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DIFFERENTIAL POTENCY OF ATROPISOMERS OF POLYCHLORINATED BIPHENYLS ON CYTOCHROME P450 INDUCTION AND UROPORPHYRIN ACCUMULATION IN THE CHICK EMBRYO HEPATOCYTE CULTURE*

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Abstract—The atropisomers of 2,2′,3,4,6-pentachlorobiphenyl (PeCB), 2,2′,3,4,4′,6-hexachlorobiphenyl (HeCB), and 2,2′,3,3′,4,4′,6,6′-octachlorobiphenyl (OCB) were studied in the chick embryo hepatocyte culture to determine if chirality plays a role in the recognition events associated with the induction of cytochromes P450 and the accumulation of uroporphyrin (URO). Concentration-related induction of cytochrome P450 content, ethoxyresorufin-*O*-deethylase (EROD) and benzphetamine *N*-demethylase (BPDM) activities were measured. The rank order of potency for total cytochrome P450 induction was HeCB > OCB ≥ PeCB. The (+)- and (−)-enantiomers of PeCB and OCB were of equal potencies as inducers of cytochromes P450, whereas the (+)-HeCB was greater than the (−)-HeCB. HeCB was a much more potent inducer of EROD activity than was either PeCB or OCB. EROD activity was induced to a much greater extent by the (+)-enantiomers of all compounds, with the (−)-enantiomers of PeCB and OCB being inactive. BPDM activity was induced by all three compounds in the order of OCB ≥ HeCB > PeCB. The (−)-enantiomers were more potent inducers of BPDM activities than were the (+)-enantiomers, except for HeCB, in which the (+)- was more potent than the (−)-enantiomer. Analysis of porphyrin accumulation in cultures treated with δ-aminolevulinic acid revealed that (+)-HeCB caused the greatest percent URO accumulation, which also correlated with the greatest increase in EROD activity. All other enantiomers caused up to 47% URO accumulation, which did not correlate with an increase in EROD activity.

There are 209 PCB¶ congeners [1] of which 78 possess axial chirality [2, 3]. Due to the presence of multiple *ortho* substituents, which limit free rotation about the phenyl—phenyl bond (racemization), only 19 of the 78 compounds were predicted to be stable to racemization at body temperature [2, 4]. We have reported previously the synthesis and purification of the stable enantiomers of 2,2′,3,4,6-pentachlorobiphenyl (PeCB), 2,2′,3,4,4′,6-hexachlorobiphenyl (HeCB) and 2,2′,3,3′,4,4′,6,6′-octachlorobiphenyl (OCB) [2, 5] (see Fig. 1).

Structure–activity studies in the rat have shown that PCBs may be classified on the basis of their ability to induce the cytochrome P450 enzymes [6–9], as being like phenobarbital (PB) or 3-methylcholanthrene (MC), or like the combination of PB and MC (mixed inducers). In the rat, racemic PeCB has been shown to be inactive as an inducer of cytochrome P450 enzymes [10]. On the other hand, racemic HeCB (a potent inducer) and racemic OCB (a weak inducer) have been shown to be PB-like inducers of cytochrome P450 enzymes [3, 10, 11]. We have reported previously that the (+)-enantiomer of HeCB in the rat was a more potent inducer of total cytochromes P450, aldrin epoxidase and aminopyrine *N*-demethylase activities than was the (−)-enantiomer [3]. The (+)- and (−)-enantiomers of OCB were of equal potency [3]. The difference in potency of the enantiomers of HeCB may have been due to an enantioselective metabolism or transport, rather than inherent induction potency [3].

In the present study, a chick embryo liver cell culture was used to study the effects of the racemic mixture and the (+)- and (−)-enantiomers of PeCB, HeCB and OCB on the induction of total cytochromes P450, ethoxyresorufin-*O*-deethylase (EROD) and benzphetamine *N*-demethylase (BPDM) activities, and the accumulation of uroporphyrin (URO). This system was chosen in order to minimize pharmacokinetic influences on cytochrome P450 induction, and also because porphyrin accumulation can be generated within hours after the addition of

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¶ Abbreviations: ALA, δ-aminolevulinic acid; BPDM, benzphetamine *N*-demethylase; DMSO, dimethyl sulfoxide; EROD, ethoxyresorufin-*O*-deethylase; HeCB, hexachlorobiphenyl; MC, 3-methylcholanthrene; OCB, octachlorobiphenyl; PB, phenobarbital; PCB, polychlorinated biphenyl; PeCB, pentachlorobiphenyl; PIA, 2-propyl-2-isopropylacetamide; PROTO, protoporphyrin; and URO, uroporphyrin.

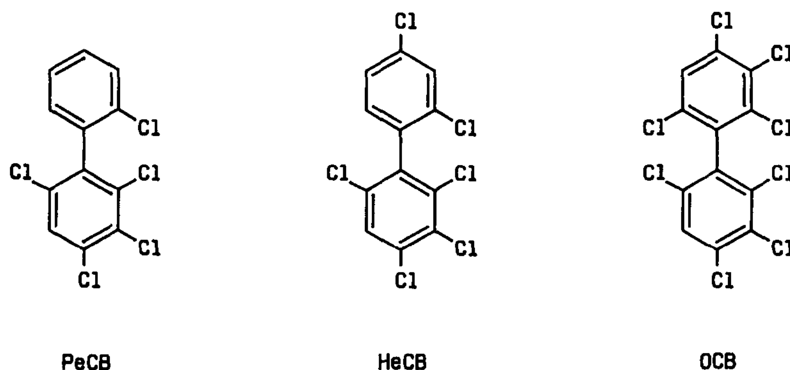


Fig. 1. Structures of 2,2′,3,4,6-pentachlorobiphenyl (PeCB), 2,2′,3,4,4′,6-hexachlorobiphenyl (HeCB) and 2,2′,3,3′,4,4′,6,6′-octachlorobiphenyl (OCB).

the PCBs. We report a difference in potency by the enantiomers for induction of cytochrome P450 enzymes and uroporphyrin accumulation. In addition, in contrast to the rat in which the PCBs were PB-like inducers, the PCB compounds demonstrated mixed induction of the cytochrome P450 enzymes. These data show that chiral differences are recognized in the induction of cytochrome P450, especially those that express EROD activity.

MATERIALS AND METHODS

Chemicals. Racemic PeCB and the corresponding enantiomers were synthesized and isolated as previously reported [5, 12]. The enantiomers and the racemate of HeCB were synthesized and isolated as described [2, 3]. Racemic OCB and the (+)- and (−)-enantiomers were synthesized and isolated by the method of Püttmann *et al.* [2]. All PCBs were characterized by proton magnetic resonance and mass spectrometry, and were >99% pure as determined by gas chromatography. The enantiomeric purities of the PCBs were determined by liquid chromatography [2, 5] and are as follows: (+)-PeCB (0.55), (−)-PeCB (1.00), (+)-HeCB (>0.8), (−)-HeCB (>0.95), (+)-OCB (1.00), and (−)-OCB (1.00).

Williams E medium was obtained from Gibco, Grand Island, NY. Triiodothyronine (T_3), insulin, bovine serum albumin, and 5-aminolevulinic acid (ALA) were purchased from Sigma, St. Louis, MO. Dimethyl sulfoxide (DMSO) and dexamethasone were obtained from Fisher Scientific, Cincinnati, OH, and Organon, West Orange, NJ, respectively. Benzphetamine·HCl was a gift from Upjohn (Kalamazoo, MI) and resorufin ethyl ether was obtained from Molecular Probes (Eugene, OR).

PCBs were dissolved in DMSO, and all additions of PCBs to the primary culture were made such that the final concentration of the test substance in the medium contributed only 2 μL of solvent vehicle per mL of medium.

Culture preparation. Primary hepatocyte cultures from 16-day-old White Leghorn chick embryos were prepared from eggs obtained from the University of Kentucky College of Agriculture. Cultures were prepared as previously described [13] and the cells were inoculated onto either 1-cm diameter multiwell or 6-cm diameter plastic culture plates. Cultures were incubated in humidified 5% CO_2:95% air at 37°. The cells were maintained for the first 24 hr in Williams E medium containing triiodothyronine (1 μg/mL), insulin (1 μg/mL), and dexamethasone (0.3 μg/mL). After 24 hr the medium was removed and fresh medium (devoid of insulin) along with the various inducers was added. The cells were harvested 18–20 hr later.

For porphyrin accumulation experiments, cultures in 1-cm wells were given 150 μM ALA 3 hr prior to harvest. Endogenously generated porphyrins (i.e. determined from cultures not given ALA) were not assayed or subtracted from total porphyrin accumulation.

Analytical techniques. Cytochrome P450 concentrations were determined from 8700 *g* supernatants of Emulgen 910 solubilized homogenates as previously described [14] by the CO difference spectral method of Omura and Sato [15]. BPDM and EROD activities of cell sonicates were determined as described [16], with the exception that methanol was used for the termination of EROD incubations. Porphyrins were analyzed spectrofluorometrically as uro-, copro-, and protoporphyrin by the method of Grandchamp *et al.* [17] and as described by Shedlofsky *et al.* [18] and Rodman *et al.* [19]. Protein concentrations, using bovine serum albumin as standard, were determined by the method of Lowry *et al.* [20].

Statistics. Statistical differences between the (+)- and (−)-enantiomers at the various concentrations for the different assays were performed by two-way ANOVA. If interaction was present in the ANOVA analysis, then a multiple comparison test was performed as described by Kleinbaum and Kupper [21]. Uroporphyrin data were arcsin transformed prior to the ANOVA test.

RESULTS

Induction of cytochrome P450 concentrations and activities. All PCBs induced total cytochrome P450 concentrations, with the most potent being HeCB

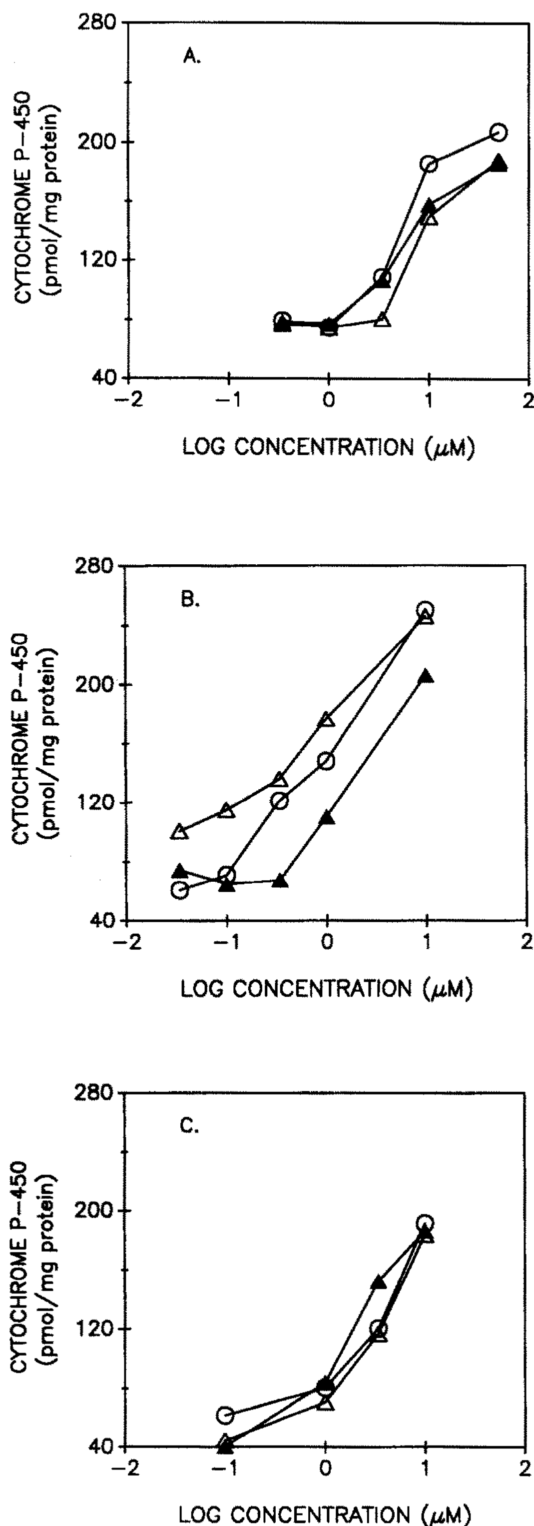


Fig. 2. Effects of (A) PeCB, (B) HeCB, and (C) OCB on the induction of total cytochrome P450 concentrations by the racemic mixture (○), (+)-enantiomer (△), and (−)-enantiomer (▲). Values are means of 3 plates with a SD < 10% of the mean (SD not shown). The total cytochrome P450 concentration for dimethyl sulfoxide controls was 40 ± 3 pmol/mg protein (N = 8). Assays and culture preparation were performed as described in Materials and Methods.

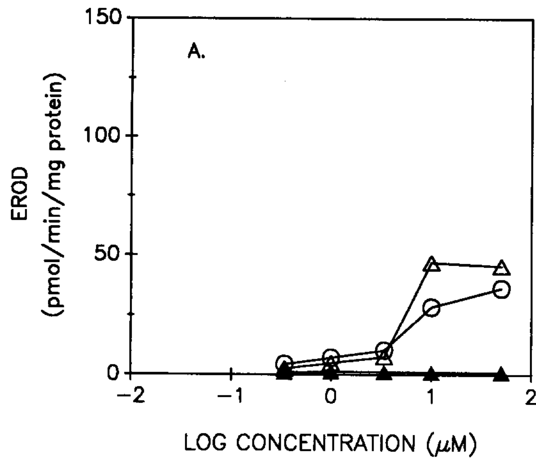
and OCB ⩾ PeCB (Fig. 2, B, C and A respectively). Although the (+)-HeCB was more potent than the (−)-HeCB, the enantiomers for PeCB and OCB were equally potent for induction of total cytochrome P450.

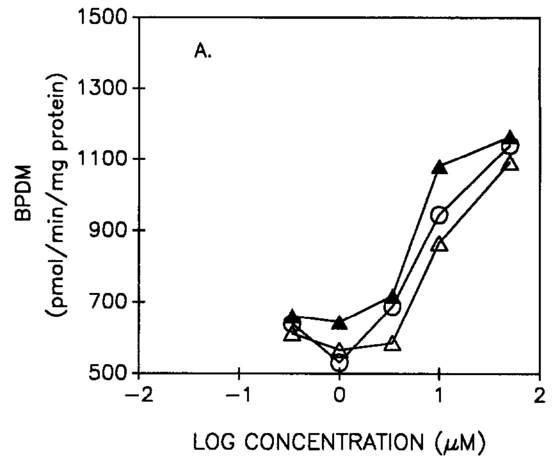
When the hepatocytes were exposed to various concentrations* of either the racemic mixture or the enantiomers of PeCB, HeCB and OCB, the most striking differences were seen for the induction of EROD activities (Fig. 3, A, B and C respectively). EROD activity was induced in the order of HeCB ≫ PeCB > OCB. The (+)-enantiomer in each case was more potent than the (−)-enantiomer. While marked EROD induction occurred with the (+)-enantiomer of HeCB, lesser EROD induction was noted for the (+)-enantiomers of PeCB and OCB. The (+)-enantiomer of HeCB had a peak inducing concentration of 0.34 μM, and higher concentrations of the HeCB caused less induction of EROD activities. *No* EROD induction was seen with the (−)-enantiomers of PeCB and OCB for all concentrations tested up to the limit of solubility (10–50 μM). The racemic mixtures induced EROD activity to levels between the (+)- and (−)-enantiomers for all compounds tested.

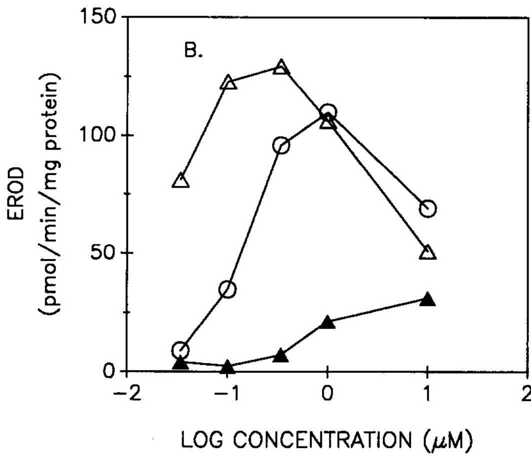
All racemates and enantiomers of PeCB, HeCB and OCB were effective inducers of BPDM activities, with the order of induction potency being OCB ⩾ HeCB > PeCB (Fig. 4, C, B and A respectively). Although the (−)-enantiomers were slightly but statistically ($P \leq 0.05$) more potent than the (+)-enantiomers for OCB and PeCB, the (+)-HeCB was more potent than the (−)-HeCB.

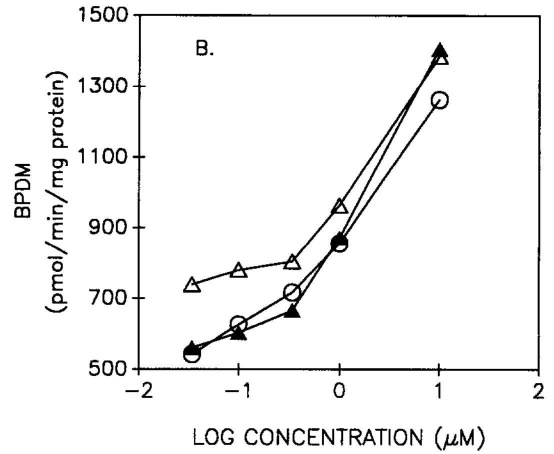
Enantiomeric effects on URO and protoporphyrin (PROTO) accumulation. Exposure of untreated chick hepatocytes to the heme precursor ALA results in porphyrin accumulation mainly as PROTO (≈88%) with little URO (≈2%) [22]. Hepatocytes pretreated with PCBs or polybrominated biphenyls, followed by ALA exposure, have a rapid increase in URO and a decrease in PROTO accumulation [19, 22]. Polyhalogenated biphenyls such as 2,3′,4,4′,5-pentabromobiphenyl [23] or 3,3′,4,4′-tetrachlorobiphenyl [24] can cause ⩾90% URO accumulation, while decreasing the PROTO accumulation to 0%. In our study, chick hepatocyte cultures exposed to various concentrations of the (+)- and (−)-enantiomers of PeCB, HeCB and OCB, followed by ALA exposure, revealed differences in URO and PROTO accumulation (Table 1). With "ALA-loading," approximately equal amounts of total porphyrins accumulated (≈30–40 pmol/well in 3 hr) in all cultures, regardless of whether they were untreated or pretreated with the PCB enantiomers or racemates. Therefore, the data are expressed as %URO or %PROTO accumulation. URO accumulation occurred only at the higher concentrations (i.e. ⩾1 μM) for PeCB and OCB, while URO accumulated at much lower concentrations for HeCB (⩾0.034 μM for the (+)-HeCB and ⩾0.34 μM for the (−)-HeCB). The greatest %URO accumulation occurred with HeCB, generating 64% URO

* The term "concentration" refers to the concentration of the PCB or inducer in the culture medium.









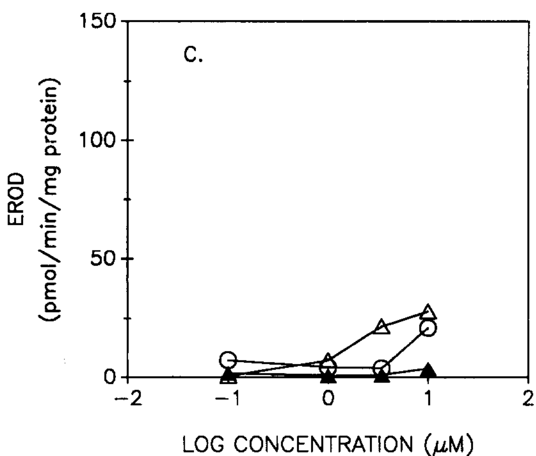


Fig. 3. Effects of (A) PeCB, (B) HeCB, and (C) OCB on the induction of ethoxyresorufin-*O*-deethylase activity (EROD) by the racemic mixture (○), (+)-enantiomer (△), and (−)-enantiomer (▲). Values are the average of 2 plates. The EROD activity for dimethyl sulfoxide controls was $<1 \pm 0$ pmol/min/mg protein (N = 5). Assays and culture preparations were performed as described in Materials and Methods.

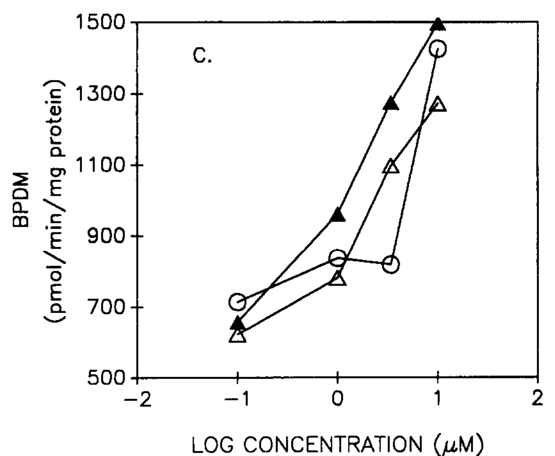


Fig. 4. Effects of (A) PeCB, (B) HeCB, and (C) OCB on the induction of benzphetamine *N*-demethylase activity (BPDM) by the racemic mixture (○), (+)-enantiomer (△), and (−)-enantiomer (▲). Values are the average of 2 plates. The BPDM activity for dimethyl sulfoxide controls was 500 ± 36 pmol/min/mg protein (N = 8). Assays and culture preparation were performed as described in Materials and Methods.

Table 1. Effects of (+)- and (−)-enantiomers of 2,2′,3,4,6-pentachlorobiphenyl (PeCB), 2,2′,3,4,4′,6-hexachlorobiphenyl (HeCB) and 2,2′,3,3′,4,4′,6,6′-octachlorobiphenyl (OCB) on percent protoporphyrin and percent uroporphyrin accumulation in the chick embryo liver cell culture

Concentration (μM)	% Protoporphyrin		% Uroporphyrin	
	(+)-PeCB	(−)-PeCB	(+)-PeCB	(−)-PeCB
0.34	90.9 ± 0.5*	88.3 ± 3.2	3.9 ± 0.3	4.2 ± 0.9
1.0	85.4 ± 1.4	87.1 ± 0.7	8.2 ± 1.2*†	5.2 ± 1.1
3.4	65.0 ± 5.7*†	83.1 ± 1.3*	17.1 ± 2.4*†	7.0 ± 0.6*
10.0	51.0 ± 2.3*†	63.2 ± 3.3*	24.5 ± 4.7*	20.7 ± 1.9*
50.0	23.7 ± 0.8*	20.5 ± 6.5*	34.1 ± 1.0*†	45.1 ± 0.8*
	(+)-HeCB	(−)-HeCB	(+)-HeCB	(−)-HeCB
0.034	85.7 ± 1.0†	89.6 ± 1.0	7.0 ± 0.6*†	4.1 ± 0.5
0.1	83.5 ± 0.7*†	89.1 ± 1.6	8.6 ± 0.1*†	4.1 ± 0.9
0.34	84.8 ± 2.3	84.6 ± 0.4*	8.5 ± 2.0*	8.0 ± 0.2*
1.0	68.3 ± 7.0*†	81.5 ± 0.3*	22.1 ± 5.5*†	10.7 ± 1.1*
10.0	19.8 ± 1.3*†	37.9 ± 1.9*	63.5 ± 1.5*†	46.6 ± 2.3*
	(+)-OCB	(−)-OCB	(+)-OCB	(−)-OCB
0.1	90.8 ± 0.1*	91.8 ± 0.3*	2.9 ± 0.5	2.6 ± 0.4
1.0	88.8 ± 1.4	91.4 ± 1.3*	4.8 ± 1.4†	2.3 ± 1.5
3.4	83.9 ± 2.1*	85.9 ± 1.0	7.5 ± 0.7*	6.3 ± 0.8*
10.0	70.8 ± 4.7*	61.0 ± 5.0*	17.6 ± 4.4*†	26.2 ± 4.7*

Hepatocyte cultures were exposed to various concentrations of the PCBs as described in Materials and Methods. For the generation of porphyrins, cells were pretreated with 150 μM δ-aminolevulinic acid (ALA) 3 hr prior to harvest. All % Protoporphyrin and % Uroporphyrin values are listed as the mean percent ± SD (N = 3). Total porphyrins in DMSO (control cultures) ranged from 52 ± 4 to 102 ± 4 pmol/well in four different experiments with values for percent protoporphyrin ranging from 88 ± 1 to 91 ± 3% and percent uroporphyrin from 3 ± 1 to 7 ± 2%.

* Significantly different from DMSO control ($P \leq 0.05$).

† Significantly different from the (−)-enantiomer at that same concentration ($P \leq 0.05$).

with the (+)-enantiomer and 47% URO by the (−)-enantiomer at the highest concentration tested. At the highest concentration tested, both PeCB (50 μM) and OCB (10 μM) caused less than 47% URO accumulation, with the (−)-enantiomers being more potent than the (+)-enantiomers for generating URO. PROTO accumulation decreased in tandem with the increases in URO accumulation. The greatest percent decrease in PROTO accumulation occurred with the (+)-enantiomer of HeCB.

DISCUSSION

We have demonstrated in the present study that PeCB, HeCB and OCB (the racemic mixtures and their respective enantiomers) have different properties of cytochrome P450 induction and cause differences in URO accumulation in chick embryo liver cells in culture. This culture system was used to minimize pharmacokinetic influences that would otherwise be present in the *in vivo* systems. Previous rat studies with these PCBs had, in fact, demonstrated that differential metabolism and distribution kinetics of the PCBs may have contributed to the differential biologic effects seen.

In the hepatocyte culture racemic PeCB, HeCB, and OCB were efficacious inducers of BPDM activity (2.2- to 2.8-fold increase) (Fig. 4). In the rat, racemic OCB and HeCB induced aminopyrine *N*-demethylase activity 1.3- and 1.5-fold respectively [3], while racemic PeCB was inactive as an inducer [10]. Therefore, in the chick hepatocyte culture, the addition of a *para* chlorine to PeCB (resulting in HeCB) produced increased BPDM activity, while the further addition of chlorines to *ortho* and *meta* positions (resulting in OCB) did not further enhance BPDM activity. In previous rat studies [25], the stepwise addition of chlorine to the *para* positions of 2,2′,5,5′-PCB to form 2,2′,4,5,5′-PCB and 2,2′,4,4′,5,5′-PCB resulted in increased concentrations of cytochromes P450b (P450IIB1) and P450e (P450IIB2). But, when chlorines occupied all *ortho* positions (i.e. 2,2′,4,4′,6,6′-PCB), concentrations of cytochromes P450IIB1 and P450IIB2 decreased. Thus, for maximal induction of those cytochromes P450 which have BPDM activity in the chick hepatocyte culture and in the rat, PCBs should have both *para* positions and two or three of the *ortho* positions occupied by chlorines. If four *ortho* positions are occupied with chlorines, the BPDM activity is diminished.

In the chick hepatocyte culture, the (−)-enantiomers were more potent inducers of BPDM activities, with the exception of HeCB, in which the (+)-enantiomer was more potent. Previous rat studies [3] have shown that the (−)-enantiomer of OCB and the (+)-enantiomer of HeCB give the best induction of aminopyrine *N*-demethylase activity [both BPDM and aminopyrine *N*-demethylase activities are associated with the same isozyme(s) of cytochrome P450]. In the chick hepatocyte culture, total cytochrome P450 concentration was a much

Table 2. Dissociation of uroporphyrin (URO) accumulation with induction of ethoxyresorufin-O-deethylase (EROD) activity in the chick embryo liver cell culture

Treatment	URO	EROD	Reference
3,3′,4,4′-PCB	++	++	[19]
3,3′,4,4′,5-PCB	++	++	[19]
2,3,3′,4,4′,5′-PCB	++	++	[19]
(+)-2,2′,3,4,4′,6-HeCB	+*	+	Present study
(−)-2,2′,3,4,6-PeCB	+	−	Present study
(−)-2,2′,3,3′,4,4′,6,6′-OCB	+	−	Present study
PIA	+	−	[19]
3-Methylcholanthrene	−	++	[19]
Phenobarbital	−	−	[19]

Abbreviations: PCB, polychlorinated biphenyl; PIA, 2-propyl-2-isopropylacetamide; PeCB, pentachlorobiphenyl; HeCB, hexachlorobiphenyl; and OCB, octachlorobiphenyl. Key: ++ (URO), ≥80% of total porphyrin accumulation is uroporphyrin; + (URO), 26–46% of total porphyrin accumulation is uroporphyrin; ++ (EROD), 162–247 pmol/min/mg protein; + (EROD), 129 pmol/min/mg protein; − (URO) and − (EROD), no uroporphyrin accumulation and no EROD induction respectively.

* URO accumulation did not occur at concentrations of (+)-HeCB (0.34 μM) in which maximal EROD induction was observed (129 pmol/min/mg protein). Concentrations of (+)-HeCB at 1 and 10 μM caused URO accumulation of 22 and 64% respectively.

less sensitive indicator of chiral effects than were EROD or BPDM activities.

The most striking chiral effects occurred with the induction of EROD activity. Of the three racemic PCBs tested, the most potent inducer of EROD activity in the chick hepatocyte culture was HeCB. The (+)-HeCB demonstrated potent EROD induction, whereas the (−)-HeCB weakly induced EROD activity. Selective EROD induction was also seen with the (+)-enantiomers of PeCB and OCB, while their (−)-enantiomers were inactive (Fig. 3, A and C). The weak EROD induction that occurred with the (−)-enantiomer of HeCB (Fig. 3B) could have been due to contamination by the more potent (+)-enantiomer of HeCB. Given that (−)-HeCB is only >95% enantiomerically pure, there is a possibility of 5% contamination by the (+)-HeCB. At a concentration of 10 μM (−)-HeCB (EROD activity = 31 pmol/min/mg), a 5% impurity would allow as much as 0.5 μM of (+)-HeCB. As shown in Fig. 3B, this amount of impurity (0.5 μM (+)-HeCB) would be an inducing concentration of EROD activity (≈129 pmol/min/mg). The enantiomeric purity of PeCB and OCB are both 100%, and both do not induce EROD activity. Therefore, it appears that chirality is a very important aspect of EROD induction in the chick hepatocyte culture.

As we have reported previously [19], those PCBs which are efficacious EROD inducers in the chick embryo liver cell culture (e.g. 3,3′,4,4′-PCB, 3,3′,4,4′,5-PCB, 2,3,3′,4,4′,5′-PCB, 2,2′,4,4′,5,5′-PCB) all have a maximum EROD-inducing concentration, above which EROD activities progressively decline. It is felt that this decline cannot be due to generalized toxicity or impairment of heme synthesis, but rather to PCB effects on cytochrome P450 gene expression [19] and/or inhibition of EROD activity *in vitro* [26, 27]. This decline in EROD activity at the higher PCB concentrations was also noted for both the racemate and the (+)-enantiomer of HeCB (Fig. 3B). Although the (−)-HeCB, (+)-PeCB, and (+)-OCB demonstrated EROD induction (Fig. 3, B, A and C respectively), solubility limitations prevented the testing of higher concentrations at which declining EROD activities may have been seen.

It has been reported that the oxidation of uroporphyrinogen to URO occurs with the MC-inducible cytochromes P450 in the chick hepatocyte culture [28, 29] and with the cytochrome P450d (P450IA2) in the rat [30]. Further, it was suggested that induction of cytochrome P450IA2 is a prerequisite for URO accumulation after PCB treatment. Evidence suggesting that URO accumulation is not totally dependent on the induction of P450IA2 in the chick embryo hepatocyte culture is demonstrated in Table 2. Previous studies have shown that PCBs such as 3,3′,4,4′-PCB, 3,3′,4,4′,5-PCB and 2,3,3′,4,4′,5′-PCB caused ≥80% URO accumulation, while maximal induction of EROD activity ranges from 162 to 247 pmol/min/mg protein [19]. In our study, the (−)-enantiomers of PeCB and OCB (both were 100% enantiomerically pure) caused 45 and 26% URO accumulation, respectively, with no EROD induction, and presumably no induction of the cytochrome P450IA2 (Table 2; Fig. 3, A and C). It has also been shown in the chick hepatocyte culture that 2-propyl-2-isopropylacetamide (PIA), a PB-like inducer in the chick embryo hepatocyte culture, causes URO accumulation (≈40%) without EROD induction [19]. These data suggest that in order to obtain maximal URO accumulation (≥80%) in the hepatocyte culture, the PCB would need to induce high EROD activities, implying induction of high concentrations of the avian form of cytochrome P450IA2. However, when chick hepatocytes are exposed to MC, EROD activity is induced to 208 ± 6 pmol/min/mg protein

[19], with no URO accumulation. Our present study demonstrated that 0.34 μM (+)-HeCB caused maximal EROD induction (129 pmol/min/mg protein), with no URO accumulation. Only at higher concentrations of (+)-HeCB (1 and 10 μM) did URO accumulation occur (22 and 64% respectively). In addition, PB causes no induction of EROD activity and no URO accumulation [19]. Thus, URO accumulation is not totally dependent on induction of the MC-inducible cytochromes P450 (P450IA2) in the chick embryo hepatocyte culture. It has been suggested by De Matteis *et al.* [24] that a mechanism involving induction of a cytochrome(s) P450 different than cytochrome P450IA2 may be involved in the URO accumulation for compounds such as PIA. It appears that those compounds that cause low levels of URO, such as PIA or the (−)-enantiomers of PeCB and OCB, may do so via a different mechanism than those PCBs in which high levels of URO are found (i.e. 3,3′,4,4′-PCB).

In a previous study on the structure–activity relationships of polyhalogenated biphenyls on URO accumulation in the chick embryo liver cell culture [31], it was concluded that URO accumulation requires 2 to 3 halogens on each phenyl ring of the biphenyl molecule at the *meta* and *para* positions with no more than one *ortho* substitution (e.g. 3,3′,4,4′,5,5′-PCB or 2,3′,4,4′-PCB). This study concluded that at a 1 to 1.5 μM concentration of the racemic PeCB, 2,2′,4,4′,5,5′-hexachlorobiphenyl, or other PCBs with halogens at the 2,2′-positions, would not cause URO accumulation in the chick hepatocyte culture. This contrasts with our studies which demonstrate that at higher concentrations than used by Sassa *et al.* both the (+)- and (−)-enantiomers of PeCB, racemic PeCB (data not shown), and 2,2′,4,4′,5,5′-hexachlorobiphenyl [19] cause URO accumulation. The difference in results most likely is due to both differences in culture conditions (Sassa *et al.* incorporated 1 μg/mL insulin in modified F12 medium) and to the fact that Sassa *et al.* did not add the porphyrin precursor ALA to their cultures. Therefore, ALA synthetase induction probably played an important role in URO accumulation in the data of Sassa *et al.*, whereas ALA synthetase induction is not so important in our system.

In summary, this study has clearly demonstrated that there are chiral differences in the recognition events associated with the induction of cytochrome P450 and accumulation of URO in the chick hepatocyte culture. Furthermore, evidence has been presented suggesting that induction of the avian form of cytochrome P450IA2 is not an absolute requirement for URO accumulation in the chick hepatocyte culture.

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