

Effects of Chemically Pure Chlorobiphenyls on the Morphology of Rat Liver¹

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Effects of Chemically Pure Chlorobiphenyls on the Morphology of Rat Liver. HANSELL, M. M. AND ECOBICHON, D. J. (1974). *Toxicol. Appl. Pharmacol.* 28, 418-427. Pure biphenyl and a series of isomerically pure mono-, di-, tetra-, hexa- and octa-chlorobiphenyls of known chemical composition were injected ip (50 mg/kg) into young male Wistar rats for 3 consecutive days and the rats were killed 4 days after the last injection. In a second experiment, rats were injected ip with 4-mono, 4,4'-di- and 2,5,2',5'-tetrachlorobiphenyl (100 mg/kg) for 7 days. The effects of the pure PCBs were compared with those caused by *o,p'*- and *p,p'*-DDT and Aroclor 1254 and 1260. Samples of liver were examined by light and electron microscopy. The major alterations observed within the hepatocytes were marked proliferation of smooth endoplasmic reticulum and increased numbers of microbodies and lipid droplets. Biphenyl and 2,2'-dichlorobiphenyl produced few morphologic effects whereas 4,4'-di-, 2,5,2',5'- and 3,4,3',4'-tetrachlorobiphenyl caused more changes. The highly chlorinated hexa- and octachlorobiphenyls produced morphological changes that were comparable to those produced by DDT, and Aroclors 1254 and 1260. The degree of ultrastructural alteration appeared to be related directly to the number of chlorine atoms and the presence of chlorine in the 4- or 4'-position.

The structure of the liver of several species of animals treated with polychlorinated biphenyls (PCBs) has been examined using light and electron microscopy. With one exception (Vos and Notenboom-Ram, 1972), all these studies have employed commercially available mixtures such as Aroclors, Phenoclers, and Kaneclors (Bennett *et al.*, 1938; von Wedel *et al.*, 1943; Miller, 1944; Nishizumi, 1970; Kimbrough *et al.*, 1972; Norback and Allen, 1972; Koller and Zinkl, 1973; Bruckner *et al.*, 1973). Some of these commercial mixtures were reported to be contaminated with impurities such as chlorinated dibenzofurans and dibenzo-*p*-dioxins (Vos *et al.*, 1970; Sissons and Welti, 1971; Zitko and Choi, 1971; Greig, 1972; Hutzinger *et al.*, 1972; Webb and McCall, 1972). Hammond (1972), in summarizing the relationship between toxicity and degree of chlorination of PCBs based on studies using commercial mixtures, found no consistent relationship valid for different species and routes of exposure. Tucker and Crabtree (1970) suggested that sublethal effects were directly correlated with chlorine content, while lethal effects were inversely correlated.

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To eliminate the effects of possible contaminants in commercial mixtures and to determine the relationship between position and degree of chlorination, and effects on the liver, a series of isomerically pure chlorobiphenyl compounds of known position and degree of chlorination was administered ip to rats. The influence of these compounds on functionally diverse hepatic drug-metabolizing enzymes and hepatic morphology were studied. The effects on various drug-metabolizing enzymes are reported elsewhere (Johnstone *et al.*, 1974).

METHODS

The methods for the preparation of the pure chlorobiphenyl compounds and the doses administered were described in detail by Hutzinger *et al.* (1971) and Johnstone *et al.* (1974). The compounds² used are listed in Table 1. A group of 7 young male Wistar rats (Woodlyn strain) weighing 50-65 g was treated ip with each agent dissolved in peanut oil, at a dosage of 50 mg/kg daily for 3 days. The rats were killed with an overdose of CHCl_3 4 days after the final injection following the determination of pentobarbital sleeping time. Control rats were treated with equivalent volumes of peanut oil and were killed by CHCl_3 overdose after pentobarbital sleeping time was performed. In a second experiment, 3 compounds (4-mono-, 4,4'-di- and 2,5,2',5'-tetrachlorobiphenyl) were injected ip at a dosage of 100 mg/kg/day for 7 days, the rats being killed 24 hr after the last injection by an overdose of CHCl_3 .

After euthanasia, the abdomen of the rats was quickly opened and samples of the liver were removed for fixation for light and electron microscopy. For light microscopy, samples were fixed in 10% buffered formalin and embedded in paraffin; sections were stained with hematoxylin and eosin. For electron microscopy, 1-mm cubes of liver were fixed in 1% osmium tetroxide in sodium cacodylate buffer at 0°C for 2 hr, dehydrated in a series of graded alcohol solutions and propylene oxide and embedded in Spurr low viscosity embedding medium. Ultrathin sections were cut on a Porter-Blum MT2B ultramicrotome stained with uranyl acetate and lead citrate and examined with a Philips 200 electron microscope. Thick plastic sections were cut from the same block and stained with methylene blue and azure A for light microscopy (Pease, 1964).

RESULTS

The results of ultrastructural evaluation and the hepatic weights for the rats treated with the agents at 50 mg/kg for 3 days are presented in Table 1. Hepatic weights were significantly increased following treatment with *p,p'*-DDT, Aroclor 1254, Aroclor 1260 and 2,3,5,2',3',5'-hexachlorobiphenyl. These compounds also induced major alterations in hepatocyte ultrastructure.

Hepatocytes of treated rats were compared with hepatocytes from control, vehicle-treated animals for the presence of these alterations. The grading system for alterations in amounts of smooth endoplasmic reticulum (SER), and numbers of lipid droplets and

² Abbreviations used: *o,p'*-DDT, 1,1,1-trichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethane; *p,p'*-DDT, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane. The nomenclature of the polychlorinated biphenyl (PCB) compounds follows the accepted form, the position of chlorines on one phenyl being indicated by numbers from 2 to 6, while chlorine positions on the second ring are designated by a number with a superscript prime, i.e., 2,2'-dichlorobiphenyl.

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microbodies is based on a comparison of the amounts present in any cell with the amount present in a control hepatocyte (value = +) and a hepatocyte having the maximum amount of change (value = ++++). The grading system reflects only relative numbers since morphometric techniques were not used for quantitation. The major ultrastructural alterations induced by the various pure chlorobiphenyls were proliferation of SER, alteration of the rough endoplasmic reticulum (RER) and increased numbers of lipid droplets and microbodies (Table 1).

TABLE 1
CHANGES IN LIVER WEIGHT, HEPATIC SMOOTH ENDOPLASMIC RETICULUM,
LIPID DROPLETS AND MICROBODIES, FOLLOWING THE ADMINISTRATION OF DDT,
AROCLOR 1254 AND 1260 AND ISOMERICALLY PURE CHLOROBIPHENYLS TO RATS^a

Compound	Liver weight ^b (g)	Smooth ER ^d	Lipid droplets	Micro- bodies
Vehicle-Treated	4.77 ± 1.14	+	+	+
<i>o,p'</i> -DDT	4.91 ± 0.46	++++	+++	++++
<i>p,p'</i> -DDT	5.59 ± 0.89 ^c	++++	+++	++++
Aroclor 1254	6.23 ± 0.30 ^c	++++	++++	++++
Aroclor 1260	6.15 ± 0.49 ^c	+++	+++	++++
Biphenyl (BP)	4.87 ± 0.11	+	++	++
4-Chloro BP	4.76 ± 0.69	++	+++	+++
2,2'-Dichloro BP	4.85 ± 0.96	+	+	+
2,4'-Dichloro BP	4.69 ± 0.34	+++	++	+++
4,4'-Dichloro BP	4.21 ± 0.44	+++	++	+++
2,5,2',5'-Tetrachloro BP	4.68 ± 0.69	++	+	++
2,4,2',4'-Tetrachloro BP	5.03 ± 0.48	+++	++	+++
3,4,3',4'-Tetrachloro BP	4.73 ± 0.47	++	+++	+++
2,4,5,2',4',5'-Hexachloro BP	4.18 ± 0.91	++++	++	+++
2,4,6,2',4',6'-Hexachloro BP	3.82 ± 1.29	++++	++	+++
2,3,5,2',3',5'-Hexachloro BP	5.80 ± 0.78 ^c	++++	++	+++
2,3,4,5,2',3',4',5'-Octachloro BP	4.53 ± 1.46	++++	++	+++

^a Animals were injected ip with a peanut oil solution at a dose of 50 mg/kg for 3 days and killed 4 days after the last injection.

^b Values presented are mean weights ± SD of 14 (vehicle-treated) and 7 (agent-treated) animals per group. Values are based on wet tissue weight.

^c Values are statistically significant from control weights at a probability level $p < 0.05$.

^d See text (Results) for grading system.

Isomerically pure hexa- and octa-chlorobiphenyls, administered at a concentration of 50 mg/kg for 3 consecutive days, caused alterations in hepatic morphology that were visible with the light microscope (Fig. 1). The changes included large numbers of hepatocytes with vacuolated cytoplasm and small foci of necrosis involving 5 or 6 cells. The necrotic foci were particularly numerous at the periphery of the lobule. Treatment with 4-mono-, 4, 4'-di and 2,5,2',5'-tetrachlorobiphenyl at 100 mg/kg for 7 days resulted in larger numbers of necrotic foci, centrilobular necrosis and proliferation of biliary ductules (Fig. 1). The lesions were most severe in rats given 4,4'-dichlorobiphenyl for 7 days.

Hepatocytes of rats treated with *o,p'*-, or *p,p'*-DDT (Fig. 2A) had increased amounts of SER in the form of vesicles (S) which tended to accumulate at the periphery of the

cell. There was a reduction in the amount of RER (R) which often displayed expanded or sinuous cisternae. The number of lipid droplets and microbodies (M) was increased. Hepatocytes from rats given the commercial Aroclors were similar to those from rats treated with DDT. Aroclor 1254 treatment (Fig. 2B) caused more pronounced alterations than Aroclor 1260 treatment. Hepatocytes of treated animals contained very abundant SER, reduced RER, increased numbers of microbodies (M) and lipid droplets (L).

Hepatocytes from rats treated with pure PCB isomers for 3 days were compared with hepatocytes from control rats and rats treated with the commercial Aroclors and DDT

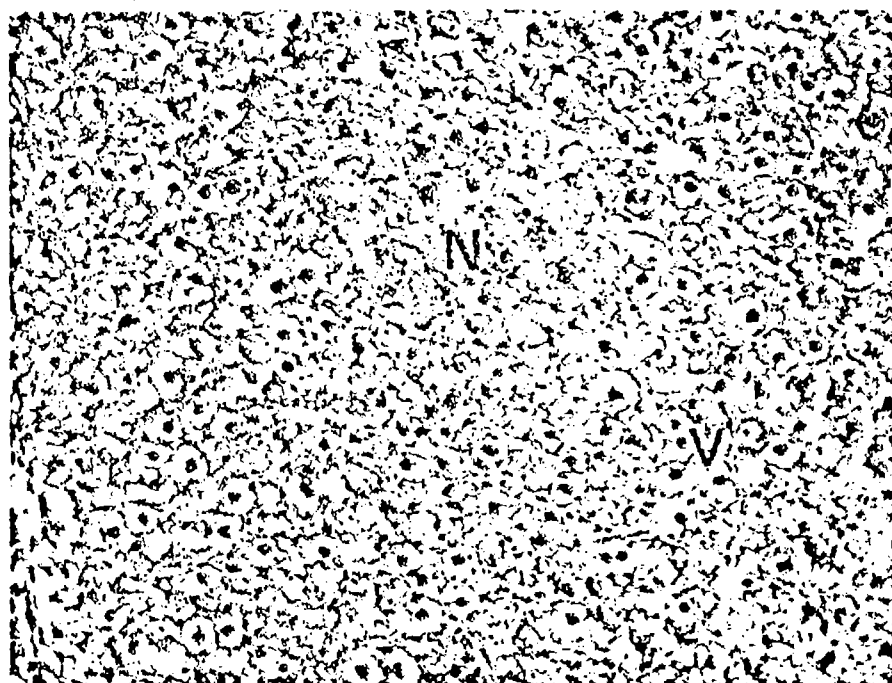


FIG. 1. Liver from a rat treated with 50 mg/kg/day of 4-chlorobiphenyl for 7 days showing hepatocytes with vacuolated cytoplasm (V) and a small area of necrosis (N). Hematoxylin and eosin. $\times 285$.

at the same dosage. The amount of hepatic smooth endoplasmic reticulum in rats treated with biphenyl and 2,2'-dichlorobiphenyl was comparable to that in control rats; but in rats treated with 4-mono-, 4,4'-di-, 2,5,2',5'- and 3,4,3',4'-tetra-chlorobiphenyl, the hepatocytes contained increased amounts of SER. All other compounds tested (2,4'-di-, 2,4,2',4'-tetra-, 2,4,5,2',4',5'-, 2,4,6,2',4',6'- and 2,3,5,2',3',5'-hexa- and 2,3,4,5,2',3',4',5'-octa-chlorobiphenyl) caused increases in the amounts of SER that were equivalent to the increases observed in hepatocytes of rats treated with DDT or Aroclor 1260 (Fig. 3A). The RER often had sinuous cisternae (Fig. 3B) and was reduced in amount in hepatocytes of rats treated with hexa- or octachlorobiphenyl (Fig. 3C). The highly chlorinated chemicals and those possessing a chlorine in the 4 and/or 4' position caused the greatest increases in the numbers of microbodies and lipid inclusions (Fig. 3C). Lesser effects were observed following treatment with biphenyl, 2,2'-di- and

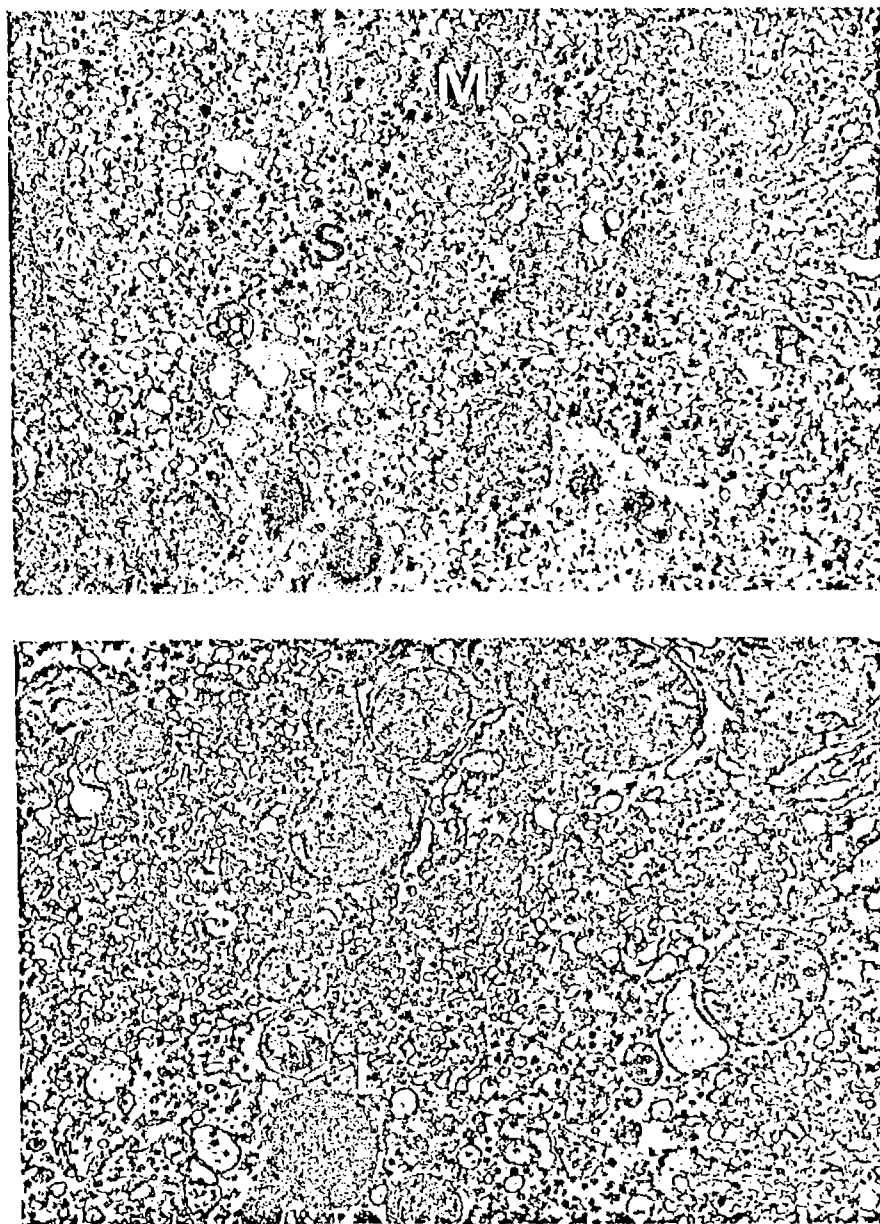


FIG. 2. Hepatocytes from rats treated with 50 mg/kg/day of *p,p'*-DDT (A) and Aroclor 1254 (B) for 3 days showing proliferation of smooth endoplasmic reticulum (S), expanded cisternae of rough endoplasmic reticulum (R) and microbodies (M) and lipid droplets (L). A, $\times 12,000$; B, $\times 14,000$.

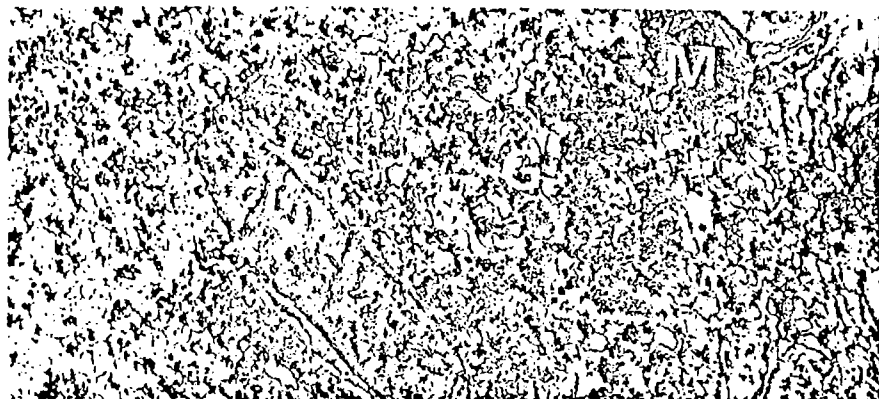


FIG. 3. Hepatocytes from different rats treated with isomerically pure chlorobiphenyls at concentrations of 50 mg/kg/day for 3 days. (A) Treated with 2,4,2',4'-tetrachlorobiphenyl, showing abundant smooth endoplasmic reticulum (S), glycogen (G) and a microbody (M) $\times 25,675$. (B) Treated with 3,4,3',4'-tetrachlorobiphenyl, showing sinuous cisternae of rough endoplasmic reticulum (R) surrounding a mitochondrion (M). $\times 23,500$. (C) Treated with 2,3,5,2',3',4'-hexachlorobiphenyl, showing proliferation of smooth endoplasmic reticulum (S), microbodies (M) and lipid droplets (L), $\times 14,100$.

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2,5,2',5'-tetrachlorobiphenyl. The number of lipid inclusions in hepatocytes was never as great as that observed following treatment with DDT and the Aroclors.

The administration of 4-mono-, 4,4'-di- and 2,5,2',5'-tetra-chlorobiphenyl at a dosage of 100 mg/kg/day for 7 days caused more pronounced alterations than those observed after treatment for 3 days (Fig. 4). The 4,4'-dichlorobiphenyl caused more drastic

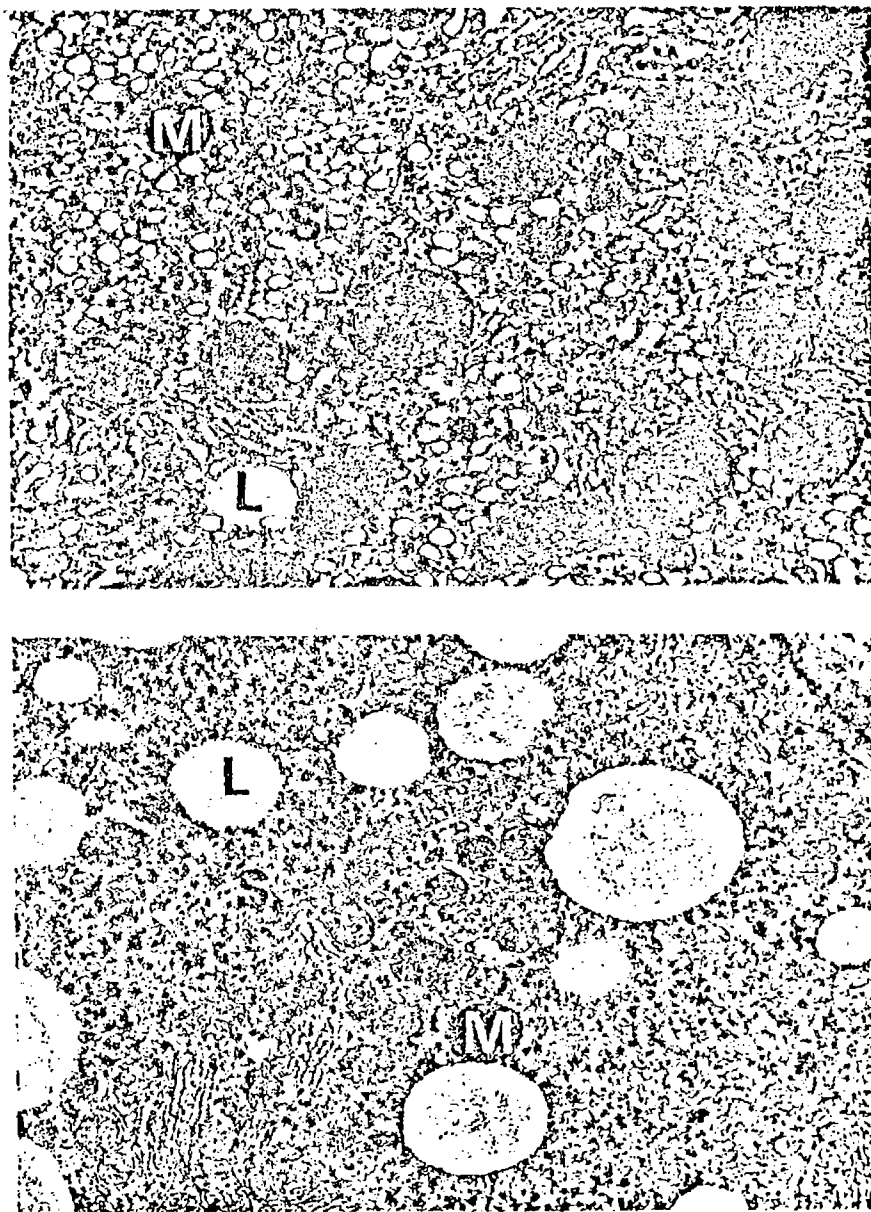


FIG. 4. Hepatocytes from rats treated with 100 mg/kg/day of 4,4'-dichlorobiphenyl for 7 days show abundant smooth endoplasmic reticulum (S), microbodies (M) and lipid droplets (L). A, $\times 14,100$; B, $\times 8580$.

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alterations than 4-monochlorobiphenyl or 2,5,2',5'-tetrachlorobiphenyl, the effects of the latter two agents being approximately the same.

DISCUSSION

Proliferation of SER is a response of the hepatocyte to a wide variety of drugs and poisons and is considered to be a structural reflection of the enhanced metabolism of foreign lipophilic compounds. The increase in SER can be related to increased amounts of drug metabolizing enzymes within the liver (Vos and Notenboom-Ram, 1972). In all previous studies, administration of PCBs has resulted in increased amounts of SER in hepatocytes (Nishizumi, 1970; Kimbrough *et al.*, 1972; Vos and Notenboom-Ram, 1972; Norback and Allen, 1972; Koller and Zinkl, 1973). In this study, the proliferation of SER was closely related to the structural characteristics of the compound used. Biphenyl and 2,2'-dichlorobiphenyl appeared not to induce extensive proliferation of SER, but with the other compounds, the proliferation appeared to be related to the degree of chlorination and particularly to the presence of a chlorine atom in the 4 and/or 4' position (Table I). The morphological alterations correlated well with the activities of drug-metabolizing enzymes within the same rats (Johnstone *et al.*, 1974). When commercial mixtures were employed, mixtures having a greater chlorine content (Aroclors 1254, 1260) had greater enzyme-inducing effects and caused more marked morphological changes than those having lower chlorine content (Villeneuve *et al.*, 1971; Bickers *et al.*, 1972; Koller and Zinkl, 1973). The more highly chlorinated compounds may have greater effect because metabolic studies show that low chlorine analogs are eliminated from tissues more rapidly than the more highly chlorinated compounds (Grant *et al.*, 1971; Bailey and Bunyan, 1972; Bickers *et al.*, 1972; Rehfeld *et al.*, 1972).

In this study and the report of Kimbrough *et al.* (1972), administration of Aroclor 1254 resulted in more severe morphological alterations than Aroclor 1260 or any of the pure compounds used. It was also a more powerful inducer of drug-metabolizing enzymes than Aroclor 1260 (Johnstone *et al.*, 1974). Aroclor 1254 was not the most highly chlorinated compound used, and the response to its administration was inconsistent with the view that increased chlorine content caused greater alterations. The failure of Aroclor 1254 to follow the rule of increased tissue response with increased chlorination might be due to the particular combination of chlorobiphenyls present in the mixture or possibly to the presence of contaminants. Chlorinated dibenzofurans and dibenzo-*p*-dioxans have been found in some commercial PCB mixtures and these compounds caused alterations in hepatocyte morphology that were similar to those caused by PCBs (Vos *et al.*, 1970; Zitko and Choi, 1971; Greig, 1972; Hutzinger *et al.*, 1972; Webb and McCall, 1972; Norback and Allen, 1972). Such contaminants are not reported to be present in Aroclor 1260 (Vos *et al.*, 1970). Kimbrough *et al.* (1972) stated that a preliminary analysis of the lot of Aroclor 1254 used in their study suggested possible contamination with a chlorinated dibenzofuran. It is likely, therefore, that increasing chlorination of the biphenyl nucleus does lead to proliferation of SER and to increased activities of drug-metabolizing enzymes. The enhanced effect observed with Aroclor 1254 can be due to some other factor than chlorination.

Treatment of rats with 4-chloro-, 4,4'-dichloro- and 2,5,2',5'-tetrachloro-biphenyl at a dosage of 100 mg/kg/day for 7 days resulted in marked proliferation of SER. The

dosages, modes of administration and duration of exposure have varied greatly in published reports of the morphological alterations produced by PCBs. With the exception of the study by Kimbrough *et al.* (1972), most studies have used much higher concentrations than the ones used in this study. Most authors have noted increased hepatic weight and hepatic cell size. In our study, the increase in hepatic weight was significant only after treatment with *p,p'*-DDT, Aroclors 1254 and 1260, and 2,3,5,2',3',5'-hexachlorobiphenyl. There was no significant increase in cell size. This result may be due to the short exposure time and relatively low doses used.

Concentric membrane arrays or whorls within the cytoplasm of hepatocytes have been noted in several studies (Nishizumi *et al.*, 1970; Norback and Allen, 1972; Koller and Zinkl, 1973). Their formation may be species-related as such structures were found in rats and mice, rarely in rabbits but not in monkeys (Norback and Allen, 1972; Nishizumi, 1970; Vos and Notenboom-Ram, 1972). One such array was observed in a hepatocyte of a rat treated with 2,4,2',4'-tetrachlorobiphenyl. Many of the pure chlorobiphenyls as well as DDT and the Aroclors resulted in a rearrangement of the rough endoplasmic reticulum into sinuous arrays which Norback and Allen (1972) believe to be the first stage of whorl production. Hepatocytes from treated rats contain increased numbers of lipid droplets, many of which were large. Norback and Allen (1972) noted that such lipid droplets were often incorporated within the membrane arrays. They suggested that the membrane arrays provided sites for storage of lipid droplets that contain lipophilic exogenous, toxic compounds, such segregation would maximize exposure to degradative enzymes in the membranes.

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