Enzyme Induction by Polychlorinated Biphenyls Relative to Known Inducing Agents (36867)

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Polychlorinated biphenyls (PCB) are mixtures of various chlorinated biphenyl molecules used in industry primarily in closed-system electrical capacitors and as heat transfer agents. These chemicals were brought to the attention of the scientific community when they were found to be contaminants of wild fish and birds (1–3). Recent interest has focused on their presence in poultry and in poultry by-products (4, 5). As a result of these disclosures, toxicological investigations have dealt largely with the effects of PCB's in poultry (6, 7) and on the toxicity of PCB's to wild birds (2, 8) and fish (9, 10). Mammalian toxicity studies have also shown that there are biochemical consequences of PCB exposure. Nishizumi (11) reported that PCB's manufactured commercially in Japan produce characteristic liver lesions in mice which are detectable by light and electron microscopy. Norback (12) treated rats with chlorinated triphenyls and correlated similar microscopic alterations with deviations from normal liver enzyme function, and Pardini (13) has demonstrated the ability of numerous PCB's to inhibit enzymes located in beef heart mitochondria. Villeneuve *et al.* (14) studied the sensitivity of oxidative enzymes from the livers of pregnant rabbits treated with PCB and reported an increase in enzyme activity. Similarly, Litterst *et al.* (15) recently reported that 30-day administration of PCB to rats produces an increase in various microsomal drug-metabolizing enzymes.

Little effort, however, has been devoted to establishing the relative ability of PCB to induce microsomal enzymes as compared with known and widely studied enzyme inducers. The present study reports the ability of equimolar dietary doses of PCB, DDT, and phenobarbital to increase the activity of drug-metabolizing enzymes in the microsomes of rat liver.

Methods. Male Osborne–Mendel rats (100–200 g) were fed a diet containing 1.5, 15, or 150 μmoles of either PCB, DDT, or phenobarbital per kg of food. Each dose was fed to six rats for a period of 30 days. The diets were prepared by adding undiluted Aroclor® 1254, p,p′-DDT (99.9%), or phenobarbital sodium to ground Purina Laboratory Chow with mixing, and serial dilutions were made until the desired test concentrations were obtained. To test for uniformity of mixing, diet samples were analyzed for DDT and PCB by gas chromatography; results showed that the concentrations were within 6–8% of the calculated concentrations. Experiments, with appropriate controls, were begun in a staggered fashion over a period of 7 days. Each rat was housed individually and weighed each week. Rats were given free access to food and water, and food consumption was monitored daily. At the end of the 30-day treatment period, rats were killed and their livers were excised. The six livers from each treatment were weighed and then pooled in groups of two for enzyme analysis. Livers were homogenized in ice-cold mannitol-sucrose buffer and the homogenate was assayed under conditions previously described (15) for the following microsomal components or enzyme reactions: hydroxylation, N-demethylation, nitroreduction, microsomal protein, and cytochrome P-450 content. Results were analyzed statistically by the Student's *t* test.

Results. Food consumption of all animals

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TABLE I. Effects of 30-Day Dietary PCB, DDT, or Phenobarbital Treatment on Parameters of Rat Liver.[a]

Chemical	Dose		Liver wt/body wt	Protein (mg/g)	Cytochrome P-450 (nmoles/mg protein)
	μmoles/kg of food	mg/kg of body wt[b]			
Control	—	—	0.032 ± 0.001	31.1 ± 1.0	0.63 ± 0.03
DDT	15	0.5	0.034 ± 0.002	34.5 ± 1.1[c]	0.73 ± 0.04[c]
	150	5.3	0.037 ± 0.003[c]	37.9 ± 0.8[d]	0.94 ± 0.06[d]
PCB	15	0.5	0.031 ± 0.003	32.3 ± 1.1	0.68 ± 0.01
	150	4.7	0.037 ± 0.001[d]	43.6 ± 0.8[d]	1.26 ± 0.03[d]
Phenobarbital	15	0.4	0.032 ± 0.001	33.0 ± 0.6	0.65 ± 0.06
	150	3.8	0.035 ± 0.002[c]	35.8 ± 1.2[d]	0.73 ± 0.04[c]

[a] Control values are the means ± SD of at least 12 determinations. Experimental data: liver wt/body wt ratios are means ± SD of 6 rats; protein and cytochrome P-450 values are means ± SD of 3 determinations. Data from rats given diets containing the chemicals at 1.5 μmoles/kg of food were not different from control values and are not included.

[b] Based on a 200-g rat consuming 20 g of food per day.

[c] Significantly different from control values, $p < 0.05$.

[d] $p < 0.01$.

fed diets with the added compounds did not vary significantly from that of controls; the average daily intake of all experimental groups was 20.0 ± 1.1 g of food per day (range, 18.7–22.4) throughout the 4 weeks of the experiment. In general, slightly higher food intake was noted during the last 7–10 days of the study.

The effect of treatment on liver weight and on protein content of liver microsomes is shown in Table I. An increase in both these parameters was seen, particularly at the

TABLE II. Effects of 30-Day Dietary PCB, DDT, or Phenobarbital Treatment on Microsomal Enzyme Activity of Rats.[a]

Chemical	Dose		Demethylation (nmoles/g/30 min)	Reduction (μg/g/30 min)	Hydroxylation (μg/g/15 min)
	μmoles/kg of food	mg/kg of body wt[b]			
Control	—	—	7447 ± 448	75 ± 4	0.14 ± 0.01
DDT	1.5	0.05	9681 ± 315[c]	82 ± 5	0.11 ± 0.02
	15	0.5	11171 ± 852[d]	88 ± 7	0.17 ± 0.02[c]
	150	5.3	14590 ± 984[d]	143 ± 19[d]	0.22 ± 0.01[d]
PCB	1.5	0.05	7092 ± 402	70 ± 5	0.17 ± 0.05
	15	0.5	7745 ± 921	83 ± 9	0.23 ± 0.01[d]
	150	4.7	14590 ± 438[d]	235 ± 19[d]	0.39 ± 0.04[d]
Phenobarbital	1.5	0.04	6832 ± 722	72 ± 14	0.15 ± 0.02
	15	0.4	9309 ± 301[c]	75 ± 8	0.17 ± 0.01[c]
	150	3.5	9607 ± 611[c]	102 ± 11[d]	0.20 ± 0.01[d]

[a] Control values are the means ± SD of 12 replicates; experimental values are the means ± SD of 3 replicates.

[b] Based on a 200-g rat consuming 20 g of food per day.

[c] Significantly different from control value, $p < 0.05$.

[d] $p < 0.01$.

highest dose level. The increase in microsomal content of cytochrome P-450 was similar to the increase seen in microsomal protein content.

Table II shows the relative ability of PCB, DDT, and phenobarbital to increase the activities of microsomal drug-metabolizing enzymes. At the highest dose level all chemicals studied caused a significant increase in enzyme activity, whereas at the lowest dose the only significant increase in activity was produced by DDT on demethylation.

Discussion. The increase in liver/body weight ratio produced by equimolar amounts of DDT, PCB, and phenobarbital (Table I) is a reflection of the ability of these chemicals to increase the absolute weight of the liver, an effect seen here only at the highest dose tested. The similar increase seen in microsomal protein content suggests that this increase in liver weight is probably attributable at least partially to an actual increase in *de novo* synthesis of hepatocyte protein. The increase in cytochrome P-450 may account for part of the increased protein synthesis and the increase in microsomal enzyme activity might be expected because of the known relation between P-450 and oxidative metabolic activity of microsomes (16). At 150 μmoles/kg, the increases in liver weight and protein content were approximately equal for the three test chemicals; however, at the same dose, phenobarbital produced a 15% increase in cytochrome P-450 content, and DDT and PCB produced 50 and 100% increases, respectively. DDT was the only enzyme-inducing compound studied that produced significant increases in protein or P-450 content at doses lower than 150 μmoles/kg of food.

Table II shows the ability of the three chemicals tested to increase the activity of specific enzymatic pathways in liver microsomes. At 150 μmoles/kg, PCB consistently produced a greater stimulation in activity than did phenobarbital and had approximately twice as great an effect on reduction and hydroxylation. PCB at this dose also produced a significantly greater increase in activity than did DDT on all but the demethylation reaction, where the effect was equal for the two inducers. At the two lower doses tested, the effect of PCB on demethylation and reduction was lower than that produced by DDT, but the effect on hydroxylation was higher even at the lowest dose tested. As seen in Table II, DDT and PCB, both highly chlorinated ecological contaminants, produced the greatest amount of enzyme stimulation. That the inducing ability of DDT appears generally to be somewhat greater than that of phenobarbital in this study is not surprising in view of the successful clinical application of DDT in cases of hyperbilirubinemia where phenobarbital was found to be ineffective (17, 18).

The usual dose of phenobarbital employed to produce maximal enzyme induction is 40–100 mg/kg of body weight administered intraperitoneally for 3 or 4 days. The results of this treatment vary quantitatively from laboratory to laboratory and range from a 15–25% increase in liver weight to a 300% increase in the activity of cytochrome P-450. Tables I and II show that the extremely small dose of phenobarbital used in this study does produce an increase in inducible components of the endoplasmic reticulum, but that the increase is far less in most cases than can be produced by phenobarbital under optimum conditions.

Summary. Enzyme induction by equimolar dietary amounts of DDT, phenobarbital, and PCB was studied in rats after 30 days of treatment. At 150 μmoles per kg of food, PCB was far more effective than phenobarbital and was at least as effective as DDT. At 15 μmoles, phenobarbital, DDT, and PCB produced substrate-specific increases in enzymatic activity.

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