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POLYCHLORINATED BIPHENYLS AS PHENOBARBITONE-TYPE INDUCERS OF MICROSOMAL ENZYMES

STRUCTURE–ACTIVITY RELATIONSHIPS FOR A SERIES OF 2,4-DICHLORO-SUBSTITUTED CONGENERS

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Abstract—Several polychlorinated biphenyl (PCB) isomers and congeners resemble phenobarbitone (PB) in their mode of induction of the hepatic drug-metabolizing enzymes; however, unlike PCBs which induce aryl hydrocarbon hydroxylase, no apparent structure–activity correlations have been reported. This study examines the effects of structure on the activity of a series of 2,4-dichloro-substituted biphenyls as inducers of several microsomal enzyme activities including dimethylaminoantipyrine *N*-demethylase, benzo[*a*]pyrene hydroxylase, aldrin epoxidase, and ethoxyresorufin *O*-deethylase. The results clearly illustrate a marked effect of structure on activity: all of the 2,4-dichloro-substituted PCBs resembled PB in their mode of induction. However, the potency of the induction response was dependent on the substitution pattern of the second phenyl ring (i.e. 2,3,4,5-tetrachloro ≥ 2,3,4,5,6-pentachloro > 2,3,4,6-tetrachloro > 2,3,5,6-tetrachloro > 2,4,6-trichloro); the structure of the lower chlorinated ring also determined induction potency since the 2,4-dichloro-substituted PCBs were generally more active than their 4-chloro-substituted analogs, whereas the 2-substituted PCB homologs were inactive. The structural factors which typify the most active PB-type inducer, 2,2′,3,4,4′,5-hexachlorobiphenyl, include the presence of two *para*-, at least two *meta*- and two *ortho*-chloro substituents. In addition to the structure–activity correlations noted for PCBs, the 2,2′,3,4,4′,5-hexachlorobiphenyl congener also elicited a dose–response induction of two PB-inducible enzymes, aldrin epoxidase and dimethylaminoantipyrine *N*-demethylase.

The cytochrome P-450-dependent monooxygenases catalyze the oxidation of both endogenous biochemicals and xenobiotics such as pollutants, drugs, and carcinogens. The terminal enzymes of this electron transfer system are a multiplicity of hemoprotein isozymes, cytochromes P-450, which have been shown to exhibit broad substrate specificity and thus are responsible for the metabolic versatility which is characteristic of this system [1–7].

The induction of one or more forms of cytochrome P-450 by an increasingly large group of structurally diverse chemicals is a well documented phenomenon. 3-Methylcholanthrene (MC) and several related polynuclear aromatic hydrocarbons, β-naphthoflavone, as well as a number of polyhalogenated aromatic hydrocarbons induce rat hepatic cytochrome P-450c (or P-450 MC) [8–14], whereas pretreatment with phenobarbitone (PB) and a wide range of structurally diverse PB-type inducers enhance rat hepatic cytochrome P-450b (P-450 PB) [8–12, 15–19]. These isozymes are distinct from each other in their spectral, catalytic, immunological and electrophoretic properties. The initial events involved in enzyme induction by MC have been well studied and appear to involve an initial noncovalent interaction of MC with a cytosolic receptor protein, the translocation of this complex into the nucleus, and the subsequent expression of the pleiotropic responses associated with this compound which include the induction of cytochrome P-450 [20–27]. Although this cytosolic receptor species has not been fully characterized, there exists excellent genetic evidence and structure–activity relationships within several series of inducers to support the proposed role of a receptor [20–24]. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is the most potent inducer of the MC-inducible cytochrome P-448-dependent monooxygenase, aryl hydrocarbon hydroxylase (AHH), and the most active halogenated aromatic hydrocarbons which exhibit this type of induction activity are approximate isostereomers of TCDD [21, 22]. Moreover, for several polychlorinated biphenyl (PCB) isomers and congeners, there exists an excellent correlation between their potencies as microsomal AHH inducers in male Wistar rats and rat hepatoma H-4-II-E cells and the avidity with which they bind to the cytosolic receptor [28–32].

The mechanism of induction by compounds which resemble PB in their mode of induction has not been determined although several models have been suggested [33, 34]. It has been proposed recently that several experimental observations are evidence for the existence of a receptor for this class of inducers.

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This evidence includes tissue specificity for the induction response, a dose-related response to the inducer, and the identification of a highly potent agonist, 1,4-bis[2-(3,5-dichloropyridyloxy)] benzene [34]. However, the lack of any apparent structure–activity relationships within or between the different classes of compounds which induce PB-type activity may be taken as indirect evidence against a receptor hypothesis. For example, relatively water soluble barbituates and highly lipophilic organochlorine pesticides (dieldrin, mirex, lindane, and *p,p'*-DDT) are all PB-type inducers [35, 36].

The PCBs represent the largest group of structurally-related compounds which have been shown to induce PB-type activity [28, 31, 32, 37–43]. The structural diversity of those congeners which have been identified as PB-type inducers is illustrated in Table 1; the summary shows that PCB congeners with highly variable *ortho*-, *meta*- and *para*-chloro substitution patterns all exhibit similar microsomal enzyme induction characteristics. Despite the apparent lack of a structure–activity relationship, those congeners which are most potent as PB-type inducers possess at least two *para*- and two *ortho*-chloro substituents and are typified by 2,2',4,4'-tetrachlorobiphenyl, 2,2',3,4,4',5-hexachlorobiphenyl, and 2,2',4,4',5,5'-hexachlorobiphenyl [28, 37, 42]. PCBs which contain a 2,4-dichloro substitution pattern on one ring of the biphenyl molecule provide an excellent model for determining the effects of structure on PB-type activity since several congeners, both symmetrical (e.g. 2,2',4,4'-tetrachlorobiphenyl) and unsymmetrical (e.g. 2,2',3,4,4',5-hexachlorobiphenyl), which contain the 2,4-dichlorophenyl moiety, exhibit this type of activity. Moreover, it is possible to synthesize individual PCB congeners in which the substitution patterns can be systematically altered in both the 2,4-dichloro-substituted phenyl ring and in the second phenyl ring. This study examines the effects of structure on the enzyme-inducing activities of several 2,4-dichloro-substituted PCBs and related compounds (Fig. 1) by comparing the enzymic, spectral and electrophoretic characteristics of the induced microsomal proteins.

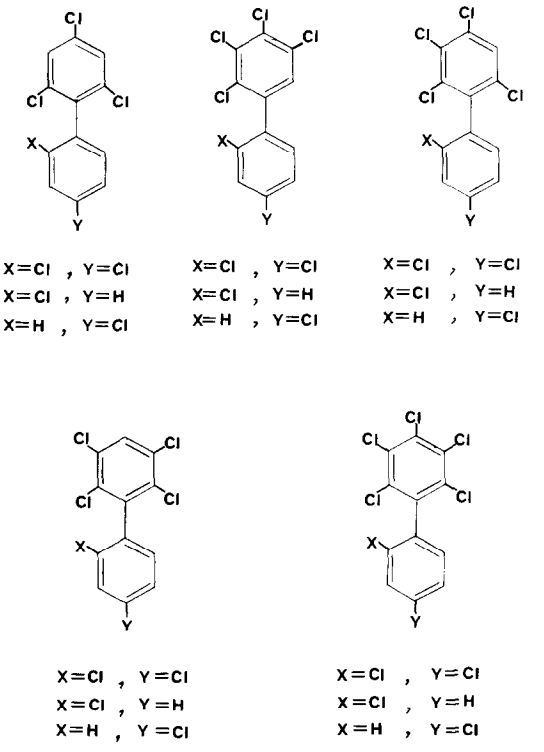

Fig. 1. Structures of PCB inducers.

MATERIALS AND METHODS

Preparation of PCB isomers and congeners. The polychlorinated biphenyl isomers and congeners used in this study were all prepared by the Cadogan coupling [28, 41, 42] of a chlorinated aniline (10–15 mmoles) in the presence of excess chlorinated benzene (100–200 mmoles) as described [28, 41, 42]. The 2,4-dichloro-, 4-chloro-, 3-chloro- and 2-chloroanilines, 1,3,5-trichlorobenzene, 1,2,3,4-, 1,2,3,5- and 1,2,4,5-tetrachlorobenzenes and pentachlorobenzene were purchased from the Aldrich Chemical Co. (Milwaukee, WI). All of the compounds were purified by column chromatography, thin-layer chromatography (TLC), and recrystallization as described [28, 41, 42]. All purities were >98% as determined by gas–liquid chromatography using a Hewlett–Packard model 5710 chromatograph equipped with a ^{63}Ni electron capture detector using a 0.6 cm × 1.2 m glass column packed with 3% OV 101 Ultrabonded Carbowax 20M (80–10 mesh, RFR Corp., Hope, RI).

Table 1. PCBs as PB-type inducers of the hepatic drug-metabolizing enzymes: effects of structure on activity

PCB congener	Number of chloro substituents *ortho*	*meta*	*para*	Ref.
2,2',4,4',6,6'-Hexachlorobiphenyl	4	0	2	38
2,2',3,4,4',5,6-Heptachlorobiphenyl	3	2	2	28
2,2',4,4',5,5'-Hexachlorobiphenyl	2	2	2	38, 37
2,3,3',4,4',5'-Hexachlorobiphenyl	1	3	2	41
3,3'-Dichlorobiphenyl	0	2	0	38, 40
2,2',3,3',5,5'-Hexachlorobiphenyl	2	4	0	39
2,3,3',4,5,5'-Hexachlorobiphenyl	1	4	1	28
2,3,3',4,4'-Pentachlorobiphenyl	1	2	2	41
2,2',4,4',6-Pentachlorobiphenyl	3	0	2	This study
Summary: positional variability	0–4	0–4	0–2	

Biochemicals. 3-Methylcholanthrene (MC), benzo[*a*]pyrene (B[*a*]P), ethylisocyanide (EIC), NADP, glucose-6-phosphate, NADPH and glucose-6-phosphate dehydrogenase were purchased from the Sigma Chemical Co. (St. Louis, MO), 4-dimethylaminoantipyrine (DMAP) and carbon monoxide (CO) were purchased from the Aldrich Chemical Co. and the Matheson Chemical Co. (East Rutherford, NJ) respectively; and ethoxyresorufin was provided by T. Sawyer. Tritiated B[*a*]P (20 Ci/mmole) was purchased from the Amersham Corp. (Arlington Heights, IL) and purified as described [28, 41, 42].

Animal treatment and isolation of microsomes. Immature male Wistar rats (average weight 100 g) were housed in wire cages and allowed free access to Purina Certified Rodent Chow, No. 5002, and water. The PCB congeners in corn oil (0.5 ml) were administered by intraperitoneal injections on days 1 and 3 to the experimental animals. The concentrations of the chemicals are summarized in Tables 2 and 3. PB, MC, PB plus MC, and corn oil (controls) were also administered to the rats as previously described [42]. All animals were fasted 24 hr prior to being killed (day 6) by cervical dislocation. The rat livers were immediately perfused by the hepatic portal vein with ice-cold isotonic saline (25 ml) supplemented with EDTA (0.1 mM) for 1 min; after determining liver weights the 100,000 *g* hepatic microsomal fraction was isolated as described [28, 41, 42].

Assays. Protein concentrations, the reduced cytochrome P-450:CO and EIC binding difference spectra, the cytochrome b_5 content, and DMAP *N*-demethylase and B[*a*]P hydroxylase activities were determined as previously described [28, 41, 42]. Microsomal ethoxyresorufin *O*-deethylase (EROD) was determined by the fluorimetric method essentially as reported by Pohl and Fouts [44]. Microsomal aldrin epoxidase activity was determined by the gas chromatographic method described by Wolff *et al.* [45]. Microsomal proteins (40 μg) were analyzed by sodium dodecyl sulfate (SDS) polyacrylamide slab gel electrophoresis as described by Laemmli [46].

The statistical significance between the means of the data obtained from the pretreated animals compared to the corn oil treated controls was determined using the method described by Dunnett [47].

RESULTS

Effects of PB, MC and their coadministration (PB + MC). The spectral, electrophoretic and enzymic characteristics of rat hepatic microsomes from animals pretreated with PB, MC and coadministration of PB plus MC are summarized in Tables 2 and 3. The data are comparable to the results previously reported for these three inducers [28, 41, 42].

Effects of 2,2′,4,4′,6-*penta-*, 2,2′,4,6-*tetra and* 2,4,4′,6-*tetrachlorobiphenyls.* The fully substituted 2,2′,4,4′,6-pentachlorobiphenyl induced some PB-type characteristics only after administration of the high dose (100 μmoles/kg) (Tables 2 and 3). Both microsomal DMAP *N*-demethylase and aldrin expoxidase activities were significantly induced compared to microsomes from corn oil treated (control) animals. However, these activities were increased to only about 50% of the maximum values induced by PB pretreatment. Administration of the high dose of 2,4,4′,6 and 2,2′,4,6-tetrachlorobiphenyl did not induce the microsomal enzymes.

Effects of 2,2′,3,4,4′,6-*hexa,* 2,2′,3,4,6- *and* 2,3,4,4′,6-*pentachlorobiphenyls.* Both 2,2′,3,4,4′,6-hexachlorobiphenyl and 2,3,4,4′,6-pentachlorobiphenyl exhibited significant PB-type induction characteristics only after administration of the high dose (100 μmoles/kg). 2,2′,3,4,6-Pentachlorobiphenyl was inactive as an inducer.

Effects of 2,2′,3,4′,5,6-*hexa-*, 2,2′,3,5,6- *and* 2,3,4′,5,6-*pentachlorobiphenyls.* Significant PB induction characteristics were elicited by 2,2′,3,4′,5,6-hexachlorobiphenyl after pretreatment with either a high (100 μmoles/kg) or low (20 μmoles/kg) dose, as reflected by the enhanced activity of both microsomal DMAP *N*-demethylase and aldrin epoxidase. Induction characteristics (Tables 2 and 3) consistent with those elicited by PB were produced by pretreatment with a high and low dose of 2,3,4′,5,6-pentachlorobiphenyl. Pretreatment with a high dose of the 2,2′,3,5,6-pentachlorobiphenyl did not induce the hepatic drug-metabolizing enzymes.

Effects of 2,2′,3,4,4′,5,6-*hepta-*, 2,2′,3,4,5,6- *and* 2,3,4,4′,5,6-*hexachlorobiphenyls.* Pretreatment with 2,2′,3,4,4′,5,6-heptachlorobiphenyl significantly induced the activity of DMAP *N*-demthylase and aldrin epoxidase and increased the microsomal cytochrome P-450 content. 2,3,4′,5,6-Hexachlorobiphenyl administered at a high and low dose exhibited PB-type induction characteristics and also induced the activity of EROD at the high dose only. The 2,2′,3,4,5,6-hexachlorobiphenyl congener was inactive as an inducer (Tables 2 and 3).

Effects of 2,2′,3,4,4′,5-*hexa-*, 2,2′,3,4,5- *and* 2,3,4,4′,5-*pentachlorobiphenyls.* Administration of the high (100 μmoles/kg) dose of 2,2′,3,4,4′,5-hexachlorobiphenyl maximally induced the activity of DMAP *N*-demethylase and aldrin epoxidase and increased the cytochrome P-450 content of the microsomes (Tables 2 and 3). Pretreatment with a low dose (20 μmoles/kg) of this congener also significantly enhanced PB-type induction characteristics. Although 2,3,4,4′,5-pentachlorobiphenyl significantly enhanced DMAP *N*-demethylase, aldrin epoxidase and cytochrome P-450 at a high dose, it was less active than the 2,2′,3,4,4′-5-hexachlorobiphenyl congener and also induced microsomal B[*a*]P hydroxylase and EROD as previously reported [28]. 2,2′,3,4,5-Pentachlorobiphenyl was inactive as an inducer. Figures 2 and 3 illustrate the electrophoretic and induction characteristics of 2,2′,3,4,4′,5-hexachlorobiphenyl and related compounds, and Fig. 4 shows a dose–response induction of DMAP *N*-demethylase and aldrin epoxidase by 2,2′,3,4,4′,5-hexachlorobiphenyl.

DISCUSSION

Pretreatment of rats with the commercial PCB mixture Aroclor 1254 enhances both PB- and MC-inducible hepatic microsomal monooxygenase activi-

Table 2. PCB congeners as inducers of hepatic drug metabolizing enzymes: effects on liver weight, liver protein and microsomal enzymes

Treatment		% Liver wt of body wt	mg protein g liver^{-1}	DMAP N-Demethylase*	B[a]P Hydroxylase†	Aldrin Epoxidase*	EROD†
Corn Oil (Control) (n=10)		5.33 ±0.53	27.6 ±5.2	5.65 ±1.08	0.406 ± 0.256	2.72 ± 0.84	117.6 ± 45.3
Phenobarbitone (PB) (n=10)		6.11 ±0.59	39.3 ±4.8[1]	14.8 ±3.2[1]	1.03 ±0.769	16.3 ± 6.0[1]	203.8 ± 107.5
3-Methylcholanthrene (MC) (n=10)		6.91 ±0.86[1]	29.4 ±6.3	6.06 ±1.57	6.84 ±2.39[1]	0.914± 0.032	4498 ±1063[1]
PB + MC (n=10)		8.12 ±1.55[1]	44.4 ±6.0[1]	13.2 ±2.9[1]	6.42 ±2.26[1]	15.1 ± 5.9[1]	5540 ±1328[1]
2,2',3,4',5,6-HexaCB	100umol/kg (n=4)	5.56 ±0.78	30.9 ±6.7	10.9 ±0.4[1]	0.610 ± 0.044	16.0 ± 0.8[1]	119.3 ± 20.3
	20umol/kg (n=4)	5.02 ±0.21	35.2 ±2.8	9.17 ±0.86[1]	0.648 ± 0.076	13.4 ± 0.9[1]	110.9 ± 57.5
2,3,4',5,6-PeCB	100umol/kg (n=4)	5.94 ±1.09	32.0 ±2.7	9.12 ±1.58[1]	0.591 ± 0.072	14.6 ± 3.5[1]	114.2 ± 27.0
	20umol/kg (n=4)	5.00 ±0.25	27.8 ±1.4	9.02 ±1.30[1]	0.267 ± 0.038	4.49 ± 0.95	83.00 ± 10.90
2,2',3,5,6-PeCB	100umol/kg (n=4)	5.75 ±1.40	23.0 ±13.6	6.04 ±0.58	0.575 ± 0.093	4.44 ± 0.62	77.30 ± 32.41
2,2',3,4,4',5-HexaCB	100umol/kg (n=4)	6.04 ±0.47	39.4 ±3.4[1]	15.4 ±2.1[1]	0.888 ± 0.082	19.3 ± 2.3[1]	463.9 ± 52.8
	20umol/kg (n=4)	6.03 ±1.48	28.1 ±6.6	10.3 ±0.5[1]	0.350 ± 0.199	6.64 ± 1.55[1]	176.9 ± 38.2
2,3,4,4',5-PeCB	100umol/kg (n=4)	9.12 ±1.73[1]	36.1 ±8.2[1]	8.45 ±0.93[1]	6.73 ±0.50[1]	13.3 ± 8.5[1]	5188 ±690[1]
	20umol/kg (n=4)	6.16 ±0.38	30.1 ±1.6	6.46 ±0.82	3.97 ±0.14[1]	2.11 ± 0.78	3446 ±758[1]
2,2',3,4,5-PeCB	100umol/kg (n=4)	4.68 ±0.42	34.4 ±3.6	4.26 ±0.55	0.465 ± 0.356	4.15 ± 1.56	205.9 ± 157.4
2,2',4,4',6-PeCB	100umol/kg (n=4)	4.67 ±0.22	28.9 ±7.8	9.41 ±0.49[1]	0.880 ± 0.440	9.20 ± 0.33[1]	140.9 ± 27.9
	20umol/kg (n=4)	5.23 ±0.59	28.8 ±3.0	5.75 ±1.22	0.175 ± 0.107	2.79 ± 0.94	53.32 ± 25.95
2,4,4',6-TetraCB	100umol/kg (n=4)	4.91 ±0.57	36.9 ±4.0	4.76 ±0.66	0.234 ± 0.122	4.98 ± 1.68	82.39 ± 35.54
2,2',4,6-TetraCB	100umol/kg (n=4)	4.82 ±0.53	32.3 ±2.1	4.70 ±0.59	0.214 ± 0.073	4.34 ± 1.68	72.47 ± 22.04
2,2',3,4,4',6-HexaCB	100umol/kg (n=4)	5.97 ±1.45	31.5 ±3.3	11.1 ±2.6[1]	0.546 ± 0.129	6.04 ± 1.60[1]	180.3 ± 76.4
	20umol/kg (n=4)	5.06 ±0.34	32.6 ±9.4	7.72 ±1.68	0.046 ± 0.089	4.06 ± 0.46	88.85 ± 24.20
2,3,4,4',6-PeCB	100umol/kg (n=4)	4.88 ±0.28	30.4 ±8.4	9.73 ±1.29[1]	0.965 ± 0.082	12.6 ± 3.7[1]	869.0 ± 210.1
	20umol/kg (n=4)	4.59 ±0.20	27.3 ±5.6	7.82 ±1.08	1.02 ±0.273	2.67 ± 0.91	160.4 ± 107.9
2,2',3,4,6-PeCB	100umol/kg (n=4)	4.76 ±0.66	28.8 ±5.3	4.75 ±1.18	0.22 ±0.030	3.18 ± 0.77	99.49 ± 38.88
2,2',3,4,4',5,6-HeptaCB	100umol/kg (n=4)	5.35 ±0.29	36.8 ±6.3[1]	10.7 ±0.9[1]	0.180 ± 0.073	18.0 ± 1.6[1]	123.6 ± 21.3
	20umol/kg (n=4)	5.92 ±1.05	29.6 ±7.9	8.91 ±0.38[1]	0.517 ± 0.052	12.3 ± 1.5[1]	83.10 ± 2.71
2,3,4,4',5,6-HexaCB	100umol/kg (n=4)	8.29 ±1.75[1]	33.2 ±5.2	9.62 ±0.97[2]	0.860 ± 0.183	11.3 ± 1.6[2]	2709 ±569[2]
	20umol/kg (n=4)	6.85 ±1.53[2]	29.1 ±6.0	10.4 ±1.4[1]	0.675 ± 0.080	14.8 ± 2.5[1]	217.3 ± 76.7
2,2',3,4,5,6-HexaCB	100umol/kg (n=4)	4.53 ±0.50	24.8 ±1.7	4.13 ±1.33	0.106 ± 0.072	3.00 ± 1.42	44.61 ± 16.9
2,2',4,4'-tetrachlorobiphenyl	100umol/kg (n=4)	5.45 ±0.57	37.06 ±6.24[2]	12.58 ±2.30[2]	0.456 ± 0.149	6.97 ± 1.75[2]	1.01 ± 0.24

* nmol product formed/mg protein/min.
† nmol substrate metabolized/mg protein/min.
[1,2] Different from control at the 5% (=0.05) and 1% (=0.01) level of significance, respectively.

Table 3. PCB congeners as inducers of hepatic drug-metabolizing enzymes: effects on cytochrome b_5 and the reduced cytochrome P-450 binding difference spectra

Treatment		Cytochrome b_5*	Cytochrome P-450* (Peak Maximum, nm)	Ethylisocyanide-Difference Spectrum Peak Maximum (nm)	Peak Maximum (nm)	Peak Height Ratio (455/428 nm)
Corn Oil (Control) (n=10)		0.244 ± 0.047	0.685 ± 0.072 (450.0 ± 0.2)	454.5 ± 0.5	428.0 ± 0.5	0.504 ± 0.076
Phenobarbitone (PB) (n=10)		0.328 ± 0.045[1]	1.69 ± 0.22[1] (450.0 ± 0.5)	455.5 ± 0.5[1]	428.0 ± 0.2	0.660 ± 0.103
3-Methylcholanthrene (MC) (n=10)		0.362 ± 0.056[1]	1.28 ± 0.11[1] (448.3 ± 0.3)	453.2 ± 0.5[1]	429.1 ± 0.4[1]	1.27 ± 0.279[1]
PB + MC (n=10)		0.326 ± 0.083[1]	2.62 ± 1.06[1] (448.7 ± 0.6)	453.8 ± 0.5[1]	428.8 ± 0.5[2]	0.972 ± 0.138[1]
2,2',3,4',5,6-HexaCB	100umol/kg (n=4)	0.215 ± 0.034	1.40 ± 0.53[2] (449.2 ± 1.1)[1]	454.4 ± 1.0	428.6 ± 0.8	1.13 ± .15[1]
	20umol/kg (n=4)	0.266 ± 0.021	1.27 ± 0.09 (450.0 ± 0.3)	455.3 ± 0.4	428.0 ± 0.0	0.771 ± 0.116[2]
2,3,4',5,6-PeCB	100umol/kg (n=4)	0.249 ± 0.031	0.964 ± 0.186 (450.3 ± 0.3)	455.1 ± 0.3	428.1 ± 0.3	0.747 ± 0.068
	20umol/kg (n=4)	0.293 ± 0.009	0.672 ± 0.162 (450.4 ± 0.4)	457.0 ± 0.5[1]	428.2 ± 0.1	0.505 ± 0.028
2,2',3,5,6-PeCB	100umol/kg (n=4)	0.249 ± 0.053	1.14 ± 0.27 (449.8 ± 0.5)	454.4 ± 1.5	427.6 ± 0.6	0.649 ± 0.127
2,2'3,4,4'5-HexaCB	100umol/kg (n=4)	0.371 ± 0.035[1]	1.99 ± 0.20[1] (450.3 ± 0.2)	455.2 ± 0.5	428.4 ± 0.3	0.687 ± 0.059
	20umol/kg (n=4)	0.286 ± 0.044	1.18 ± 0.02 (450.3 ± 0.6)	455.2 ± 0.8	428.0 ± 0.0	0.653 ± 0.092
2,3,4,4',5-PeCB	100umol/kg (n=4)	0.361 ± 0.053[1]	1.93 ± 0.31[1] (448.6 ± 0.4)[1]	453.8 ± 0.3[2]	429.0 ± 0.2[1]	0.824 ± 0.028[1]
	20umol/kg (n=4)	0.344 ± 0.026[1]	1.39 ± 0.12[2] (448.2 ± 0.3)	453.7 ± 0.2	428.9 ± 0.2[1]	0.660 ± 0.096[1]
2,2',3,4,5-PeCB	100umol/kg (n=4)	0.218 ± 0.006	0.703 ± 0.089 (449.6 ± 0.4)	454.6 ± 0.4	428.4 ± 0.4	0.584 ± 0.109
2,2',4,4',6-PeCB	100umol/kg (n=4)	0.316 ± 0.035	0.911 ± 0.145 (450.1 ± 0.2)	455.4 ± 1.2	428.3 ± 0.4	0.509 ± 0.037
	20umol/kg (n=4)	0.189 ± 0.052	0.563 ± 0.098 (450.4 ± 0.5)	454.4 ± 0.8	429.1 ± 0.5[1]	0.547 ± 0.056
2,4,4',6-TetraCB	100umol/kg (n=4)	0.234 ± 0.014	0.630 ± 0.117 (450.1 ± 0.4)	454.2 ± 0.6	427.9 ± 0.4	0.448 ± 0.069
2,2',4,6-TetraCB	100umol/kg (n=4)	0.226 ± 0.023	0.649 ± 0.079 (449.8 ± 0.4)	454.3 ± 1.1	428.0 ± 0.3	0.469 ± 0.067
2,2',3,4,4',6-HexaCB	100umol/kg (n=4)	0.346 ± 0.025[1]	1.19 ± 0.253 (450.5 ± 0.4)	455.8 ± 0.5[1]	428.4 ± 0.3	0.497 ± 0.057
	20umol/kg (n=4)	0.295 ± 0.040	0.906 ± 0.106 (449.6 ± 0.8)	455.6 ± 0.4	428.5 ± 0.2	0.450 ± 0.019
2,3,4,4',6-PeCB	100umol/kg (n=4)	0.237 ± 0.020	0.998 ± 0.114 (449.3 ± 0.4)[2]	455.0 ± 0.4	429.2 ± 0.2[1]	0.626 ± 0.045
	20umol/kg (n=4)	0.231 ± 0.041	0.719 ± 0.083 (449.9 ± 0.2)	454.8 ± 0.4	428.3 ± 0.2	0.571 ± 0.040
2,2',3,4,6-PeCB	100umol/kg (n=4)	0.202 ± 0.020	0.622 ± 0.091 (450.0 ± 0.6)	455.0 ± 0.5	429.3 ± 0.2[1]	0.525 ± 0.044
2,2',3,4,4',5,6-HeptaCB	100umol/kg (n=4)	0.275 ± 0.050	1.53 ± 0.07[2] (450.0 ± 0.5)	454.5 ± 0.2	428.1 ± 0.3	0.687 ± 0.059
	20umol/kg (n=4)	0.252 ± 0.026	0.647 ± 0.121 (450.2 ± 0.5)	455.0 ± 0.8	428.0 ± 0.3	0.653 ± 0.092
2,3,4,4',5,6-HexaCB	100umol/kg (n=4)	0.281 ± 0.041	1.64 ± 0.44[1] (449.5 ± 0.5)	454.2 ± 0.6	428.6 ± 0.3	0.824 ± 0.028[1]
	20umol/kg (n=4)	0.236 ± 0.011	1.08 ± 0.05 (450.0 ± 0.5)	455.6 ± 0.4[1]	427.6 ± 0.5	0.660 ± 0.096
2,2',3,4,5,6-HexaCB	100umol/kg (n=4)	0.206 ± 0.032	0.708 ± 0.141 (449.8 ± 0.5)	455.0 ± 1.0	428.0 ± 1.0	0.584 ± 0.109
2,2',4,4'-tetrachlorobiphenyl	100umol/kg (n=4)	0.278 ± 0.051	0.935 ± 0.192[2] (450.0 ± 0.2)	453.5 ± 0.7[2]	429.6 ± 0.3[2]	0.693 ± 0.118

* nmol/mg protein.

[1,2] Different from control at the 5% (=0.05) and 1% (=0.01) level of significance, respectively.

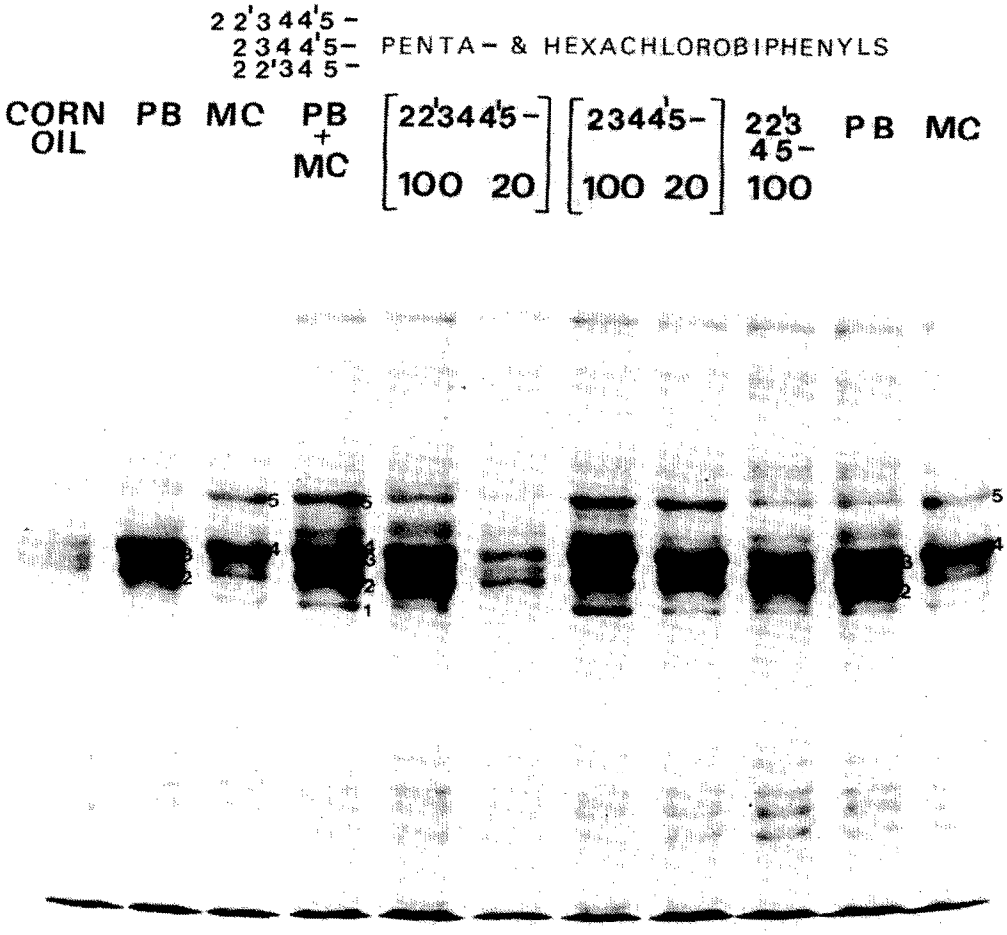

Fig. 2. SDS slab gel electrophoresis of hepatic microsomal proteins from immature male Wistar rats pretreated with corn oil, PB, MC, PB plus MC (coadministered), 2,2',3,4,4',5-hexachlorobiphenyl (100 and 20 μmoles/kg), 2,3,4,4',5-pentachlorobiphenyl (100 and 20 μmoles/kg, 2,2',3,4,5-pentachlorobiphenyl (100 μmoles/kg), PB and MC respectively.

ties and increases the hepatic content of the major cytochrome P-450 isozymes induced by these two xenobiotics [8–11, 48]. Not surprisingly, a host of individual PCB isomers and congeners exhibit a mode of induction activity similar to that elicited by PB, MC, or a mixture of the two [28–32, 37–42]. PCBs which elicit a pattern of induction consistent with that induced by MC (e.g. AHH) are defined by precise structure–activity rules, and there is an excellent correlation between the potency of individual congeners as AHH inducers and the affinity with which they bind to the cytosolic receptor protein [32]. No such structure–activity relationship has been observed for those PCBs which elicit PB-type induction characteristics (see Table 1). This paper summarizes a structure–activity study for 2,4-dichloro-substituted biphenyls in which both the substitution patterns and the degree of chlorination on both phenyl rings were systematically varied.

All of the 2,4-dichloro-substituted PCB congeners were active as PB-type inducers as evidenced by enhanced DMAP *N*-demethylase and aldrin epoxidase activity, increased microsomal P-450 content, and the intensification of an electrophoretic staining band at 52,000 daltons. The SDS slab gel electrophoresis of 2,2',3,4,4',5-hexa-, 2,3,4,4',5-penta- and 2,2',3,4,5-pentachlorobiphenyl-induced microsomal proteins, illustrated in Fig. 2, shows the intensification of the PB-inducible band 3 in microsomes from animals pretreated with the former two PCB congeners. The results also demonstrate that the potency of the PCB congeners was markedly dependent on the substitution pattern of the second phenyl ring (i.e. 2,3,4,5-tetrachloro ≥ 2,3,4,5,6-pentachloro > 2,3,4,6-tetrachloro > 2,3,5,6 tetrachloro > 2,4,6-trichloro). Clearly, the most active inducers contain at least two *ortho*-chloro and two *para*-chloro substituents. The effects of introducing a third *ortho*-chloro substituent may be examined by comparing the PB-type inducing characteristics of 2,2',4,4'-tetrachlorobiphenyl and 2,2',3,4,4',5-hexachlorobiphenyl with those of 2,2',4,4',6-penta-

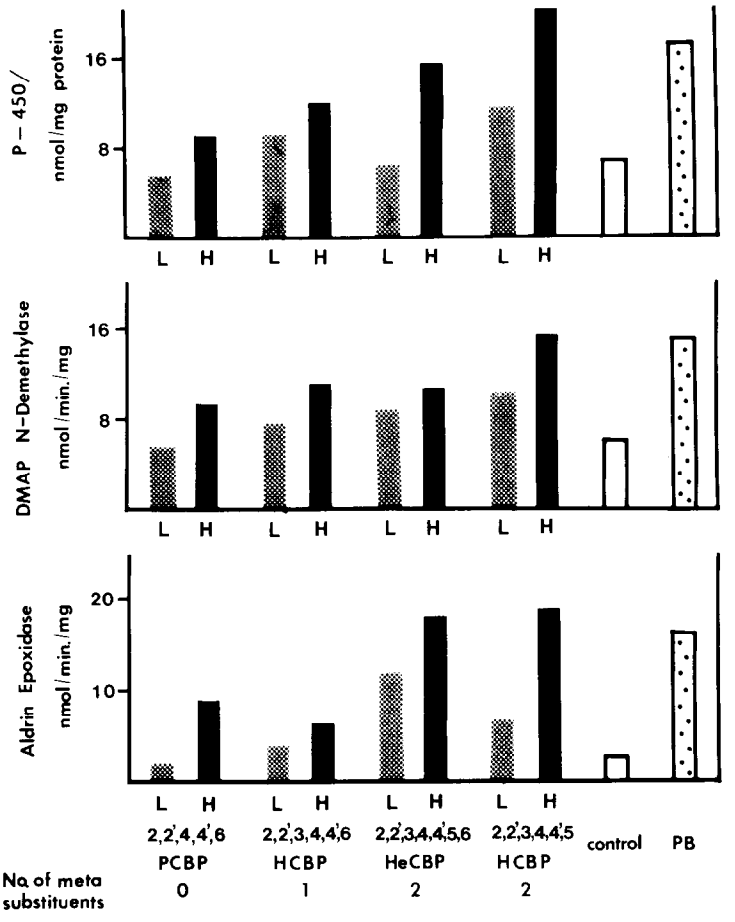

Fig. 3. Effects of structure on the activity of PCB isomers and congeners as inducers of hepatic microsomal cytochrome P-450, DMAP *N*-demethylase and aldrin epoxidase. L = low dose; H = high dose.

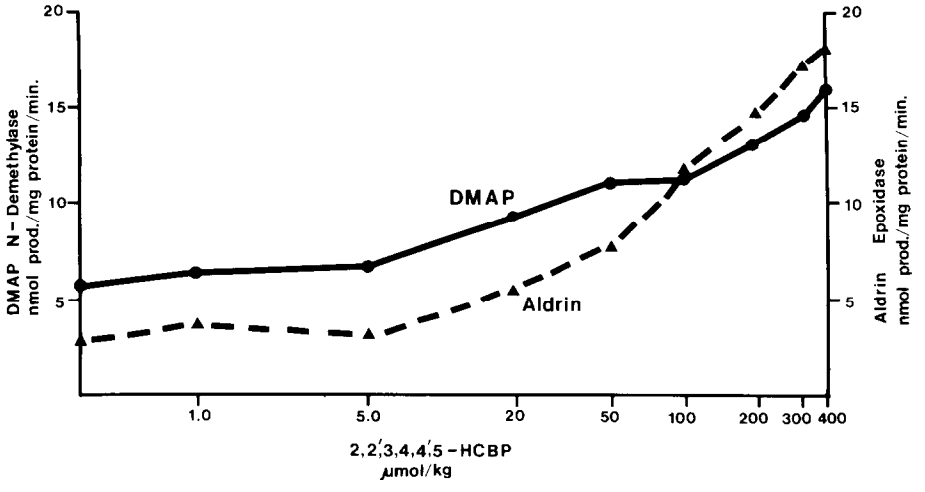

Fig. 4. Dose–response induction of hepatic microsomal aldrin epoxidase and DMAP *N*-demethylase in immature male Wistar rats.

chlorobiphenyl and 2,2′,3,4,4′,5,6-heptachlorobiphenyl respectively. There was a diminution in the PB-type inducing activity of the resultant PCB when a third *ortho*-chlorosubstituent was added to the 2,2′,4,4′-tetrachlorobiphenyl nucleus to make 2,2′,4,4′,6-pentachlorobiphenyl. Similarly 2,2′,3,4,4′,5-hexachlorobiphenyl was more active than 2,2′,3,4,4′,5,6-heptachlorobiphenyl as an inducer of DMAP *N*-demethylase (at 100 μmoles/kg and 20 μmoles/kg) and aldrin epoxidase (at 100 μmoles/kg) and confirms that maximum-inducing activity for these PCBs is obtained with congeners containing two *ortho*-chloro substituents.

The results also illustrate the pronounced effects of *meta*-chloro substituents (see Fig. 3). With only a few exceptions, the introduction of *meta*-chloro substituents into the PCB nucleus resulted in an increase in the induction of microsomal DMAP *N*-demethylase, aldrin epoxidase, and cytochrome P-450 content.

Although the hepatic residues of the 2,4-dichloro-substituted PCBs were not determined, it is unlikely that metabolic or bioconcentration factors play a role in determining relative enzyme induction potencies for the following reasons. First, all of the compounds contain the 2,4-dichloro substitution pattern on the lower chlorinated phenyl ring. Second, the more highly chlorinated phenyl rings do not contain adjacent unsubstituted carbon atoms and all of the 2,4-dichloro-substituted PCBs would not be readily metabolized [49]. Third, with the exception of 2,2′,3,4′,5,6-hexachlorobiphenyl, all of the remaining 2,4-disubstituted analogs contain two *para*-chloro substituents which are the prime sites of PCB hydroxylation [50]. Fourth, comparative bioconcentration studies in the rat with several hexachlorobiphenyls which contain two *para* substituents and variable *ortho*/*meta*-chloro substituent ratios exhibited only minor differences in their hepatic residue levels [51].

A comparison of the activities of the 2,4-dichloro, 4-chloro, and 2-chloro analogs within each of the five groups of PCBs (Fig. 1) clearly illustrate the effects of structural variations in the lower chlorinated phenyl ring. The 2,4-dichloro-substituted analogs were more active than the 4-chloro analogs, whereas all of the 2-chloro-substituted PCBs were inactive as inducers of DMAP *N*-demethylase, aldrin epoxidase and cytochrome P-450 content. Furthermore, pretreatment with a high dose (100 μmoles/kg) of two of the 4-chloro analogs, 2,3,4,4′,5,6-hexachlorobiphenyl and 2,3,4,4′,5-pentachlorobiphenyl, significantly enhanced the activity of EROD, an MC-inducible monooxygenase activity. Both of these AHH-inducing congeners possess only one *ortho*-chloro substituent, and the introduction of the second *ortho* substituent to give the 2,4-dichloro substitution pattern serves to reduce the coplanar conformation of the resultant PCBs and eliminates the induction of cytochrome P-448-dependent monooxygenases.

The results clearly show that, for fifteen structurally-related PCB isomers and congeners, there is a marked effect of structure on their microsomal DMAP *N*-demethylase and aldrin epoxidase-inducing activities. The most active congener, 2,2′,3,4,4′,5-hexachlorobiphenyl, is substituted at both *para*, two *ortho* and at least two *meta* positions. Elimination of one or both of the *para*-chloro substituents or the addition of a third *ortho*-chloro group tended to diminish this type of induction activity. It is apparent that a 2,4-dichloro substitution pattern on one of the phenyl rings is also an important structural determinant in this type of induction activity. Figure 4 illustrates that 2,2′,3,4,4′,5-hepa CB also elicited a dose-related response for the induction of the PB-inducible microsomal DMAP *N*-demethylase and aldrin epoxidase activities (Fig. 4).

This study thus confirms that within a group of structurally-related PCBs at least two of the criteria proposed for the existence of a receptor as mediator of the induction response are satisfied: namely, a structure–activity relationship within a series of compounds and a dose-related response to one of the inducers. Current research in our laboratory is focused on the enzyme-inducing activities of modified substituted biphenyls and their use as probes for delineating the mechanism of induction of PB-inducible monooxygenases.

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