

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 ECTORICHON, 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, Phenoclor, 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; Saxons 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 I. 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 I. 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-(*p*-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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AROCOR 1254 AND 1260 AND ISOMERICALLY PURE CHLOROBIPHENYLS TO RATS^a

Compound	Liver weight ^b (g)	Smooth ER ^c	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 ^d	++++	+++	++++
Aroclor 1254	6.23 ± 0.30 ^e	+++++	++++	++++
Aroclor 1260	6.15 ± 0.49 ^e	+++	+++	+++
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 ^e	++++	++	+++
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^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.

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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 1). 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

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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 1). 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

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- HUTZINGER, O., SAIF, S. AND ZITKO, V. (1971). Polychlorinated biphenyls: synthesis of some individual chlorobiphenyls. *Bull. Environ. Contam. Toxicol.* 6, 209-219.
- HUTZINGER, O., NASH, D. M., SAIF, S., DEFREITAS, A. S. W., NORSTROM, R. J., WILDISH, D. J. AND ZITKO, V. (1972). Polychlorinated biphenyls: Metabolic behavior of pure isomers in pigeons, rats and brook trout. *Science* 178, 312-314.
- JONKATONE, G. J., ECOMICHON, D. J. AND HUTZINGER, O. (1974). The influence of pure polychlorinated biphenyl compounds on hepatic function in the rat. *Toxicol. Appl. Pharmacol.* 28, 66-81.
- KIMBROUGH, R. D., JINDER, R. E. AND GAINES, T. B. (1972). Morphological changes in livers of rats fed polychlorinated biphenyls. *Arch. Environ. Health* 25, 354-364.
- KOLLER, L. D. AND ZINKL, J. G. (1973). Pathology of polychlorinated biphenyls in rabbits. *Amer. J. Pathol.* 70, 363-373.
- MILLER, J. W. (1944). Pathologic changes in animals exposed to a commercial chlorinated biphenyl. *U.S. Public Health Rep.* 59, 1085-1093.
- NISHIZUMI, M. (1970). Light and electron microscope study of chlorobiphenyl poisoning in mouse and monkey liver. *Arch. Environ. Health* 21, 620-632.
- NORBACK, D. J. AND ALLIN, J. R. (1972). Chlorinated aromatic hydrocarbon induced modification of hepatic endoplasmic reticulum: Concentric membrane arrays. *Environmental Health Perspectives* 1, 137-143.
- PIANI, D. C. (1964). *Histological Techniques for Electron Microscopy*. Academic Press, New York.
- RIMMELD, B. M., BRADLEY, R. L. AND SUNDE, M. L. (1972). Toxicity study of polychlorinated biphenyls in the chick. 2. Biochemical effects and accumulation. *Poultry Sci.* 51, 488-493.
- SIMONS, D. AND WELT, D. (1971). Structural identification of polychlorinated biphenyls in commercial mixtures by gas-liquid chromatography, nuclear magnetic resonance and mass spectrometry. *J. Chromatogr.* 60, 15-32.
- TUCKER, R. K. AND CRANFILL, D. G. (1970). In: *Handbook of Toxicity of Pesticides to Wildlife*. U.S. Dept. of Interior, Bureau of Sport Fisheries and Wildlife. Pub. 84.
- VILLIENOT, D. C., GRANT, D. L., PHILLIPS, W. E. J., CLARK, M. L. AND CLEGG, D. J. (1971). Effects of PCB administration on microsomal enzyme activity in pregnant rabbits. *Bull. Environ. Contam. Toxicol.* 6, 120-128.
- VOS, J. G. AND KUFMAN, J. H. (1970). Comparative toxicologic study with polychlorinated biphenyls in chickens with special reference to porphyria, edema formation, liver necrosis, and tissue residues. *Toxicol. Appl. Pharmacol.* 17, 656-668.
- VOS, J. G. AND NOTENBOOM-RAM, E. (1972). Comparative toxicity study of 2,4,5,2',4',5'-hexachlorobiphenyl and a polychlorinated biphenyl mixture in rabbits. *Toxicol. Appl. Pharmacol.* 23, 563-577.
- VOS, J. G., KUFMAN, J. H., VAN DER MAAS, H. L., TEN NOEVER DE BRAUW, M. G. AND DE VOS, R. H. (1970). Identification and toxicological evaluation of chlorinated dibenzofuran and chlorinated naphthalene in two commercial polychlorinated biphenyls. *Food Cosmet. Toxicol.* 8, 625-655.
- WIBB, R. G. AND MCCALL, A. C. (1972). Identities of polychlorinated biphenyl isomers in Aroclors. *J. Ass. Offic. Anal. Chem.* 55, 746-752.
- VON WILDI, H., HOLLA, W. A. AND DENTON, J. (1943). Observations on the toxic effects resulting from exposure to chlorinated naphthalene and chlorinated phenyls with suggestions for prevention. *Rubber Age* 53, 419-425.
- ZITKO, V. AND CHOI, P. M. K. (1971). PCB and other industrial halogenated hydrocarbons in the environment. *Fish. Res. Bd. Can. Tech. Rep.* 272, 1-64.