PCB Enzymes

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A Comparative Study of Two Polychlorinated Biphenyl Mixtures (Aroclors 1242 and 1016) Containing 42% Chlorine on Induction of Hepatic Porphyria and Drug Metabolizing Enzymes[1]

JOYCE A. GOLDSTEIN, PATRICIA HICKMAN, VIRLYN W. BURSE[2] AND HINDA BERGMAN

Environmental Protection Agency, National Environmental Research Center, Research Triangle Park, North Carolina 27711

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A Comparative Study of Two Polychlorinated Biphenyl Mixtures (Aroclors 1242 and 1016) Containing 42% Chlorine on Induction of Hepatic Porphyria and Drug-Metabolizing Enzymes. GOLDSTEIN, J. A., HICKMAN, P., BURSE, V. W. AND BERGMAN, H. (1975) *Toxicol. Appl. Pharmacol.* **32**, 461–473. Aroclor 1242 and Aroclor 1016 are polychlorinated biphenyl (PCB) mixtures with similar chlorine content (42 vs 41%), but Aroclor 1242 contains 9% biphenyl homologs with five or more chlorines while Aroclor 1016 contains only 1%. The effects of Aroclor 1242 and Aroclor 1016 on induction of hepatic porphyria and drug-metabolizing enzymes were compared in female rats fed 100 ppm or 500 ppm of each. At 1 wk, Aroclor 1242 markedly increased liver weight and all drug-metabolizing pathways tested including cytochrome P-450, liver weight, *N*-demethylase, nitroreductase, aniline hydroxylase, and glucuronyl transferase, while Aroclor 1016 had produced only very minimal effects. At 6 mo, however, 500 ppm of either Aroclor markedly increased drug-metabolism, while at the lower dose, Aroclor 1016 was much less effective than Aroclor 1242. Both doses of Aroclor 1242 produced porphyria, but only the higher dose of Aroclor 1016 was porphyrogenic. The porphyria occurred after a lag of 6 mo and was characterized by excretion and hepatic storage of uroporphyrins. Aroclor tissue concentrations were similar in rats fed equal doses of the two mixtures. Therefore, the marked differences in the biological effects of Aroclor 1016 and Aroclor 1242 cannot be explained by differences in absorption, metabolism, or excretion.

Polychlorinated biphenyls (PCBs) are industrial chemicals which have been widely used and are widespread contaminants of the environment (Risebrough *et al.*, 1968). In the United States, PCBs are sold under the trademark Aroclor. They are mixtures containing various percentages of organically bound chlorine by weight. PCBs have been found in human tissue and were responsible for an outbreak of chloracne in

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[2] Present address: Consumer Products Safety Commission, 1330 W. Peachtree NW, Atlanta, Ga. 30309.

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Japan (Yobbs, 1972; Kuratsune *et al.*, 1972). Chronic exposure to PCBs results in porphyria resembling hexachlorobenzene poisoning, characterized by delayed onset and massive increases in urinary and liver uroporphyrins (Goldstein *et al.*, 1974a). PCBs are also known to induce the activity of the hepatic mixed-function oxidase system which metabolizes foreign chemical and endogenous steroids (Litterst *et al.*, 1972). These authors reported that effects on the microsomal mixed function oxidase system correlated directly with the percent chlorine. It has been reported that biphenyls with higher numbers of chlorine per molecule are retained in tissues for longer periods of time, while biphenyls with fewer numbers of chlorines appear to be preferentially metabolized and excreted (Weigel and Smith, 1974; Curley *et al.*, 1971). Preferential retention of highly chlorinated biphenyls could explain the greater biological effectiveness of highly chlorinated PCB mixtures compared with mixtures with lower percent chlorination.

At present, sale of American-made PCBs is limited largely to uses as dielectric fluids in capacitors and transformers. Currently, the major PCB mixture being marketed in the United States is Aroclor 1016, a mixture containing 41.3% chlorine with a very low percentage (1%) of biphenyls with more than four chlorines per molecule. Only one study on the biological effects of Aroclor 1016 has been published. This study compared the effects of Aroclor 1016 with Aroclor 1254 (a biphenyl mixture containing 54% chlorine) on certain pathways of drug metabolism (cytochrome P-450, ethylmorphine *N*-demethylase, aniline hydroxylase, zoxazolamine, and hexobarbital metabolism) after administration to rats of 25 mg/kg day for 6 days (Hickers *et al.*, 1972). Aroclor 1254 produced up to a 3-fold increase in some drug-metabolizing enzymes, while the maximum increase produced by Aroclor 1016 was 40%. Effects of chronic administration of Aroclor 1016 were not determined. Litterst *et al.* (1972) reported up to threefold increases in nitroreduction of *p*-nitrobenzoic acid and hydroxylation of pentobarbital by Aroclor 1242, a mixture containing approximately 42% chlorine. Because of the reports that, in general, biphenyls with greater numbers of chlorines are excreted, it appeared possible that the lesser effects of Aroclor 1016 could be due to greater metabolism and/or excretion because of its very low content of highly chlorinated biphenyls. In addition, it was not known whether Aroclor 1016 or Aroclor 1242 were less porphyrogenic than other Aroclor mixtures. Therefore, the present study was undertaken to compare the effects of Aroclor 1016 with another chlorinated biphenyl mixture, Aroclor 1242 (with similar chlorine content 42%) but containing 9% biphenyls with five or more chlorines vs 1% for Aroclor 1016. Effects of two relatively high doses of Aroclor 1016 and Aroclor 1242 were compared at both 1 wk and 6 mo on a large variety of drug-metabolizing pathways as well as porphyrin accumulation and excretion. In addition, total PCB content of liver and fat were compared after administration of comparable doses of the two compounds.

METHODS

Thirty 1-mo old female Sherman rats were divided into five groups of six each and fed ground Purina laboratory chow *ad libitum* containing 100 or 500 ppm Aroclor 1016 (lot No. KH-06-75f) (Monsanto Chemical Co., St. Louis, Mo.), or plain chow. Rats were sacrificed by decapitation after 1 wk. An additional 30 rats were treated in a

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similar fashion and sacrificed at 6 mo. Urine was collected in metabolic cages for the 24-hr period preceding sacrifice.

After sacrifice, liver homogenates, 9000 *g* supernates and microsomes were prepared as previously described (Goldstein *et al*., 1974a). δ-Aminolevulinic acid (ALA) synthetase was assayed in whole liver homogenates (Maver *et al*., 1966a). *o*-Aminophenol glucuronyl transferase was assayed in microsomes equivalent to 50 mg wet wt of liver (resuspended in 1.15% KCl) in the presence of 0.3% digitonin and 2.7 mol/ml of UDPGA (Goldstein and Taurog, 1968). Aminopyrine *N*-demethylase, aniline hydroxylase, and nitroreductase were assayed in 9000 *g* supernates (Goldstein *et al*., 1973b). Aniline hydroxylase was assayed in the dark. *N*-demethylase was measured by determination of formaldehyde produced (Cochin and Axelrod, 1959), and aniline hydroxylase by determination of *p*-aminobenzoic acid from *p*-nitrobenzoic acid (Coutts and Brodie, 1957) with the following modification.

Porphyrin in liver fractions from porphyric rats interfered with analysis of products of drug metabolizing enzymes by producing high blanks. In addition, porphyrins interfered specifically with analysis of *p*-aminophenol which is coupled with phenol in alkaline solution to form an indophenol dye and measured spectrophotometrically (Kato and Gillette, 1965). The reaction product was unstable in the presence of porphyric tissue, disappearing variably during the reaction period. Therefore, we routinely removed porphyrin from all protein-free supernatant fractions of drug-metabolizing reactions by the addition of 50 mg talc to the incubation mixtures after the reactions were stopped with acid. The talc, which adsorbed the porphyrins in acid conditions, was precipitated by centrifugation at 2000 *g* for 15 min, and the porphyrin-free supernates were analyzed for *p*-aminophenol, formaldehyde, *p*-aminobenzoic acid, or *o*-aminophenol glucuronide as described above. Recoveries of all compounds were unaffected by talc.

Microsomal cytochrome P-450 and protoheme were determined by the methods of Omura and Sato (1964a,b) using an Aminco DW-2 spectrophotometer. Microsomal protein (Lowry *et al*., 1951), tissue porphyrins (Doss, 1967), urinary porphyrins (Goldstein *et al*., 1974a), and urinary ALA and porphobilinogen (PBG) (Maver *et al*., 1966b) were measured as previously described. Tissue residues of PCBs were analyzed by gas chromatography (GC) using a Micro Tek 415-220 and quantitated by comparing the sum of the peak heights with the sum of total peak heights in the corresponding Aroclor standard (Curley *et al*., 1971).

Results were analyzed by analysis of variance followed by Duncan's multiple range test (Steel and Torrie, 1960).

RESULTS

At 4 wk, 500 ppm of Aroclor 1242 markedly increased cytochrome P-450 (4-fold), *N*-demethylase (2.8-fold), aniline hydroxylase (6.8-fold), nitroreductase (3-fold), glucuronyl transferase (4-fold), and microsomal protoheme (2.6-fold), and produced moderate increases in ALA synthetase (36%) and liver weight (28%) (Fig. 1). In contrast, 500 ppm Aroclor 1016 produced only a 50% increase in most enzymes. Effects of 500 ppm Aroclor 1016 were significantly lower than those of 500 ppm Aroclor 1242 on all parameters except nitroreductase. The smaller dose of 100 ppm Aroclor 1242

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caused significant increases in cytochrome P-450, 12-fold), aniline hydroxylase (2.5-fold), glucuronyl transferase (2-fold), ALA synthetase (22-fold), microsomal protein (60%), and liver weight (9%), while 100 ppm Aroclor 1016 had no effects. The peak of the CO-aldehyde spectrum of cytochrome P-450 was shifted from 450 to 449 nm by Aroclor 1242 treatment but not by Aroclor 1016.

At 6 mo, 500 ppm Aroclor 1242 had lesser effects on cytochrome P-450 and *N*-demethylase than at 1 wk, but aniline hydroxylase, microsomal protein, and liver weight

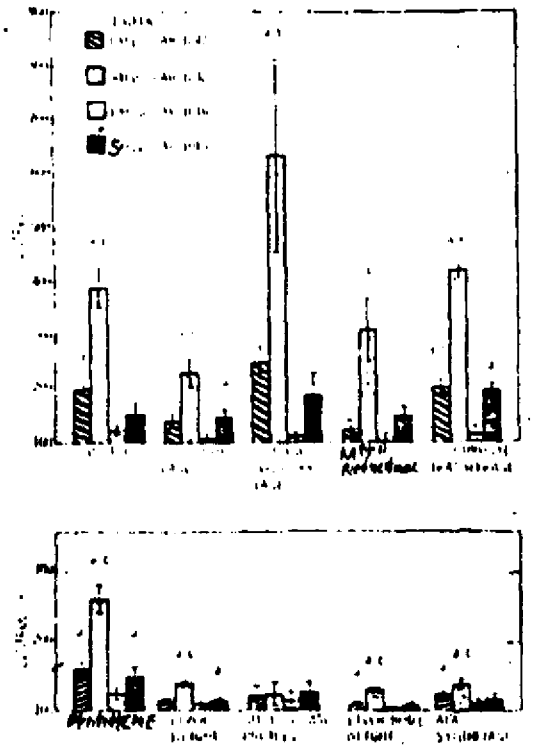

FIG. 1. Effect of Aroclors 1016 and 1242 on cytochrome P-450, drug metabolizing enzymes, porphyrins, liver weight, liver microsomal protein, and ALA synthetase activity at 1 wk. Groups of six female rats were fed 100 or 500 ppm of Aroclors 1016 or 1242 and sacrificed at 1 wk. Values represent means ± SE. a: Significantly greater than controls at $p < 0.05$. b: 100 ppm Aroclor 1242 significantly greater than 100 ppm Aroclor 1016, $p < 0.05$. c: 500 ppm Aroclor 1242 significantly greater than 500 ppm Aroclor 1016, $p < 0.05$.

remained elevated (Fig. 2 vs Fig. 1). In contrast, the effects of Aroclor 1242 on glucuronyl transferase (13-fold vs 4-fold) and ALA synthetase (5- to 9-fold vs 1.3-fold) were much greater at 6 mo than at 1 wk. In addition, the effects of 500 ppm of Aroclors 1016 and 1242 were roughly comparable at 6 mo; 500 ppm Aroclor 1016 caused marked increases in glucuronyl transferase (11-fold), ALA synthetase (7-fold), micro-

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reductase (2-fold), aniline hydroxylase (2-fold), and liver weight (29%) (Fig. 2). However, the effects of 500 ppm Aroclor 1016 were significantly lower than the effects of 500 ppm Aroclor 1242 on aniline hydroxylase, nitroreductase, and glucuronyl transferase. At the low dose of 100 ppm, Aroclor 1016 had relatively slight effects, in

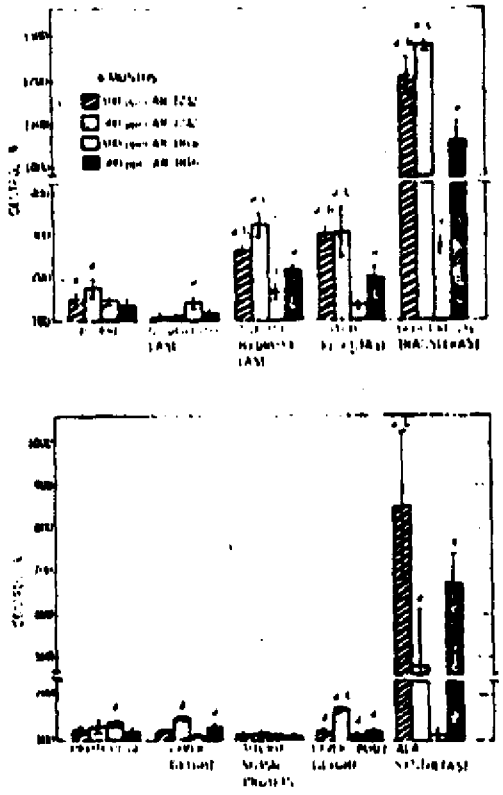

FIG. 2. Effect of Aroclors 1016 and 1242 on cytochrome P-450, drug-metabolizing enzymes, porphyrins, liver weight, liver microsomal protein, and ALA synthetase activity at 6 mo. Groups of six female rats fed 100 or 500 ppm of Aroclors 1016 and 1242 were sacrificed at 6 mo. Values represent means ± SE. a: Significantly greater than controls at $p < 0.05$. b: 100 ppm Aroclor 1242 significantly greater than 100 ppm Aroclor 1016, $p < 0.05$. c: 500 ppm Aroclor 1242 significantly greater than 500 ppm Aroclor 1016, $p < 0.05$.

contrast to the marked effects of Aroclor 1242. These differences were statistically significant for aniline hydroxylase, nitroreductase, glucuronyl transferase, and ALA synthetase.

Weight gain was significantly decreased only by the high dose of Aroclor 1242 at 6 mo (151 ± 11 vs 196 ± 7 g). At 6 mo, the dose of Aroclor consumed was 6 mg/kg/day for rats fed 100 ppm Aroclor 1242 or 1016 and 30 mg/kg/day for rats fed 500 ppm

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Aroclor 1242 or 1016. The uterine horns of most rats on both doses of Aroclors 1016 and 1242 were swollen at 1 wk and markedly swollen at 6 mo.

Porphyria

At 1 wk, neither Aroclor 1016 or Aroclor 1242 had any effects on liver or urinary porphyrins or PBG (Figs. 3 and 4) (Table 1). A small increase in urinary ALA was seen in rats fed 500 ppm Aroclor 1242, but the significance of this increase is questionable. At 6 mo, however, both doses of Aroclor 1242 and 500 ppm 1016 produced large increases in hepatic porphyrins (1000-fold) (Table 1) and urinary uroporphyrins (200-fold) (Fig. 4) and moderate increases in coproporphyrin (8-fold), urinary ALA and PBG (approx. 3- and 10-fold) (Fig. 3). The porphyrin found in the livers of

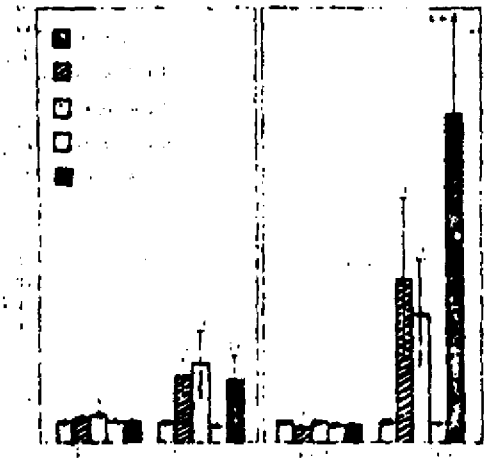

FIG. 3. Effects of Aroclors 1016 and 1242 on urinary ALA and PBG. Rats were treated as described in Figs. 1 and 2, and urine was collected in individual metabolic cages for 24 hr preceding sacrifice. Values represent means ± SE. The numbers above the bars represent the proportion of animals whose individual values were outside the 99% confidence limits of the controls at $p < 0.01$. a - Significantly different from controls, $p < 0.05$.

PCB-treated rats was identified as 8- and 7-carboxyporphyrins. None of the rats fed 100 ppm Aroclor 1016 showed any evidence of increased liver or urinary porphyrins. Analysis of urinary ALA, PBG, and porphyrins at 12 days, 2 mo, 4 mo, and 6 mo showed that porphyrinuria occurred after 2–6 mo. There was no clear indication of a different time course in the development of porphyria in Aroclor 1242 vs Aroclor 1016 treated rats.

Tissue PCB Content

Comparing the GC profile of tissue PCBs from Aroclor 1242 and Aroclor 1016 fed animals with the GC profile of the appropriate Aroclor standard, we found disappearance of a number of peaks, changes in the ratios of the peaks, and possible emergence

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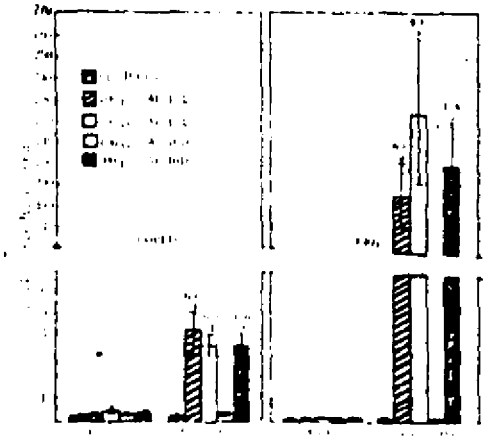

FIG. 4. Effects of Aroclors 1016 and 1242 on urinary coproporphyrin and uroporphyrin excretion. Treatment is described in Fig. 1. Values represent means ± SE. The numbers above the bars represent the proportion of animals whose individual values were outside the 99% confidence limits of the controls at $p < 0.01$. a Significantly different from controls, $p < 0.01$.

TABLE 1

EFFECT OF AROCLORS 1016 AND 1242 ON LIVER PORPHYRINS IN THE RAT*

Treatment	1 Week		6 Months	
	7-COOH	8-COOH	7-COOH	8-COOH
Control	Trace*	Trace*	Trace*	Trace*
100 ppm Aroclor 1242	Trace*	Trace*	>164 ± 27	>534 ± 61
500 ppm Aroclor 1242	Trace*	Trace*	227 ± 59	669 ± 146
100 ppm Aroclor 1016	Trace*	Trace*	<Trace*	<Trace*
500 ppm Aroclor 1016	Trace*	Trace*	89 ± 23	343 ± 65

* Porphyrins were methylated and analyzed by thin-layer chromatography. Female rats were treated as described in Fig. 1. Results are given as the mean ± SE.
* <1 μg/g.

of additional late-eluting peaks (Figs. 5 and 6). The GC residue profiles of all three tissues from Aroclor 1242 fed rats contained 11 major peaks, while those from Aroclor 1016 fed rats contained five major peaks. The total PCB content was similar in liver and fat of animals fed comparable doses of Aroclor 1242 or Aroclor 1016 (Fig. 7). In particular, rats fed 100 ppm of either Aroclor for 6 mo had comparable liver, blood, and fat PCB concentrations despite markedly different biological effects. At the high dose of 500 ppm, the PCB content of livers from rats fed Aroclor 1242 for 1 wk was significantly higher than liver PCB residues in rats fed Aroclor 1016; however, fat content was comparable.

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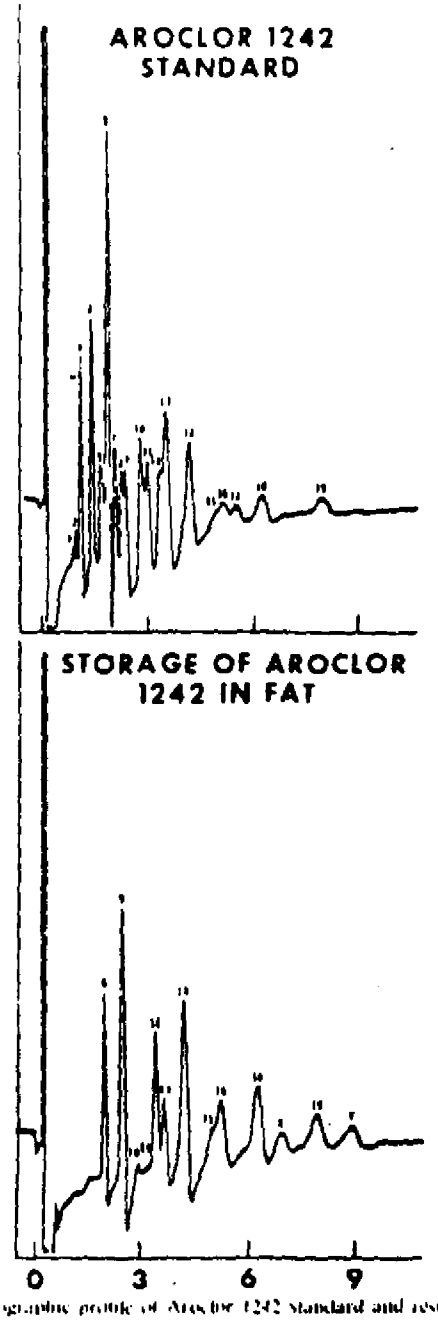

FIG. 5. Gas chromatographic profile of Aroclor 1242 standard and residue from fat of rats fed 100 ppm Aroclor 1242 for 6 mo. Peaks were numbered consecutively according to retention time. Two peaks labeled 7 and 9 were found in extracts of tissues from Aroclor 1242-fed animals but not in the Aroclor 1242 standard.

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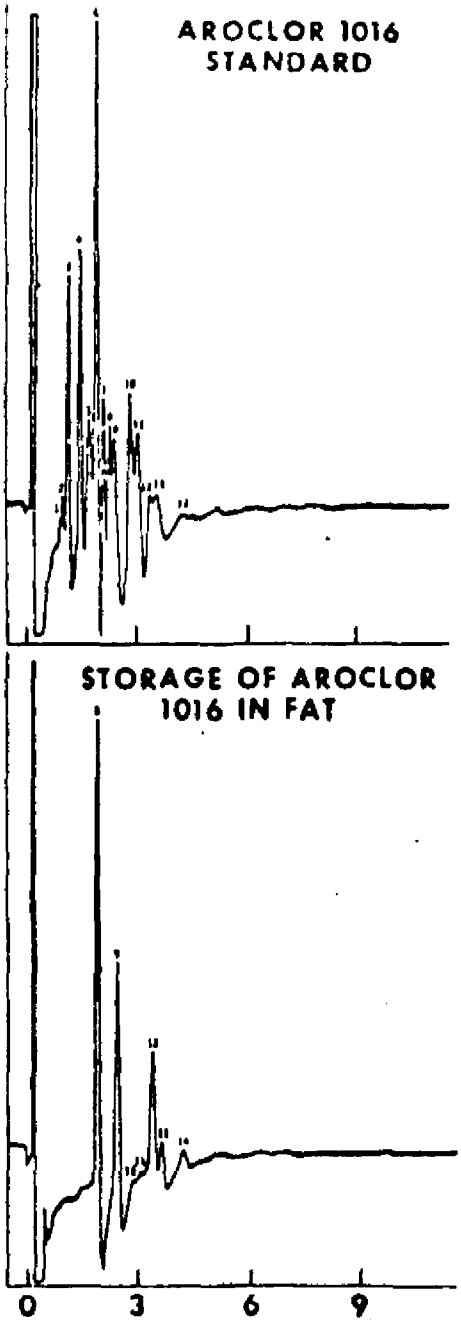

FIG. 6. Gas chromatographic profile of Aroclor 1016 standard and residue from fat of rats fed 100 ppm Aroclor 1016 for 6 mo. Peaks were numbered consecutively according to retention time

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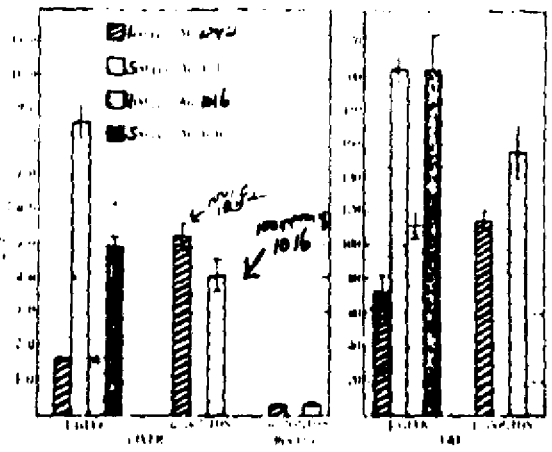

FIG. 7. Tissue PCB content of female rats fed Aroclor 1016 or 1242. Rats were treated as in Fig. 1. Values represent means ± SE. a = 500 ppm Aroclor 1016 significantly different from 500 ppm Aroclor 1242, b = 100 ppm Aroclor 1016 significantly different from 500 ppm Aroclor 1242.

DISCUSSION

We show that Aroclor 1242 markedly increases liver weight, cytochrome P-450, *N*-demethylase, aniline hydroxylase, nitroreductase, and glucuronyl transferase activity when fed to female rats for 1 wk, while Aroclor 1016 has only very slight effects on liver drug-metabolizing enzymes and no effect on liver weight. Bickers *et al.* (1972) similarly found only 40% increases in cytochrome P-450 and ethylmorphine *N*-demethylase and no increases in liver weight after six daily doses of Aroclor 1016 (25 mg/kg/day). However, he compared Aroclor 1016 with Aroclor 1254, a mixture with higher chlorine content, and concluded that the lesser effects of Aroclor 1016 probably related to the lower chlorine content. Similarly, Litterst *et al.* (1972) showed that the potency of PCB mixtures as inducers of drug-metabolizing enzymes increased with increasing percentage of chlorination. We show that Aroclor 1016 is much less effective than Aroclor 1242, despite similar chlorine content. Therefore, the ability of PCBs to induce drug-metabolizing enzymes does not depend simply on percentage of chlorination. Aroclor 1242 may be a more potent inducer than Aroclor 1016 because it contains a higher concentration of penta- and hexachlorobiphenyls, which may be the most active inducers in the mixture.

In contrast to its minimal effects at 1 wk, 500 ppm Aroclor 1016 markedly increased glucuronyl transferase, aniline hydroxylase and nitroreductase activity as well as liver weight and caused porphyria at 6 mo. These results were completely unexpected in view of the earlier study of Bickers *et al.* (1972) which reported minimal effects of Aroclor 1016 on drug metabolism and liver weight, apparently because they studied only a 6 day dosage period. The present study demonstrates that the effects of chronic exposure cannot be predicted by short-term dosage. In particular, the porphyria produced by Aroclor 1016 could not have been predicted on the basis of short-term studies. The effects of Aroclor 1242 also differed with length of administration. Some

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effects were less noticeable at 6 mo than at 1 wk; other pathways were increased comparably or even more markedly after long-term administration. We have previously noted that effects of piperonyl butoxide on glucuronyl transferase were much more pronounced after 2 mo, while most other drug-metabolizing enzymes were maximally increased after 1 wk (Goldstein *et al.*, 1973b).

Aroclor 1242 shifted the maximum of the CO-difference spectrum from 450 to 449 nm, but Aroclor 1016 had no discernable effect, possibly because Aroclor 1016 increased cytochrome P-450 content only minimally. A similar shift of the CO-difference spectrum and an increase in the 455:430 ratio of the peaks of the ethyl isocyanide difference spectrum has been noted after Aroclor 1254 administration (Alvares *et al.*, 1973). These changes are similar to those produced by polycyclic hydrocarbons; however, PCBs, unlike polycyclic hydrocarbons, stimulate a variety of pathways.

Both Aroclors 1016 and 1242 produced swelling of the uterine horns. DDT and PCBs with less than 54% chlorination have been reported to have estrogenic effects on the rat uterus (Bitman and Cecil, 1970). Aroclor 1016 appears at least as effective as Aroclor 1242 in the present study. At present, no studies of the effects of Aroclor 1016 on reproduction have been reported.

Previously, we showed that 100 ppm Aroclor 1254 increased urinary excretion and hepatic accumulation of uroporphyrins and induced ALA synthetase, the rate-limiting step in heme synthesis, after a lag of several months (Goldstein *et al.*, 1974a). Both 100 ppm and 500 ppm Aroclor 1242 produced similar effects after 6 mo, while only the high dose of Aroclor 1016 was effective. Apparently, the lag in development of PCB-induced porphyria did not depend on greater tissue accumulation of PCBs with time, since rats fed 100 ppm Aroclor 1242 for 6 mo were porphyric, while rats fed 500 ppm Aroclor 1242 were not porphyric at 1 wk despite higher tissue PCB levels. A similar unexplained lag has been observed in hexachlorobenzene-induced porphyria (Ockner and Schmid, 1961), in contrast to porphyrogenic drugs such as allylisopropylacetamide which increase excretion of porphyrins and their precursors within hours (De Matteis and Prior, 1962).

The lesser effects of Aroclor 1016 than 1242 could not be explained by greater metabolism and excretion, since tissue concentrations were comparable. Other workers in our laboratory found plasma, kidney, brain, liver, and fat content of PCBs were generally higher in rats fed 100 ppm Aroclor 1016 than in rats fed Aroclor 1242 for 0.5, 1, 2, 4, 6, 8, or 10 mo (Burse *et al.*, 1974). It is probable that induction of liver enzymes and hepatic porphyria by PCBs depends on both the number and position of chlorine atoms on the PCB molecule. Significantly, Bush *et al.* (1974) found high correlation between occupation of the 4,4' positions, tissue retention, cytochrome P-450, and aniline hydroxylase activity after administration of Aroclor 1254. Compounds with one or both *p*-positions open were not persistent in tissue.

Two European commercial preparations of PCBs have been reported to be contaminated with 5–20 ppm of 2,3,7,8-tetrachlorodibenzofuran (TCDF) (Vos *et al.*, 1970). The related compound, 2,3,7,8-tetrachlorodibenzodioxin (TCDD), induces ALA synthetase in the chick embryo at doses as low as 0.5 *pg*/kg (Poland and Glover, 1973), induces hepatic porphyria in the mouse at doses of 100 *pg*/kg (Goldstein *et al.*, 1973a), and increases hepatic cytochrome P-450, glucuronyl transferase, and a number of drug-metabolizing enzymes at doses as low as 1 *pg*/kg (Lucier *et al.*, 1973). Vos

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et al. (1970) could not detect TCDF in American-made PCBs at a sensitivity limit of 1 ppm, but Curley *et al.* (1972) reported contamination of Aroclor 1254 with a trace of TCDF (less than 1 ppm). TCDD was not detected. It is doubtful whether contamination of Aroclors with less than 1 ppm TCDF could attribute appreciably to the hepatic effects of PCBs, since a number of synthetically prepared hexachlorobiphenyls induce ALA synthetase, cytochrome P-450, glucuronyl transferase, and porphyria in the chick, while TCDF does not induce porphyria, even at lethal doses, has little effect on cytochrome P-450, and no effect on glucuronyl transferase or ALA synthetase (Goldstein *et al.*, 1974b). Similarly, TCDF did not induce porphyria in the mouse at 40-times the porphyrogenic dose of TCDD under identical conditions (Goldstein *et al.*, 1974b). Therefore, it is probable that differences in the effects of various PCB mixtures on the liver are due to the effectiveness of the various chlorinated biphenyl homologs in each mixture. Aroclor 1016 is less potent in its effects on the liver, probably because it contains fewer of the biphenyls which are the most active inducers of liver drug-metabolizing enzymes.

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