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STRUCTURE-DEPENDENT INDUCTION OF CYP2B BY POLYCHLORINATED BIPHENYL CONGENERS IN FEMALE SPRAGUE–DAWLEY RATS

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Abstract—The dose–response induction of hepatic microsomal pentoxyresorufin *O*-dealkylase (PROD) activity by phenobarbital (PB) and several polychlorinated biphenyl (PCB) mixtures and congeners was determined in the immature female Sprague–Dawley rat. At a dose of 75 mg/kg/day of PB for 3 days, the microsomal PROD activity was 2154 pmol/min/mg protein. Aroclors 1260, 1254, 1242, and 1016 did not induce maximal PROD activity at doses up to 500 mg/kg, and only Aroclor 1016 induced > a half-maximal response at the 500 mg/kg dose. The relative potencies of eighteen different PCB congeners were also determined, and the structures of these compounds differed with respect to the degree of chlorination (tri- to octochloro) and substitution patterns. The relative potencies of these compounds were estimated by comparing their induced activities at the high dose (150 or 100 mg/kg) with that of PB. The most potent inducers were 2,3,3′,4′,5,6-hexaCB and 2,2′,3,4′,5,5′,6-heptaCB; at a dose of 150 mg/kg, the PROD activity induced by 2,2′,3,4′,5,5′,6-heptaCB was comparable to that observed for PB. 2,3,3′,4′,5,6-HexaCB was the most potent inducer, and hepatic PROD activity in rats treated with 150 mg/kg was 4202 pmol/min/mg; this value was higher than that observed for PB at a dose of 75 mg/kg. A second group of congeners including 2,2′,3,4,4′,5,5′-heptaCB, 2,2′,4,4′,5,5′-hexaCB, 2,2′,3,3′,4,4′,5,5′-octaCB, 2,2′,4,4′-tetraCB, 2,2′,4,5,5′-pentaCB, 2,2′,3,4,4′,5′,6-heptaCB, 2,2′,4,4′,5-pentaCB and 2,2′,3,3′,4′,5,5′,6-octaCB induced PROD activity ≥1090 pmol/min/mg at the 150 mg/kg dose, and this value was >50% of the maximal response observed for PB. The remaining compounds, namely 2,4,4′-triCB, 2,2′,3,4′-tetraCB, 2,2′,5,5′-tetraCB, 2,3′,4,4′,5-pentaCB, 2,3,3′,4,4′-pentaCB, 2,2′,4,4′,5,6′-hexaCB, 2,3,3′,4,4′,5,5′-heptaCB and 2,2′,3,-3′,4,4,5-heptaCB were all relatively weak inducers of hepatic microsomal PROD activity (<450 pmol/min/mg). In parallel experiments, western blot analysis of immunoreactive CYP2B1 and CYP2B2 proteins showed that PB, the PCB mixtures, and congeners induced both proteins. Previous studies have identified a *cis*-acting DNA element that plays a role in regulating *CYP2B1/B2* gene expression and binds nuclear *trans*-acting factor(s) induced by PB. The results of gel electrophoretic mobility shift assays with nuclear extracts showed that both PB and 2,2′,3,4′,5,5′,6-heptaCB induce formation of a common retarded band using a ^{32}P-labeled oligonucleotide corresponding to the *cis*-acting DNA promoter sequence. Both PB and PCBs appear to induce CYP2B1/B2 via a common mechanism. Although the results of this study do not define structure–induction (CYP2B1/B2) relationships for PCBs, two compounds, namely 2,3,3′,4′,5,6-hexaCB and 2,2′,3,4′,5,5′,6-heptaCB, were identified as highly potent inducers.

Key words: CYP2B; hepatic induction; PCBs, structure-dependent; Sprague–Dawley rats

Commercial PCB§ mixtures, such as the Aroclors, elicit a number of toxic and biochemical responses in laboratory animals and mammalian cells in culture. Some of the toxic responses include immunosuppressive effects, hepatotoxicity, dermal toxicity, neurodevelopmental and behavioral effects, developmental and reproductive problems, carcinogenesis, and tumor promoter activity [reviewed in Refs. 1 and 2]. PCB mixtures also induce several biochemical responses in laboratory animals, and these include induction of phase I and phase II drug-metabolizing enzymes such as CYP1A and CYP2B proteins and/or dependent enzyme activities and glutathione *S*-transferase, epoxide hydrolase, and glucuronosyl transferase activities [1]. Aroclor 1254 was characterized initially as a mixed-type inducer of drug-metabolizing enzymes resembling the induction pattern observed after coadministration of both PB and MC [3]. Subsequent studies have characterized individual PCB congeners that exhibit both MC- and mixed-type activity [1, 2, 4–9]; these compounds also competitively bind to the cytosolic Ah receptor [10] and are responsible for some of the toxic responses associated with the commercial PCBs. The nonortho coplanar and monoortho coplanar PCB congeners are relatively minor components of commercial and environmental PCB mixtures, and several studies have reported that other congeners elicit diverse Ah receptor-independent responses [1, 11–16]. 2,2′,4,-4′,5,5′-Hexachlorobiphenyl (hexaCB, No. 153) is the dominant PCB congener in most environmental samples [17, 18], and this compound resembles PB as an inducer of *CYP2B* gene expression in rodent liver [9, 19–21].

The effects of structure on the activity of PCB congeners as PB-like inducers of cytochrome P450-dependent enzyme activity were investigated previously using the relatively insensitive aldrin expoxidase and dimethylaminoantipyrine *N*-demethylase assays [22]. The study

† Corresponding author. Tel. (409) 845-5988; FAX (409) 862-4929.§ Abbreviations: Ah, aryl hydrocarbon; CB, chlorinated biphenyl; MC, 3-methylcholanthrene; PB, phenobarbital; PCBs, polychlorinated biphenyls; and PROD, pentoxyresorufin *O*-dealkylase.

investigated a series of 2- and 4-chloro- and 2,4-dichloro substituted compounds that contained different chlorine substitution patterns on the second phenyl ring. The 2,4-dichlorosubstituted biphenyls were the most active inducers, and the induction potency varied with the substitution pattern on the second phenyl ring, namely 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. PROD activity has been identified as a sensitive indicator of CYP2B induction by PB-like inducers [23]. This study reports the activity of four PCB mixtures and eighteen congeners (Fig. 1) as inducers of hepatic microsomal PROD activity in female Sprague–Dawley rats. At least five congeners were more active than 2,2′,4,4′,5,5′-hexaCB as inducers of PROD activity, and 2,3,3′,4′,5,6-hexaCB was the most potent inducer.

MATERIALS AND METHODS

Chemicals and biochemicals

The commercial Aroclors were a gift of Dr. B. J. Camp, Texas A&M University. The PCB congeners (>98% pure as determined by gas chromatographic analysis) were synthesized in this laboratory as previously described [24]. Horseradish peroxidase-conjugated goat anti-rabbit IgG, goat anti-sheep IgG and T4-polynucleotide kinase were purchased from the Promega Corp. (Madison, WI) and the Sigma Chemical Co. (St. Louis, MO), respectively. The ECL detection system was obtained from the Amersham Corp. (Arlington Heights, IL). The BCA protein assay reagent was obtained from Pierce (Rockford, IL). Nitrocellulose was purchased from Schleicher & Schuell (Keene, NH), while all other materials for electrophoresis were obtained from Bio-Rad Laboratories (Richmond, CA). Acrylamide:bisacrylamide solution was purchased from National Diagnostics (Atlanta, GA). Ethoxyresorufin and pentoxyresorufin were synthesized in this laboratory, and all other chemicals were purchased from the Sigma Chemical Co. or were the highest quality available from the commercial vendors.

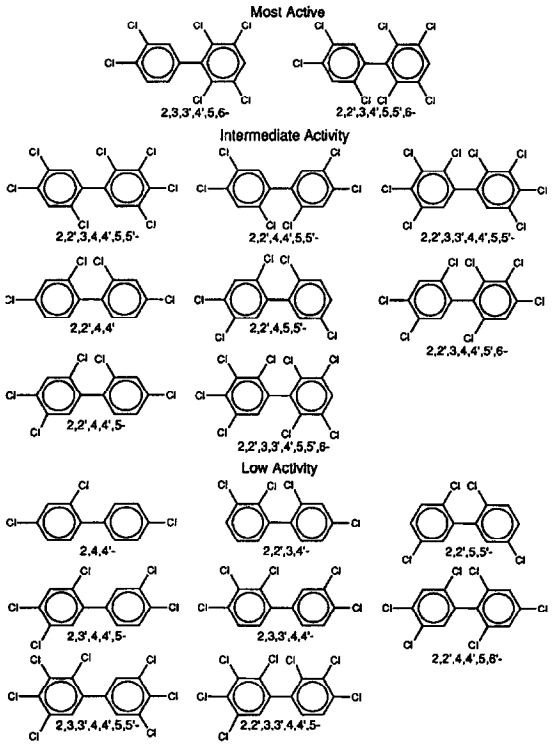

Fig. 1. Structures of PCB congeners used to investigate structure–induction (CYP2B1/B2) relationships.

Animal treatment

Female Sprague–Dawley rats weighing 120–150 g were housed in plastic cages and allowed free access to Purina Laboratory Chow and water. The rats (4–5 per treatment group) were injected intraperitoneally with a PCB congener or an Aroclor mixture in corn oil (10 mL/kg) at doses of 0, 0.5, 5, 25, 50, 100 or 150 mg/kg body weight. Some animals received PB in saline on 3 consecutive days at doses of 25 or 75 mg/kg. Three days after initiating treatment, the animals were killed by CO_2 asphyxiation, and the livers were perfused with isotonic saline and removed. Gas-chromatographic analysis of PCB levels in liver samples from rats treated with 50 mg/kg 2,2′,3,4′,5,5′,6-heptaCB and 2,3,3′,4′,5,6-hexaCB were carried out by the Geochemical and Environmental Research Group (College Station, TX) as previously described [25].

Preparation of microsomal suspensions

Liver samples were homogenized in a sucrose/EDTA (0.25 M sucrose/0.1 M EDTA) solution (pH 7.6) using a stainless steel Polytron homogenizer. The homogenate was centrifuged at 10,000 *g* for 15 min at 4°. The resulting supernatant was further centrifuged at 105,000 *g* for 1 hr to yield the cytosolic (soluble) and microsomal (pellet) fractions. The pellet was washed twice with the sucrose/EDTA solution, resuspended in the same buffer, and the suspension was diluted to 1 mg protein/mL. Suspensions were stored at −80° until required for enzyme assays.

PROD assay

PROD activity was measured by fluorimetric methods as previously described [23, 26]. Incubation mixtures consisted of 0.1 mg NADH, 0.1 mg NADPH, 1.5 mg BSA, 0.7 mg $MgSO_4$ in 1.15 mL of 0.1 M *HEPES* (pH 7.6). Ten-minute reactions were initiated with the addition of 10 μL of 500 μM pentoxyresorufin and terminated with 2.5 mL methanol. The production of resorufin was measured with a Perkin–Elmer fluorimeter (model 650-10M) with excitation and emission wavelengths of 550 and 585 nm, respectively. Protein was measured by the method of Bradford [27].

Immunoblot analysis

Polyclonal antisera (IgG fractions) specifically recognizing CYP2B1/CYP2B2 were generated in the laboratory of Dr. Colin Jefcoate, as described elsewhere [28]. Microsomal proteins were subjected to SDS-PAGE (3% acrylamide stacking gel, 7.5% acrylamide separating gel), followed by immunoblot analysis [29, 30]. Immunoreactive proteins were visualized using the ECL method of detection, according to the manufacturer's protocol.

Gel mobility shift analysis

Sections of liver weighing 1 g were homogenized in ice-cold TEGD buffer (25 mM Tris–Cl, 1.5 mM EDTA, 10% glycerol, 1 mM dithiothreitol, pH 7.4), and nuclear fractions were prepared according to the method of Blobel and Potter [31] and extracted with a 1-hr incubation in 0.5 M KCl/TEGD. Extracts equal to 10 mg protein were incubated with 1 mg of poly[d(I-C)], and ^{32}P-labeled oligonucleotide derived from a *cis*-acting DNA element [32] in HEGD buffer (25 mM HEPES, 1.5 mM EDTA, 1 mM dithiothreitol, 10% glycerol, pH 7.6) for 15 min at 20°. The oligonucleotide (5′-GAGGAGT-GAATAGCCAAAGCAGGAGGCGTG-3′, −98 to −69 upstream of the rat *CYP2B* gene) was annealed to its complement and ^{32}P-labeled at the 5′ end using T4-polynucleotide kinase and [γ-^{32}P]ATP. Incubations were electrophoresed for 2.5 hr at 125 V on a 6% polyacrylamide gel (acrylamide:bisacrylamide, 30:0.8%, w/v) and analyzed as described [33].

Statistical analysis

Results are expressed as means ± SD for at least 4 animals for each treatment group. Statistical differences were determined by Student's *t*-test or by ANOVA using the Duncan New Multiple Range Test.

RESULTS

The data presented in Table 1 summarize the dose–response induction of hepatic microsomal PROD activity by Aroclors 1260, 1254, 1242, and 1016 in female Sprague–Dawley rats. Significant induction was observed for the four PCB mixtures at a dose of 50 mg/kg, and, at this dose, the induced activity was lowest for Aroclor 1016. At a dose of 500 mg/kg, Aroclor 1016 induced a significantly higher response (1708 pmol/min/mg) than the other mixtures.

The results in Table 2 summarize the induction of hepatic microsomal PROD activity in female Sprague–Dawley rats by eighteen PCB congeners. At the lowest dose (0.5 mg/kg), the induction response was minimal or not significant for these congeners; at a dose of 5 mg/kg, less than a 2.5-fold induction response was observed for the following congeners: 2,4,4′-triCB, 2,2′,5,5′-tetraCB, 2,2′,4,4′-tetraCB, 2,2′,4,4′,5,6′-hexaCB, 2,2′,3,3′,4,4′,5-heptaCB, and 2,2′,3,3′,4,4′,5,5′-octaCB. The shape of the dose–response curves for the congeners was highly variable, and many of the ED_{50} values calculated by log probit analysis (data not shown) exhibited low *r* values. The relative potencies of the PCB congeners were estimated by comparing their induced activities at the high dose (150 mg/kg for all congeners except 2,3,3′,4,4′,5,5′-heptaCB and 2,3′,4,4′,5-pentaCB in which 100 mg/kg doses were used) with that observed for PB (75 mg/kg/day for 3 days; PROD activity, 2154 ± 110 pmol/min/mg). The most active inducers were 2,3,3′,4′,5,6-hexaCB and 2,2′,3,4′,5,5′,6-heptaCB; at a dose of 150 mg/kg, the PROD activity induced by 2,2′,3,4′,5,5′,6-heptaCB was comparable to that observed for PB. 2,3,3′,4′,5,6-HexaCB was the most potent inducer, and hepatic PROD activity in rats treated with 150 mg/kg was 4202 pmol/min/mg; this value was higher than observed for PB at a dose of 75 mg/kg. A second group of congeners including 2,2′,3,4,4′,5,5′-heptaCB, 2,2′,4,4′,5,5′-hexaCB, 2,2′,-3,3′,4,4′,5,5′-octaCB, 2,2′,4,4′-tetraCB, 2,2′,4,5,5′-pentaCB, 2,2′,3,4,4′,5′,6′-heptaCB, 2,2′,4,4′,5-pentaCB and 2,2′,3,3′,4′,5,5,6-octaCB induced PROD activity ≥ 1090 pmol/min/mg at the 150 mg/kg dose, and this value was >50% of the maximal response observed for PB. The remaining compounds, namely 2,4,4′,triCB, 2,2′,3,4′-tetraCB, 2,2′,5,5′-tetraCB, 2,3′,4,4′,5-pentaCB, 2,3,3′,4,4′-pentaCB, 2,2′,4,4′,5,6′-hexaCB, 2,3,3′,4,4′,5,5′-heptaCB and 2,2′,3,3′,4,4′,5-heptaCB were all relatively weak inducers of hepatic microsomal PROD activity (<450 pmol/min/mg). The data summarized in Table 2 show that SD values were relatively high for some congeners. This was further investigated by comparing the induced PROD activity versus liver PCB levels for two congeners, namely 2,3,3′,4′,5,6-hexaCB and 2,2′,3,4′,5,5′,6-heptaCB, at the 50 mg/kg dose. The SD values for these compounds were >50% of the mean PROD activity. The results presented in Fig. 2 illustrate that there was a correlation between the tissue PCB levels of these congeners and induced PROD activity ($r = 0.72$). Sprague–Dawley rats are outbred, and these data illustrate that there could be considerable intra-individual variability in hepatic uptake of these compounds.

Polyclonal antisera (IgG fractions), which specifically recognize CYP2B1 and CYP2B2, were utilized to determine the induction of immunoreactive proteins. The results presented in Fig. 3 illustrate the western blot analysis of immunoreactive hepatic CYP2B1 and CYP2B2 induced by 2,2′,4,4′,5-pentaCB, 2,2′,4,5,5′-pentaCB,

Table 1. Induction of hepatic microsomal PROD activity in female Sprague-Dawley rats by Aroclors 1260, 1254, 1242, and 1016*

	PROD activity (pmol/min/mg)					
	Dose					
Inducer	0 mg/kg	0.5 mg/kg	5 mg/kg	50 mg/kg	150 mg/kg	500 mg/kg
Aroclor 1260	10.5 ± 0.9	9.9 ± 0.8	30 ± 15†	252 ± 103†	536 ± 260†	566 ± 197†
Aroclor 1254	11 ± 1.6	14 ± 2.2	27 ± 10†	373 ± 140†	567 ± 225†	438 ± 275†
Aroclor 1242	17 ± 2.0	20 ± 1.8	27 ± 15	115 ± 72†	441 ± 230†	516 ± 193†
Aroclor 1016	15 ± 2.5	15 ± 1.5	15 ± 0.9	30 ± 13†	248 ± 211†	1708 ± 802†

* The PCBs in corn oil were administered as a single dose by intraperitoneal injection, and after 3 days hepatic microsomes were isolated by differential centrifugation as described in Materials and Methods. PROD activity was determined fluorimetrically [24, 25]. PB in saline was administered on 3 consecutive days, and hepatic microsomal PROD activity was determined as described in Materials and Methods. PROD activity observed at a PB dose of 75 mg/kg for 3 days was 2154 ± 110 pmol/min/mg (means ± SD), and this value was used as the maximal induced dose. Results are means ± SD with at least four animals for each treatment group.

† Significantly higher ($P < 0.05$) than in control animals.

Table 2. Induction of hepatic microsomal PROD activity in female Sprague-Dawley rats by eighteen PCB congeners*

Congener (IUPAC No.)	PROD activity (pmol/min/mg) Dose 0 mg/kg	0.5 mg/kg	5 mg/kg	25 mg/kg	50 mg/kg	150 mg/kg	% of PB-Induced activity at high dose
Octa-CBs							
2,2',3,3',4,4',5,5'-OctaCB (194)	4.0 ± 2.5	6.0 ± 0.8	9.0 ± 0.7†	658 ± 587†	846 ± 630†	1352 ± 602†	63
2,2',3,3',4',5,5',6-OctaCB (201)	10 ± 2.0	11 ± 1.7	212 ± 212†	560 ± 384†	717 ± 421†	1090 ± 97†	51
Hepta-CBs							
2,2',3,4,4',5,5'-HeptaCB (180)	17 ± 4.5	17 ± 0.6	38 ± 34	ND	625 ± 485†	1584 ± 624†	74
2,2',3,4,4',5',6-HeptaCB (183)	10 ± 2.5	9.1 ± 1.2	71 ± 92†	393 ± 240†	385 ± 344†	1198 ± 969†	56
2,2',3,4',5,5',6-HeptaCB (187)	6.6 ± 0.3	12 ± 1.8	234 ± 136†	ND	1587 ± 861†	2194 ± 842†	102
2,2',3,3',4,4',5-HeptaCB (170)	7.8 ± 0.5	8.1 ± 0.5	15 ± 1.5†	ND	144 ± 77†	441 ± 244†	20
2,3,3',4,4',5,5'-HeptaCB (189)	13 ± 0.7	19 ± 3.2	60 ± 16†	206 ± 103†	302 ± 59†	367 ± 59†	17
Hexa-CBs							
2,2',4,4',5,5'-HexaCB (153)	11 ± 2.7	11 ± 1.7	27 ± 10†	ND	550 ± 180†	1377 ± 355†	64
2,2',4,4',5,6'-HexaCB (154)	18 ± 3.5	14 ± 3.5	15 ± 6.5	62 ± 66[b]	236 ± 140[b]	360 ± 339[b]	17
2,3,3',4',5,6,-HexaCB (163)	8.2 ± 2.2	9.9 ± 1.1	28 ± 23†	437 ± 315†	1287 ± 681†	4202 ± 245†	195
Penta-CBs							
2,3,3',4,4'-PentaCB (105)	10 ± 4.3	23 ± 8.3	56 ± 24†	ND	116 ± 21†	277 ± 83†	13
2,2',4,4',5-PentaCB (99)	8.0 ± 0.3	10 ± 2.3	46 ± 40†	668 ± 362†	1139 ± 342†	1129 ± 547†	52
2,2',4,5,5'-PentaCB (101)	17 ± 1.9	18 ± 1.5	168 ± 90†	180 ± 101†	771 ± 687†	1243 ± 630†	58
2,3',4,4',5-PentaCB (118)	16 ± 1.3	26 ± 16	41 ± 6†	180 ± 72†	350 ± 91†	384 ± 136†	18
Tetra/Tri-CBs							
2,2',4,4'-TetraCB (47)	7.1 ± 2.7	15 ± 5.4	10 ± 1.5	ND	360 ± 235†	1310 ± 147†	61
2,2',5,5'-TetraCB (52)	9.9 ± 2.4	13 ± 3.0	11 ± 0.9	106 ± 124†	108 ± 69†	363 ± 330†	17
2,2',3,4'-TetraCB (42)	15 ± 0.5	16 ± 1.6	36 ± 4.2†	ND	76 ± 40†	96 ± 50†	4
2,4,4'-TriCB (28)	14 ± 2.0	15 ± 1.8	16 ± 2.3	21 ± 13	18 ± 524	290 ± 156†	13

* The treatment protocols utilized for the PCB mixtures (Table 1) and conteners were identical. The high doses of 2,3,3',4,4',5,5'-heptaCB and 2,3',4,4',5-pentaCB were 100 mg/kg due to limited availability. ND = not done at this dose. Results are means ± SD with at least four animals for each treatment group.

† Significantly higher ($P < 0.05$) than in control animals.

2,2',4,4',5,5'-hexaCB, 2,2',3,4',5,5',6-heptaCB, 2,3,-3',4',5,6-hexaCB and 2,2',3,3',4,4',5,5'-octaCB. Both CYP1B1 and CYP2B2 were induced by the PCB congeners, and CYP2B1 was the major inducible protein. Similar results were observed for PB and the other PCB congeners and mixtures (data not shown).

Previous studies have identified a *cis*-acting DNA element that plays a role in regulating CYP2B1/B2 gene expression and binds nuclear *trans*-acting factor(s) induced by PB [32]. The results in Fig. 4 illustrate that after incubation of [^{32}P]oligonucleotide with nuclear extracts from PB-treated animals, an intense retarded band was detected in a gel electrophoretic mobility shift assay. This retarded band was also observed in hepatic nuclear extracts from control (corn oil-treated) animals, and the band was also induced by 2,2',3,4',5,5',6-heptaCB. The retarded band was not observed after incubating hepatic nuclear extracts from 2,2',3,4',5,5',6-heptaCB-treated animals in the presence of ^{32}P-labeled oligonucleotide plus a 500-fold excess of unlabeled oligonucleotide.

DISCUSSION

Commercial PCB mixtures have been identified as "mixed-type" inducers of drug-metabolizing enzymes in several animal models, and the induced responses resemble those observed after cotreatment of the animals with PB plus MC [16]. The nonortho and monoortho coplanar PCB congeners have been characterized extensively as Ah receptor agonists and are primarily respon-

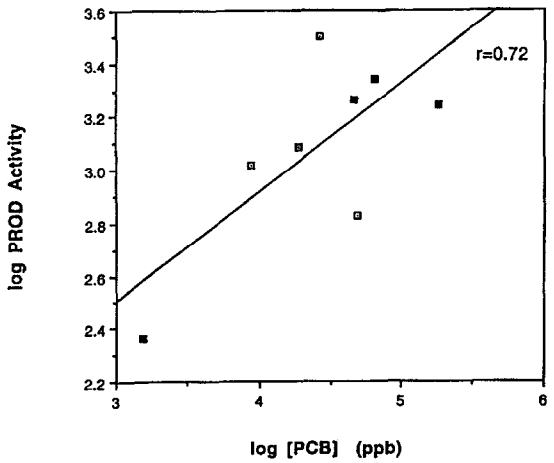

Fig. 2. Correlation between log PROD activity versus log PCB congener levels. Female Sprague–Dawley rats (4 per group) were treated with 50 mg/kg of 2,2′,3,4′,5,5′,6-heptaCB (■) or 50 mg/kg of 2,3,3′,4′,5,6-hexaCB (⊡), and hepatic microsomal PROD activity and residue levels were determined as described in Materials and Methods. The results obtained for the individual animals were plotted in this figure.

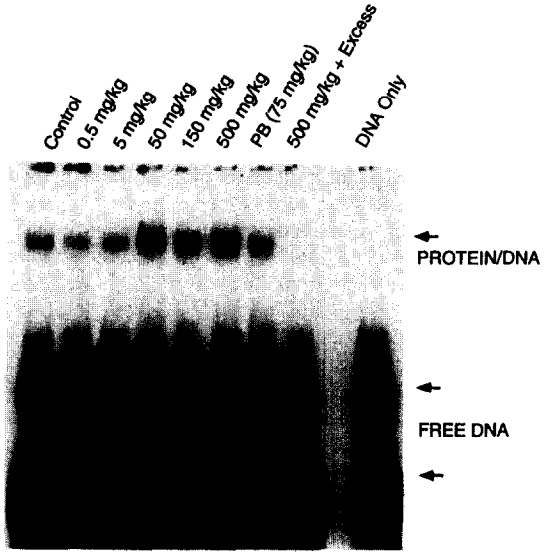

Fig. 4. Gel electrophoretic mobility shift assay of nuclear extracts from treated animals with [^{32}P]oligonucleotide derived from the CYP2B2 5′-promoter sequence [32]. Nuclear extracts from animals treated with corn oil (control), 2,2′,3,4′,5,5′,6-heptaCB (0.5, 5, 50, 150 and 500 mg/kg), or PB (75 mg/kg/day for 3 days) were incubated with [^{32}P]oligonucleotide and analyzed in a gel electrophoretic mobility shift assay. The specifically bound retarded band (←protein/DNA) was not observed after incubation with a 500-fold excess of unlabeled oligonucleotide.

sible for the MC-type induction of CYP1A1 and CYP1A2 [4–9]. The monoortho coplanar PCB congeners are mixed-type inducers, whereas PCBs that primarily induce CYP2B1/B2 and resemble PB usually contain two or more ortho-chloro substituents [9, 19–22]. 2,2′,4,4′-TetraCB and 2,2′,4,4′,5,5′-hexaCB are two compounds that have been characterized previously as prototypical PB-type inducers. This study has focused on the induction of hepatic microsomal PROD activity and immunoreactive CYP2B1/B2 in female Sprague–Dawley rats by commercial PCB mixtures and eighteen congeners that contain 1–3 ortho-chloro substituents.

Several criteria were utilized to select the eighteen congeners used in this study. The chlorination levels (tri- to octachloro) and substitution patterns were highly variable, and all congeners were substituted with at least one ortho-chloro group. In addition, many of the congeners were either major components (>5%) in commercial Aroclors and/or environmental samples [17, 18, 24], and these include 2,2′,4,4′,5,5′-hexaCB, 2,3′,4,4′,5-pentaCB, 2,3,3′,4,4′-pentaCB, 2,2′,4,4′,5-pentaCB, 2,4,4′-triCB, and 2,2′,3,34,4′,5-heptaCB.

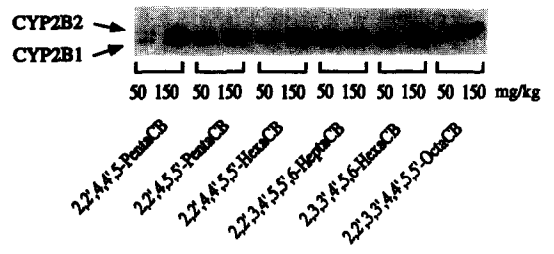

Fig. 3. Western blot analysis of hepatic microsomal protein from PCB-treated rats. Rats were treated with different doses of 2,2′,4,4′,5-pentaCB, 2,2′,4,4′,5,5′-pentaCB, 2,2′,4,4′,5,5′-hexaCB, 2,2′,3,4′,5,5′,6-heptaCB 2,3,3′,4′,5,6-hexaCB and 2,2′,3,3′,4,4′,5,5′-octaCB, microsomes were isolated, and western blot analysis was determined as described in Materials and Methods [28–30]. The CYP2B1/B2 induction pattern was observed for PB and all PCB congeners and mixtures (data not shown).

The results in Table 1 summarize the induction of PROD activity by Aroclors 1260, 1254, 1242, and 1016. The threshold for induction of hepatic PROD activity was 5.0, 5.0, 50 and 50 mg/kg, respectively, for these mixtures, and, at a dose of 500 mg/kg, only Aroclor 1016 induced PROD activity greater than the half-maximal response for PB (1077 pmol/min/mg). Aroclor 1260 was a more potent inducer of PROD activity than Aroclors 1254, 1248, 1242, and 1232 in male Wistar rats [34], whereas in the present study the activity of Aroclor 1260 was comparable to that of Aroclors 1254 and 1242. In contrast, Aroclor 1016 was the most active mixture in female Sprague–Dawley rats; the reason for the strain-dependent differences in PROD inducibility by the PCB mixtures is unknown.

The results in Table 2 summarize the dose-dependent induction of hepatic microsomal PROD activity by eighteen different PCB congeners (Fig. 1), which vary in degree of chlorination (3 to 8) and substitution pattern. All of the PCB congeners exhibited a threshold dose (0.5 mg) for induction of hepatic microsomal PROD activity. Three lower chlorinated congeners, namely 2,2′,4,4′-tetraCB, 2,2′,4,5,5′-pentaCB, and 2,2′,4,4′,5-pentaCB, induced PROD activity > 1000 pmol/min/mg at the 150 mg/kg dose level, and these compounds all contained the 2,4,5-trichloro or 2,4-dichloro substitution pattern in at least one of the phenyl rings. These results were consistent with previous structure–induction studies with PCB congeners as PB-type inducers [9, 22].

The structure–induction relationships for the higher chlorinated congeners (hexa- to octachloro) showed that all ten congeners induced hepatic microsomal

PROD activity and, at a dose of 150 or 100 mg/kg, only three compounds, namely 2,2′,4,4′,5,6′-hexaCB, 2,3,3′,-4,4′,5,5′-heptaCB and 2,2′,3,3′,4,4′,5-heptaCB, induced PROD activity that was <500 pmol/min/mg. Previous studies have demonstrated that these congeners induce CYP2B1/B2 and related enzyme activities in immature male Wistar and Long Evans rats [4–9]; however, their relative potencies were not determined. The remaining hexa- to octaCBs exhibited structure-dependent potencies as inducers of hepatic microsomal PROD activity, and the most active compounds were 2,2′,3,4′,5,5′,6-heptaCB and 2,3,3′,4′,5,6-hexaCB. 2,3,3′,4′,5,6-HexaCB is a minor component of commercial PCB mixtures and environmental extracts; however, both 2,2′,4,4′,5,5′-hexaCB and 2,2′,3,4′,5,5′,6-heptaCB are relatively major components in commercial mixtures and environmental samples [17, 18]. The two most active PCB congeners contain either a 3,4-dichloro or 2,4,5-trichloro substitution pattern on one phenyl ring and the 2,3,5,6-tetrachloro substitution on the second phenyl ring. However, the results also show that several compounds that contain a 2,3,4,5-tetrachloro substitution pattern on one phenyl ring (i.e. 2,2′,3,3′,4,4′,5,5′-octaCB, 2,2′,3,4,4′,5,5′-heptaCB and 2,2′,3,3′,4′,5,5′,6-octaCB) also induced hepatic microsomal PROD activity > 1000 pmol/min/mg at the 150 mg/kg dose. In contrast, 2,3,3′,4,4′,5,5′,-heptaCB, a monoortho coplanar PCB congener that has been identified previously as a mixed-type inducer [9], induced relatively low levels of PROD activity at the 100 mg/kg dose. The reduced activity of this congener appears to be associated not with the 2,3,4,5-substitution pattern but with the presence of only a single orthochloro substituent. Rearrangement or addition of a single ortho-chloro group (i.e. see Fig. 5) converts the 2,3,-3′,4,4′,5,5′-heptaCB into 2,2′,3,4,4′,5,5′-heptaCB or 2,2′,3,3′,4,4′,5,5′-octaCB, which were significantly more active as inducers of PROD activity (Table 2). The relatively low activity of monoortho coplanar PCBs as inducers of PROD activity in female Sprague–Dawley rats

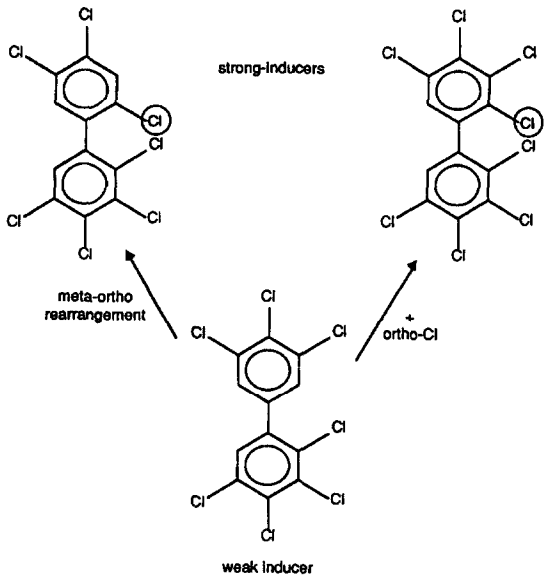

Fig. 5. Effects of structural modifications of 2,3,3′,4,4′,5,5′-heptaCB on the activity of the resulting congeners as PB-type inducers.

is also observed with both 2,3,3′,4,4′-pentaCB and 2,3,4,4′,5′-pentaCB. The induction of CYP2B1/B2 by these compounds in male Long Evans rats was more pronounced [9]; however, a quantitative comparison of induction responses in the two rats strains is not possible since the data obtained in Long Evans rats were derived from a single dose (500 μmol/kg) of these congeners [9].

The results presented in Fig. 3 show that several of the PCB congeners used in this study induce immunoreactive CYP2B1 and CYP2B2, as indicated by western blot analysis. These induction patterns were also observed for PB and the other PCB congeners and mixtures (data not shown); however, a quantitative comparison of the immunologic and induced PROD activity was not determined. Additional confirmation that PB and PCBs induce CYP2B1/B2 via common pathways was obtained using gel electrophoretic mobility shift assays of hepatic nuclear extracts from rats treated with PB or a different dose of 2,2′,3,4′,5,5′,6′-heptaCB. Previous studies have identified *cis*-acting DNA sequence in the 5′-flanking region of the *CYP2B2* gene, which appears to be important for induction of *CYP2B2* gene expression by PB [31]. This DNA element binds to PB-induced nuclear factors to form a DNA–protein complex that can be detected *in vitro* by gel electrophoretic mobility shift assays. The results in Fig. 4 illustrate that both PB and 2,2′,3,4′,5,5′,6-heptaCB induce formation of the same retarded band (protein/DNA; Fig. 4), and in competition experiments with a 500-fold excess of unlabeled oligonucleotide the radiolabeled retarded band was eliminated. Thus, PB and 2,2′,3,4′,5,5′,6-heptaCB induce *trans*-acting factors that specifically bind to the CYP2B2 DNA element, which is required for transactivation of the *CYP2B2* gene. These data are in contrast to studies by Poornima and coworkers [32], who showed that the electrophoretic mobilities of the DNA–protein complexes from control and PB-treated Wistar rats were different. Strain-dependent DNA–protein complex formation from control and induced rats should be investigated further.

The structure–induction relationships for PCBs as PB-type inducers demonstrate that several structurally diverse congeners induce hepatic microsomal PROD activity in female Sprague–Dawley rats. Analysis of the results does not reveal an obvious PCB structure–activity relationship since several substitution patterns on the two phenyl rings will confer activity on PCBs as PB-type inducers. However, this study has identified several compounds that appear to be more active than 2,2′,4,4′,5,5′-hexaCB as inducers of PROD activity. Moreover, at least one compound, namely 2,2,3′,4′,5,6-hexaCB, was a "super" inducer in which the PROD activity observed at the 150 mg/kg dose (4202 pmol/min/mg) was significantly higher than the maximally induced response observed for PB (2154 ± 110 pmol/min/mg) at a dose of 75 mg/kg/day for 3 days. There appeared to be intra-individual differences in the induction response for some PCB congeners. For example, the SD values were >50% of the mean induced PROD response for both 2,2′,3,4′,5,5′,6-heptaCB and 2,3,3′,4′,5,6-hexaCB at the 50 mg/kg dose. The results presented in Fig. 2 show that there were intra-individual differences in hepatic levels of these congeners, and this may be due to the genetics of Sprague–Dawley rats, which are an outbred strain. Despite the variability in hepatic levels of these congeners, there was a linear

correlation ($r = 0.72$) between log tissue concentration versus log PROD activity for the individual animals in the treatment groups.

Previous studies have demonstrated that halogenated biphenyl mixtures and some PB-type inducers are liver tumor promoters [11–14], and it has been suggested that induction responses may be associated with tumor promoting activity [35–38]. A recent study by Rice and coworkers [38] with a series of barbiturates has shown that both duration of sedative action and hepatic CYP2B induction potency correlate with parameters related to liver tumor promoting activity in F344/NCr male rats. In contrast, for a series of structurally related 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) analogs, their potency as liver tumor promoters in male Sprague–Dawley rats did not correlate with their activity as inducers of hepatic CYP2B [39]. The results of the present study have defined a series of PCB congeners with variable activities as CYP2B inducers, and future studies will utilize some of these congeners to investigate possible correlations between CYP2B induction and liver tumor promoter activities for PCB congeners and mixtures.

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