 ± 1.25	0.038 ± 0.003[c]
	5	259 ± 20	7.87 ± 0.68	0.030 ± 0.002
	0.5	262 ± 15	8.38 ± 1.23	0.032 ± 0.004
	0	272 ± 22	8.02 ± 0.89	0.029 ± 0.001
1260	500	267 ± 22	15.90 ± 2.56	0.060 ± 0.009[c]
	50	285 ± 15	11.96 ± 1.54	0.042 ± 0.004[c]
	5	278 ± 22	9.11 ± 0.85	0.033 ± 0.003
	0.5	259 ± 23	8.21 ± 0.65	0.031 ± 0.002
	0	286 ± 21	8.65 ± 0.98	0.030 ± 0.002

[a] Results are given as the mean of 6 rats ± SD.
[b] Polychlorinated biphenyl compounds containing 42% (1242) to 60% (1260) by weight of chlorine, fed in the diet for 4 wk.
[c] Significantly different from control group at $P < 0.001$ as judged by the student t test.
[d] $P \leq 0.05$.

Figure 2 presents the effect of PCB on the triglyceride content of the liver. The peak triglyceride concentrations were reached with the Aroclor containing 48% chlorine and decreased with increasing chlorine content. An increase in triglyceride concentration was seen with increasing doses of each individual Aroclor.

Table 2 shows the effects of Aroclor treatment on demethylase activity in 9000 g supernatants of liver homogenates. PCB induced the activity of demethylase(s) in liver microsomes and, with few exceptions, the products formed from all 3 substrates

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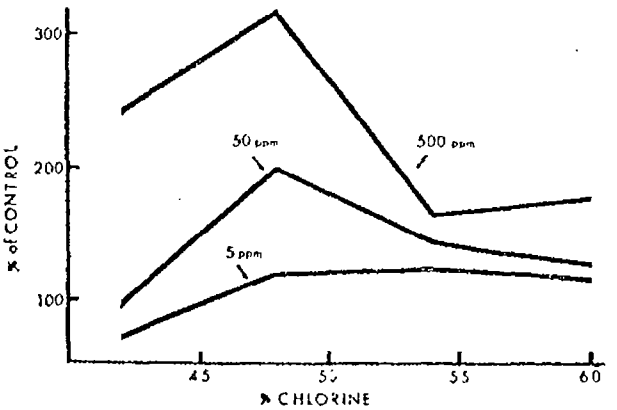


FIG. 2. Effect of dose and chlorine content of Aroclors on hepatic triglyceride concentrations in rats after 4 wk. Each point represents the mean of 3 replicates. With one exception all SD values were less than 10% of their respective means (SD of the 5 ppm dose of Aroclor 1248 was 18% of its mean).

TABLE 2

EFFECT OF PCB TREATMENT ON DEMETHYLATION ACTIVITIES IN HEPATIC MICROSOMES OF RATS[a]

		Substrate					
		Aminopyrine		Codeine		Ethylmorphine	
Aroclor No.[b]	Dose (ppm)	Units[c]	% of control	Units[c]	% of control	Units[c]	% of control
1242	500	14.2 ± 0.4[d]	240	22.4 ± 0.3[d]	245	15.9 ± 0.1[d]	219
	50	9.6 ± 0.1[d]	163	15.1 ± 0.4[d]	165	11.2 ± 0.3[d]	155
	5	7.6 ± 0.4	129	11.6 ± 0.2	127	8.7 ± 0.2	121
	0.5	9.3 ± 0.2[e]	108	9.1 ± 0.2	99	7.8 ± 0.1	108
	0	5.9 ± 0.2	100	9.1 ± 0.1	100	7.2 ± 0.3	100
1248	500	17.4 ± 0.5[d]	272	24.8 ± 0.4[d]	258	17.6 ± 0.5[d]	243
	50	10.4 ± 0.4[d]	163	16.5 ± 0.4[d]	171	12.1 ± 0.5[d]	166
	5	7.6 ± 0.3	120	11.5 ± 0.3[e]	119	9.6 ± 0.4[d]	133
	0.5	5.1 ± 0.2[e]	79	8.5 ± 0.3[e]	88	7.4 ± 0.3	102
	0	6.4 ± 0.4	100	9.6 ± 0.1	100	7.2 ± 0.1	100
1254	500	19.3 ± 0.2[d]	336	25.3 ± 0.3[d]	264	17.9 ± 0.5[d]	236
	50	16.6 ± 0.2[d]	289	24.4 ± 0.5[d]	253	17.9 ± 0.4[d]	236
	5	8.9 ± 0.3[d]	155	13.2 ± 0.3[d]	138	11.1 ± 0.2[d]	146
	0.5	7.2 ± 0.2[d]	127	12.2 ± 0.2[d]	127	9.6 ± 0.2[e]	127
	0	5.8 ± 0.2	100	9.6 ± 0.2	100	7.6 ± 0.3	100
1260	500	22.2 ± 0.2[d]	274	28.5 ± 0.5[d]	197	17.4 ± 0.3[d]	162
	50	18.6 ± 0.3[d]	229	26.6 ± 0.3[d]	183	18.0 ± 0.2[d]	167
	5	13.0 ± 0.3[d]	160	20.7 ± 0.3[d]	143	15.9 ± 0.2[d]	148
	0.5	9.6 ± 0.3[d]	118	14.9 ± 0.3	103	12.4 ± 0.3[e]	116
	0	8.1 ± 0.1	100	14.5 ± 0.1	100	10.7 ± 0.2	100

[a] Each value is the mean ± SD of 3 replicates.
[b] Polychlorinated biphenyl compounds containing 42% (1242) to 60% (1260) by weight of chlorine, fed in the diet for 4 wk.
[c] Expressed as μmoles of product formed/g liver/30 min.
[d] Significantly different from control group at $P \leq 0.01$ as judged by the student *t* test.
[e] $P < 0.05$.

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(representing *O*-methyl, *N*-methyl and *O*-dimethyl substitution), were increased approximately to the same degree. Although a dose of 5 or 50 ppm of most Aroclors was necessary before induction became evident, the 0.5 ppm dose of Aroclors 1254 and 1260 appeared to produce an increase in enzyme activity. Two significant points relative to the PCB effect on demethylase activity can be seen in Table 2: (1) the increase in amounts of product formed with increased PCB concentrations suggests that a dose-response relationship exists between any one specific Aroclor and this enzyme; and (2) the fact that the data seem to reach a peak at the 54% chlorine content and then

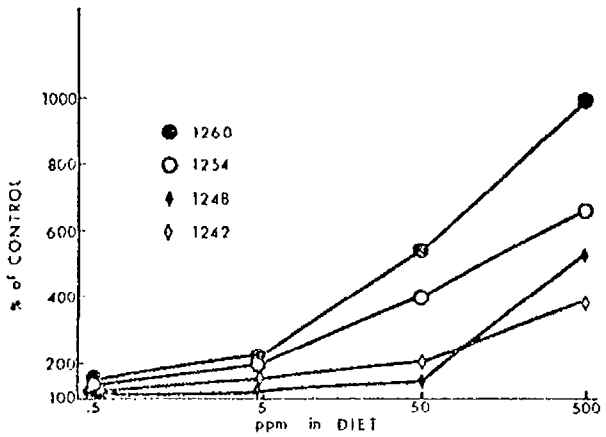


FIG. 3. Dose-response relationship of 4 Aroclors on nitroreduction of *p*-nitrobenzoic acid in rat liver microsomes. Each point represents the mean of 3 replicates. The SD values were all less than 5% of their respective means.

decrease in amplitude with Aroclor 1260, however, suggests that there is no linear increase in the effect of PCB as the chlorine content of the molecule increases.

Table 2 also shows that even at the highest dose of all Aroclors tested, enzyme activities do not exceed a level of 2–3 times the control values. As shown in Fig. 3, the dose-response relationship was strikingly different in the nitroreduction of *p*-nitrobenzoic acid. The maximum values for this reaction seemed to be limited only by the number of concentrations and the number of different Aroclors tested. The activity of the nitroreductase in rats fed 500 ppm of Aroclor 1260 was 10 times the control values, while all 4 Aroclors at 0.5 ppm caused a 50–70% increase in activity. The slope of the curve was increased with increasing chlorine content of the Aroclor.

The effect of the chlorine content and the dose of the test compounds on the production of hydroxylated metabolites of pentobarbital is shown in Fig. 4. As with the triglyceride data (Fig. 2), hydroxylation appeared to decrease at intermediate chlorine levels, but then unlike the triglycerides, increased as the chlorine content increased further. This increase in activity was for the most part linear and may be limited only by the limit of chlorination studied. An increasing effect with increasing doses was observed, as indicated by the upward shift for the curves of the higher test doses.

Table 3 shows the relationship between microsomal protein content and cytochrome P-450 levels in PCB treated rats. A consistent effect on microsomal protein did not

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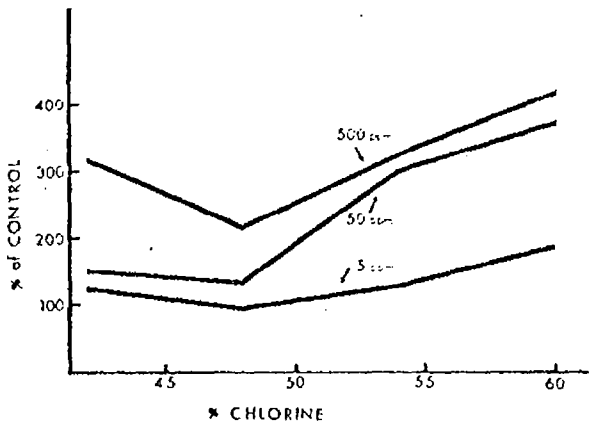


FIG. 4. Effect of dose and chlorine content of Aroclors on hydroxylation of pentobarbital in rat liver microsomes. Each point represents the mean of 3 replicates. With two exceptions, all SD values were less than 5% of their respective means (SD of 500 ppm of Aroclors 1248 and 1254 were 7% and 9%, respectively, of their means).

TABLE 3

EFFECT OF PCB TREATMENT ON LIVER MICROSOMAL PROTEIN AND CYTOCHROME P-450 CONTENT OF RATS[a]

Aroclor No.[b]	Dose (ppm)	Protein[c]	P-450[d]
1242	500	51 ± 2	1.26 ± 0.09[e]
	50	43 ± 4	0.69 ± 0.05
	5	45 ± 4	0.42 ± 0.03
	0.5	40 ± 3	0.51 ± 0.03
	0	45 ± 5	0.46 ± 0.04
1248	500	84 ± 2[e]	1.29 ± 0.11[e]
	50	54 ± 4	0.71 ± 0.04[e]
	5	54 ± 5	0.46 ± 0.02
	0.5	51 ± 4	0.59 ± 0.04
	0	66 ± 7	0.37 ± 0.05
1254	500	60 ± 3[e]	1.42 ± 0.12
	50	60 ± 4	0.88 ± 0.06
	5	45 ± 2	0.71 ± 0.04
	0.5	45 ± 2	0.61 ± 0.04
	0	40 ± 3	—
1260	500	100 ± 8[e]	1.24 ± 0.09[e]
	50	73 ± 4[e]	1.20 ± 0.10[e]
	5	60 ± 5	0.71 ± 0.04
	0.5	53 ± 3	0.54 ± 0.03
	0	47 ± 3	0.57 ± 0.02

[a] Values are the results ±SD of duplicate determinations conducted on a microsomal pellet from six pooled livers.

[b] PCB compounds containing from 42% (1242) to 60% (1260) by weight of chlorine, fed in the diet for 4 wk.

[c] Expressed as mg microsomal protein/g liver.

[d] Expressed as nmoles of cytochrome P-450/g of microsomal protein.

[e] Significantly different from control values at $P \leq 0.05$ as judged by the student *t* test.

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become evident until chlorine content of the biphenyl molecule reached 60%, although an increase in protein was seen at the highest dose level with Aroclors of lower chlorine content. An increase in cytochrome P-450 levels was observed with all PCB mixtures at dietary levels of 50 and 500 ppm. In addition, an apparent increase in P-450 was seen at 5 ppm of Aroclors containing 54 or 60% chlorine.

TABLE 4

EFFECT OF PCB TREATMENT ON GLUCOSE-6-PHOSPHATASE ACTIVITY IN RAT LIVER HOMOGENATES[a]

	Aroclor No.[b]							
	1242		1248		1254		1260	
Dose (ppm)	Units[c]	% of control	Units[c]	% of control	Units[c]	% of control	Units[c]	% of control
0	21.6 ± 0.3	100	14.9 ± 0.4	100	15.3 ± 0.1	100	13.7 ± 0.1	100
0.5	18.2 ± 0.2[d]	84	14.6 ± 0.2	97	15.5 ± 0.3	101	13.8 ± 0.1	101
5	18.7 ± 0.5	86	14.7 ± 0.2	98	14.3 ± 0.1[d]	93	14.0 ± 0.3	102
50	14.7 ± 0.5[d]	68	12.0 ± 0.1[d]	80	13.0 ± 0.4[d]	85	11.8 ± 0.2[d]	86
500	12.6 ± 0.2[d]	58	9.6 ± 0.2[d]	64	9.1 ± 0.3[d]	59	10.7 ± 0.4[d]	78

[a] Each value is the mean ± SD of 3 replicates.
[b] PCB compounds containing 42% (1242) to 60% (1260) by weight of chlorine, fed in the diet for 4 wk.
[c] Expressed as μmoles of product formed/g liver/min.
[d] Significantly different from control value at $P < 0.01$ as judged by the student t test.

Table 4 shows the effect of increasing dose on activity of G-6-Pase in crude liver homogenates. This enzyme was the only parameter studied that showed a decrease in activity, an effect that appeared to increase as the dose was increased.

DISCUSSION

The inability of the four Aroclors tested in this study to affect the weight gain of rats during a 4 wk test period is in essential agreement with data of Street *et al.* (1969), who showed that 25, 100 or 200 ppm of Aroclors given to female rats in the diet for 15 days had no effect on weight gain, food consumption or utilization efficiency. The increased weight of livers from rats treated with 50 or 500 ppm of all Aroclors suggested that an effect on liver enzyme function might also be detectable at these levels. This was indeed the case, as evidenced by our data which show disruptions in normal enzyme activity even at dose levels which had no effect on liver weights or on liver:body weight ratios

Perhaps the most interesting result of this study was the striking increase in reductase activity in Aroclor-treated rats seen at all doses tested and with all four Aroclors. The effect was not only dose related, but it was also related to the chlorine content of the biphenyl molecule and increased in magnitude with an increase in chlorine content. This finding assumes even greater importance in view of the role of reductases in steroid

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metabolism (McGuire and Tomkins, 1960) and the suggestion by Lincer and Peakall (1970) that steroid metabolism in animals may be altered by exposure to PCB. Although our experiments monitored only a reductase that may or may not play a part in the metabolism of endogenous steroid compounds, the results nonetheless suggest a possible relationship with effects on steroid metabolism reported by other workers.

The decline in G-6-Pase activity shown in Table 4 may appear inconsistent with the other results presented here; all other microsomal enzymes studied showed striking increases in activity. The reverse effect shown by G-6-Pase, is however, consistent with observations that this enzyme can be decreased concomitantly with an increase in other microsomal enzymes from livers of phenobarbital treated rats (Pandhi and Baum, 1970) or CCl_4 treated rats (Feuer and Golberg, 1967).

Both demethylase activity and the level of cytochrome P-450 in liver microsomes appear to reach a maximum level of induction that seems to be unaffected by increasing the amount of chlorine in the test chemical. However, the hydroxylation of pentobarbital (Fig. 4), another oxidative reaction, does not seem to be limited by chlorine content. This lack of linear relationship between chlorine content and effect is also seen with liver triglyceride concentrations in the PCB treated rats (Fig. 2). Street *et al.* (1969), using *p*-nitroanisole as substrate, showed that maximum demethylation activity in the microsomes occurred at about 50% chlorination of the biphenyl molecule and then decreased at higher levels of chlorination, a result that agrees well with our present study.

Although Aroclor 1260 is an exception, it appears that the increase in levels of cytochrome P-450 is dose-related, with a maximum stimulatory dose greater than 500 ppm.

In an attempt to observe effects on enzyme activity that might be related to protein content of microsomes, all data were replotted on a dose-effect basis, using activity per mg of microsomal protein to express enzyme activity. These curves essentially paralleled those shown and are therefore not presented.

The data reported here suggest that a chronic exposure to low dose of PCB might exert an effect on enzyme activity in human liver. The detection of PCB both in human fat (Biros *et al.*, 1970) and in human breast milk (Acker and Shulte, 1970) indicated that humans are exposed to these chemicals from some source(s) as yet unidentified. The level of PCB in the average human diet is unknown and represents a possible source of human exposure. Recent newspaper disclosures of PCB contaminated poultry products, packaging materials and dried cereal products suggest routes by which these chemicals could contaminate man. In addition, there have been reports of PCB contaminated marine fauna from European waters (Koeman *et al.*, 1969) and some freshly caught samples of edible crustaceans and fish from U.S. waters have been reported to contain 1.0–7.0 ppm and 4.5–20.0 ppm, respectively, of PCB (Duke *et al.*, 1970), although later studies using more specific analytical techniques indicate that actual levels in fish may be lower. At any rate, the dietary levels utilized in the present study are consistent with PCB concentrations being detected in certain raw products which may eventually find their way into the human diet, and our treatment levels offer a good approximation of dietary levels that may be expected in some human diets. The long range hazard to man and animals from diets of PCB contaminated food is unknown and represents an area for continued investigation.

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