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Marked differences in the inductive effects of two symmetrical hexachlorobiphenyls and the corresponding unsymmetrical isomer on hepatic monooxygenases

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Polychlorinated biphenyls (PCBs) produce a mixed type of induction of hepatic mixed-function oxidases similar to that produced by a mixture of the two classical types of inducers, phenobarbital and 3-methylcholanthrene (3-MC) [1]. The cytochromes induced by PCBs are catalytically, spectrally, electrophoretically, and immunologically identical to those induced by combination of phenobarbital and 3-MC (generally referred to as cytochromes P-450 and P-448 respectively) [2]. Individual isomers, however, have been separated into two groups: those which induce cytochrome(s) P-450 (phenobarbital-type), and those which induce cytochrome P-448 (3-MC-type induction) [3, 4].

Isomers that induce cytochrome P-448 are isosteric with the very potent inducer, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). They contain halogens in the *meta* and *para* positions of both rings (3,4,3′,4′-tetra-; 3,4,5,3′,4′-penta-; and 3,4,5,3′,4′,5′-hexa-) but not in the *ortho* positions. These compounds interact with the cytosolic protein believed to be the receptor for 3-MC and TCDD [4]. From this data, it has been hypothesized that the optimum configuration for binding to the TCDD receptor is a planar rectangle 3×10 Å, with halogens in at least three of the four corners. Presumably, *ortho* halogenation inhibits binding because it increases the energy barrier to rotation [5], which must be overcome for the biphenyl rings to assume a coplanar configuration.

Most PCB isomers are phenobarbital-type inducers, or they are inactive [3]. The structural requirements for phenobarbital-type induction are not clear; however, we noted earlier that, in general, potency seems to be inversely related to the rate of metabolism [3]. Highly chlorinated congeners are more potent than less chlorinated congeners [3], and they are metabolized more slowly than the less chlorinated congeners [6]. Moreover, the presence of two adjacent, unsubstituted carbon atoms decreases potency, possibly by increasing the rate of metabolism. *Para* substitution increases potency among the less chlorinated isomers, but it is not important for highly chlorinated isomers since 2,3,5,2′,3′,5′-hexachlorobiphenyl is as active as 2,4,5,2′,4′,5′-hexachlorobiphenyl [7]. *Para* substitution also decreases the rate of metabolism of the less chlorinated isomers, but would not be expected to have much effect when the compound does not have two adjacent unsubstituted carbon atoms [6].

Most of this work has involved symmetrical PCB isomers. Recently, Dannan *et al.* [8] reported that 2,4,5,3′,4′,5′-hexabromobiphenyl was a mixed inducer, suggesting that the presence of only one *ortho* halogen did not completely block interaction with the TCDD receptor. More recently, Parkinson *et al.* [9, 10] synthesized a number of unsymmetrical congeners containing a 3,4- or 3,4,5-halogenation pattern in one ring and a 2,3,4-; 2,4,5-; or 2,3,4,5-pattern in the second ring. With the exception of 2,4,5,3′,4′,5′-hexachlorobiphenyl, which was a phenobarbital-type inducer in their hands, several of these unsymmetrical isomers (2,4,5,3′,4′-penta-; 2,3,4,3′,4′-penta-; 2,3,4,3′,4′,5′-hexa-; 2,3,4,5,3′,4′,-hexa; and 2,3,4,5,3′,4′,5′-heptachlorobiphenyls) were mixed inducers. The discrepancy between the effects reported for 2,4,5,3′,4′,5′-hexachlorobiphenyl [10] and the corresponding brominated analog [8] has not been explained.

In the present study, we synthesized and examined the inducing properties of 2,3,5,3′,4′,5′-hexachlorobiphenyl (HCB), which contains a 3,4,5,-halogenation pattern in one ring and a 2,3,5-pattern in the other ring. 2,3,5,2′,3′,5′-HCB is a phenobarbital-type inducer [7] whereas 3,4,5,3′,4′,5′-HCB is the most potent of the 3-MC type inducers [3]. This compound, therefore, differs from most other PCB isomers tested in that it is: (1) unsymmetrical; (2) contains one-half of the phenobarbital-induc-

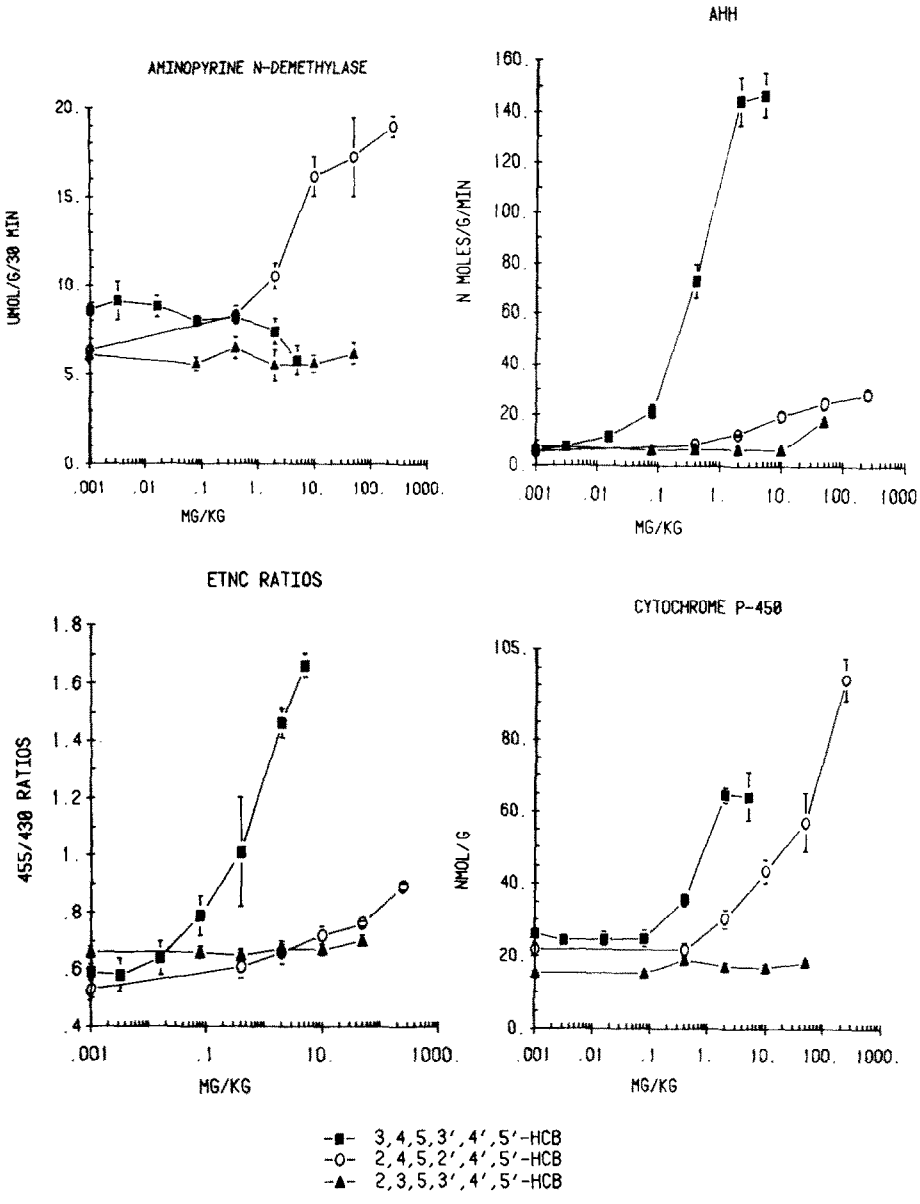

Fig. 1. Comparison of dose–response curves for induction of cytochrome P-450 and P-448-mediated enzymes by 2,3,5,3',4',5'-HCB versus 2,4,5,2',4',5'-HCB and 3,4,5,3',4',5'-HCB. Female rats were injected i.p. daily for 3 days at the designated dose and killed 24 hr later. Each value is the mean ± S.E for four rats.

ing pattern in one ring and one-half of the 3-MC-inducing pattern in the other ring; and (3) contains only one halogen in the *para* position.

2,3,5,3',4',5'-HCB was prepared by the standard Ullmann coupling of 2,3,5- and 3,4,5-trichloroiodobenzenes (prepared from the corresponding trichloroanilines) using activated copper dust. The reaction yielded a mixture of two known symmetrical hexachlorobiphenyl isomers (2,3,5,2',3',5'- and 3,4,5,3',4',5'-) and a third product with the expected chromatographic properties of the unsymmetrical isomer (2,3,4,3',4',5'-). The third product was purified by repeated recrystallization (from acetone then methanol) followed by sequential column chromatography from 1:1 Darco charcoal: Celite 545, then Woelm alumina (activity grade I). The compound was identified as 2,3,5,3',4',5',-HCB by its chromatographic properties (its retention index matched the theoretical value on four g.c. columns) and by its spectroscopic properties (MS: M+ = 358, $C_{12}H_4Cl_6^{35}$; n.m.r.: δ in deuterated chloroform relative to tetramethylsilane, $H^4 = 7.529$, $H^6 = 7.206$, $H^{2',6'} = 7.419$, $J_{4,6} = 2.42$ Hz) The sample was determined to be 99.6 per cent pure by g.c. on Dexil 410 at 200°. The trace impurities were: 2,3,5,3',5'-pentaCB (0.3%), 2,3,4,5,6,3',4',5'-octaCB (120 ppm), 2,3,4,5,6,2',3',5',6'-nonaCB (100 ppm), 2,3,4,5,6,2',3',4',5'-nonaCB (80 ppm), 2,3,5,3',5'- + 3,4,5,2',5'- pentacBs (80 ppm), 3,4,5,2',3'-pentaCB (30 ppm) and an unidentified nonPCB impurity (300 ppm). We did not detect any 3,4,3',4'-tetraCB or 3,4,5,3',4',5'-HCB at a detection limit of < 60 ppm.

Female Sprague–Dawley rats (3 to 4 weeks-old) were injected i.p. for 3 days with various doses of 2,3,5,3',4',5'-; 2,4,5,2',4',5'-; 3,4,5,3',4',5'- or 2,3,5,2',3',5'-HCBs in corn oil. Animals were killed 24 hr

after the last dose in the first experiment and 72 hr in a later experiment.

Enzyme and cytochrome P-450 assays were performed as described earlier [3]. To analyze the amount of PCB in the liver, a portion of the liver was spiked with 25 μg of 2,3,6,2′,3′,6′-HCB in dimethylsulfotide (DMSO) as an internal standard, extracted according to the method of Folch *et al.* [11], and the extract cleaned up as described previously [12]. The fraction that eluted with 2% CH_2Cl_2 was analyzed for parent compound by g.l.c. on Dexsil 410 at 210° using a hydrogen flame ionization detector. Peak areas were corrected for relative molar response.

Figure 1 compares the dose–response curves of 2,3,5,3′,4′,5′-HCB with those of 3,4,5,3′,4′,5′- (3-MC-type) and 2,4,5,2′,4′,5′-HCBs (phenobarbital-type inducer), whereas 2,3,5,2′,3′,5′-HCB was studied only at a single maximally inducing dose (Fig. 2). Three doses of 50 mg/kg of 2,3,5,3′,4′,5′-HCB had no effect on aminopyrine *N*-demethylase activity or cytochrome(s) P-450 content. In contrast, similar doses of 2,4,5,2′,4′,5′-HCB (Fig. 1) and 2,3,5,2′,3′,5′-HCB (Fig. 2) produced maximum induction of both. Moreover, 2,3,5,3′,4′,5′-HCB produced only a very slight increase in aryl hydrocarbon hydrogenase (AHH) activity compared to 3,4,5,3′,4′,5′-HCB, even when administered at much larger doses (Fig. 1). 3,4,5,3′,4′,5′- and 2,3,5,2′,3′,5′-HCBs increased the hepatic contents of cytochromes P-448 and P-450, respectively [3, 7], as shown here by the substrate specificities, the λ_{max} of the CO difference spectrum (448 and 450 nm respectively), and the increase in the ratio of the 455 to 430 nm peaks of the ethyl isocyanide (ETNC) difference spectra (Fig. 2). The relative heights of the 455 and 430 nm peaks of the ETNC difference spectrum are pH-dependent; however, the intercept for the 3-MC-induced cytochrome is lower than the intercept for the control or phenobarbital-induced cytochromes [13]. In contrast, 2,3,5,3′,4′,5′-HCB did not increase the amount of cytochrome(s) P-450 or alter its spectral properties. When the livers of these animals were analyzed for PCB content (Table 1), those treated with 2,3,5,3′,4′,5′-HCB contained 50–60 per cent less parent compound than those treated with 2,3,5,2′,3′,5′- or 3,4,5,3′,4′,5′-HCB. This difference, however, is not enough to explain the absence of induction in the 2,3,5,3′,4′,5′-HCB-treated animals, since response decreased as a function of the log of the dose. Induction was seen with a 125-fold lower dose of 3,4,5,3′,4′,5′-HCB and a 25-fold lower dose of 2,4,5,2′,4′,5′-HCB (Fig. 1).

Because a slight increase in AHH was observed with 2,3,5,3′,4′,5′-HCB, we did a second experiment with very high doses of this isomer. The results are shown in Table 2. Even at high doses (200 mg/kg × 3), 2,3,5,3′,4′,5′-HCB had little effect. It produced minimal induction of AHH, less than that seen with lower doses of either of the symmetrical compounds, and small, variable increases in cytochrome P-450 and aminopyrine *N*-demethylase.

The inability of 2,3,5,3′,4′,5′-HCB to appreciably induce enzymes dependent on either cytochrome(s) P-450 or P-448 was surprising. Two factors, the presence of an *ortho* chlorine and the absence of a chlorine in one of the *para*

Table 1. Analysis of hepatic content of PCB*

Dose	PCB (μg/g liver)
50 mg/kg 2,3,5,2′,3′,5′-HCB	35.6 ± 4.2
10 mg/kg 3,4,5,3′,4′,5′-HCB	45.7 ± 3.8
50 mg/kg 2,3,5,3′,4′,5′-HCB	18.0 ± 3.7

* Animals were treated as described in Fig. 2. Each value is the mean ± S.E. (N = 4).

Table 2. Effects of a high dose of 2,3,5,3′,4′,5′-hexachlorobiphenyl on mixed-function oxidases*

Treatment						
Compound	Daily dose	AHH (pmoles·mg^{-1}·min^{-1})	Aminopyrine *N*-demethylase [μmoles·g^{-1}·(30 min)$^{-1}$]	Cytochrome(s) P-450 (nmoles/g)	A_{max}	ETNC ratios ($A_{455-430}$)
Control		3.0 ± 0.2	4.3 ± 0.3	9.3 ± 0.5	449.9 ± 0.1	0.682 ± 0.003
2,3,5,2′,3′,5′-HCB	50 mg/kg	17.8 ± 3.1†	14.4 ± 2.5†	36.5 ± 6.5†	449.7 ± 0.1	0.607 ± 0.032
2,3,5,3′,4′,5′-HCB	200 mg/kg	9.2 ± 1.2†	5.1 ± 0.7	16.9 ± 3.4	449.2 ± 0.1	0.603 ± 0.039
3,4,5,3′,4′,5′-HCB	10 mg/kg	138.0 ± 21†	3.1 ± 0.2	66.0 ± 7.7†	448.2 ± 0.1	1.845 ± 0.094

* Female rats were given three daily i.p. doses of 2,3,5,2′,3′,5′-HCB, 2,3,5,3′,4′,5′-HCB or 3,4,5,3′,4′,5′-HCB and killed 3 days after the last dose. Each value is the mean ± S.E. (N = 4). ETNC = ethylisocyanide.

† Significantly different from controls, $P < 0.05$.

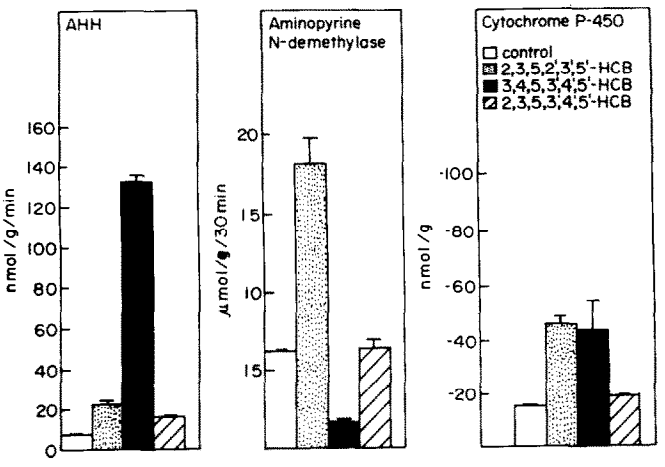

Fig. 2. Comparison of the effects of 2,3,5,2′,3′,5′-HCB (50 mg/kg) 3,4,5,3′,4′,5′-HCB (10 mg/kg), and 2,3,5,3′,4′,5′-HCB (50 mg/kg) on drug-metabolizing enzymes. Female rats were injected i.p. daily for 3 days at the designated dose and killed 24 hr later. Each value is the mean ± S.E. for four rats.

positions, could explain the absence of 3-MC-type induction. Parkinson *et al.* [9, 10] have recently reported that a number of unsymmetrical PCB isomers containing one *ortho* chlorine were "mixed-type" inducers. All of these isomers, however, contained a 3,4- or 3,4,5-halogenation pattern in one ring and chlorines in *both* of the *para* positions. The fact that 2,3,5,3′,4′,5′-HCB was almost inactive as a phenobarbital-type inducer is more difficult to explain. This compound is highly polarizable, and this polarizability may prevent it from inducing the phenobarbital-type cytochrome. Heretofore, the inactivity of certain PCB isomers as inducers was explained by their susceptibility to metabolism and short half-lives. The results with this compound show that not all highly chlorinated isomers are inducers, suggesting the involvement of other electronic and/or steric factors.

National Institute of Environmental Health Sciences
Research Triangle Park NC 27709, *U.S.A.*

JOYCE A. GOLDSTEIN*
PATRICIA LINKO
JAMES D. MCKINNEY
PHILLIP W. ALBRO

* Address all correspondence to: Dr. Joyce A. Goldstein, NIEHS, P.O. Box 12233, Research Triangle Park, NC 27709.

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