Biochemical Pharmacology, Vol. 38, No. 8, pp. 1345–1352, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00

CHIRAL EFFECTS IN THE INDUCTION OF DRUG-METABOLIZING ENZYMES USING SYNTHETIC ATROPISOMERS OF POLYCHLORINATED BIPHENYLS (PCBs)

MICHAEL PÜTTMANN*†, ALBRECHT MANNSCHRECK,‡ FRANZ OESCH† and LARRY ROBERTSON§‖

†Institute of Toxicology, University of Mainz, D-6500 Mainz, FRG; ‡Department of Organic Chemistry, University of Regensburg, D-8400 Regensburg, FRG; and §Graduate Center for Toxicology, University of Kentucky, Lexington, KY 40506-0054, U.S.A.

(*Received* 23 *February* 1988; *accepted* 19 *August* 1988)

Abstract—Atropisomers of the polychlorinated biphenyls 2,2′,3,4,4′,6-hexachlorobiphenyl (II) and 2,2′,3,3′,4,4′,6,6′-octachlorobiphenyl (III), stable to racemization under physiological conditions, were administered to immature male Sprague–Dawley rats. The racemic hexachlorobiphenyl (II) was found to be a potent (phenobarbital-type) inducer, whereas (+)-II and (−)-II, administered at 100 μmol/kg, showed clearly differing potencies as inducers with (+)-II enhancing aminopyrine *N*-demethylase, aldrin epoxidase and cytochrome P-450 content more potently than (−)-II. In contrast, the racemic octachlorobiphenyl (III) and its individual enantiomers were only weak phenobarbital-type inducers of cytochrome P-450, and the enantiomers of III were equally (weakly) potent. Separate studies conducted to investigate pharmacokinetic influences on the differential potency of the enantiomers of II showed that after 5 days the concentration of (+)-II in the liver was twice as high as that of its antipode. Therefore, enantioselectivity in disposition as well as in recognition may be responsible for the differential potency seen.

PCBs¶ are ubiquitous environmental contaminants which, in addition to their carcinogenic potential, elicit numerous toxic effects, including immunosuppression with thymic atrophy, edema, hyperkeratosis, hepatotoxicity and lethality. PCBs are also particularly potent as inducers of drug-metabolizing enzymes [1, 2]. Many of the above effects have been observed after exposure to commercial preparations of PCBs (e.g. Aroclor, Kanechlor) which are themselves complex mixtures of many individual isomers and congeners (209 are theoretically possible [3]). It was through studies with single components that characterization of the differential activities of PCBs and the definition of structure–activity relationships were possible.

Accordingly, PCBs with chloro substitution in two para and in two meta positions (and without ortho substitution), e.g. 3,3′,4,4′-tetrachlorobiphenyl, will induce a pattern of drug-metabolizing enzymes resembling that seen after treatment with MC. This pattern is characterized by increases in cytochromes P-450c and P-450d [4], by greatly enhanced benzo[*a*]pyrene hydroxylase activity, and by a shift in the absorption maximum of the CO-binding difference spectrum to 448 nm [5]. If one or more ortho chlorines are introduced into the MC-type PCB, these characteristics are reduced or lost completely [5]. The probable explanation for this observation is that the assumption of a coplanar conformation by the biphenyl molecule is made more difficult by increasing ortho chlorine substitution. Only in a coplanar conformation can a molecular geometry similar to that of TCDD be attained, with the resulting binding avidity to the TCDD (Ah) receptor. This in turn controls the induction of the cytochromes P-450c and P-450d [6].

Those PCBs which induce a pattern of drug-metabolizing enzymes like PB are less well defined. PB-type induction is characterized by an increase in cytochromes P-450b and P-450e [4], a proliferation of the liver endoplasmic reticulum (increase in microsomal protein per gram liver), and an increase in aminopyrine *N*-demethylase and aldrin epoxidase activities. The absorption maximum of the CO-binding difference spectrum remains at 450 nm [5]. Potent PB-type inducers are typically those PCBs which possess two ortho and two para chlorine substituents, e.g. 2,2′,4,4′-tetrachlorobiphenyl or 2,2′,4,4′,5,5′-hexachlorobiphenyl. However, other structures may also elicit this type of response, e.g. 4,4′-dichlorobiphenyl [2]. Recently, several PB-type inducers have also been shown to induce cytochrome P-450p [7]. A receptor for PB-type inducers, analogous to the TCDD receptor, has not been found, although several positive indications for such a receptor exist. These include dose dependency and tissue specificity of induction as well as the existence of an extremely potent PB-type inducer [8]. However, it

* This work is a part of the M.D. thesis of M.P.

‖ Address all correspondence to: Dr. L. W. Robertson, Graduate Center for Toxicology, 204 Funkhouser Building, University of Kentucky, Lexington, KY 40506-0054.

¶ Abbreviations: PBCs, polychlorinated biphenyls; MC, 3-methylcholanthrene; PB, phenobarbital; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TAC, triacetyl cellulose; EIC, ethyl isocyanide; and CO, carbon monoxide.

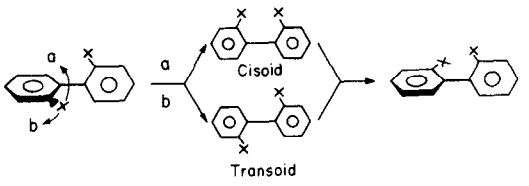
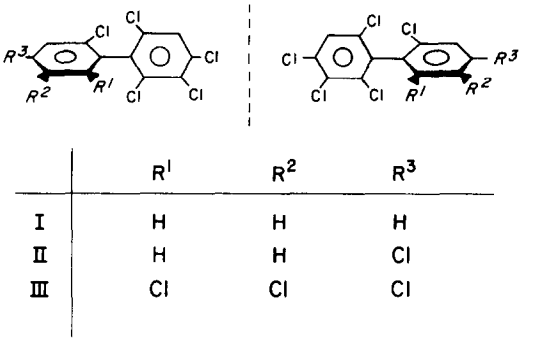


Fig. 1. Racemization of PCB derivatives with two ortho chlorine atoms by rotation around the central C—C bond.



	R^1	R^2	R^3
I	H	H	H
II	H	H	Cl
III	Cl	Cl	Cl

Fig. 2. Structures of the investigated PCB atropisomers.

is not clear how such structurally diverse molecules as the water-soluble PB and lipophilic PCBs and organochlorine pesticides (dieldrin, p,p'-DDT, chlorinated benzenes) bind to a single receptor [9, 10].

Previous studies on the structure–activity relationships of PCBs have not considered that at least 78 out of the possible 209 PCB isomers and congeners can exist as rotational isomers which are enantiomeric to each other. The requirement for chirality is that neither phenyl ring possesses a plane of symmetry which passes through the central C—C bond perpendicular to the plane of the ring. Rotation around the central C—C bond takes place via coplanar transition states, where the number of ortho substituents and their size (e.g. Br > Cl) increase the steric hindrance and consequently the energy barrier to racemization. PCB derivatives with only two ortho chlorine atoms racemize quickly at room temperature because of an energetically favorable transoid transition state, as outlined in Fig. 1. However, only cisoid transition states are possible for PCBs with three or four ortho chlorines, greatly increasing the energy barrier to racemization. Kaiser [11] predicted, therefore, that 19 PCBs, at least 9 of which are components of commercial PCB formulations (Table 1), will exist as stable atropisomers at room temperature [13]. Atropisomers are separable, single bond torsion isomers of which a more general discussion and examples are presented in Ref. 14.

We have recently confirmed this prediction and have reported the chromatographic enrichment [15] (I and II on TAC) and the separation [13] (III via synthetic diastereomers) of PCB atropisomers for the first time (see Fig. 2). These compounds possess sufficient thermal stability such that they are well-suited for biologic studies, i.e. no racemization will take place at body temperature. Previous studies with the racemates of I and II showed that I, administered at a dose of 100 μmol/kg, was inactive as an inducer of drug-metabolizing enzymes whereas II, at 100 μmol/kg, was a PB-type inducer [2]. The effects of III as an inducer have not been investigated previously.

Those PCBs which form stable atropisomers cannot be MC-type inducers because the transition to the necessary coplanar conformation would require more than 120 kJ/mol [15]. Even PCBs with four ortho H atoms possess an energy barrier to rotation of about 12 kJ/mol and more than 99% of these molecules reside in a non-planar conformation [6].

The present study demonstrates the different activities of the PCB enantiomers II and III as inducers of drug-metabolizing enzymes. In addition, liver PCB concentrations were measured in order to look at the influence of pharmacokinetic parameters on the induction potency.

Table 1. PCBs that will form enantiomers stable to racemization at room temperature

Ring substitution	Ring substitution -2,3,6	-2,3,4,6	
-2	2,2',3,6 (45)	2,2',3,4,6 (88)	[I]
-2,3	2,2',3,3',6 (84)	2,2',3,3',4,6 (131)*	
-2,4	2,2',3,4',6 (91)	2,2',3,4,4',6 (139)	[II]
-2,5	2,2',3,5',6 (95)*	2,2',3,4,5',6 (144)*	
-2,3,4	2,2',3,3',4,6' (132)*	2,2',3,3',4,4',6 (171)	
-2,3,5	2,2',3,3',5,6' (135)*	2,2',3,3',4,5',6 (175)	
-2,3,6	2,2',3,3',6,6' (136)*	2,2',3,3',4,6,6' (176)	
-2,4,5	2,2',3,4',5',6 (149)*	2,2',3,4,4',5',6 (183)	
-2,3,4,5	2,2',3,3',4,5,6' (174)*	2,2',3,3',4,4',5,6' (196)*	
-2,3,4,6	2,2',3,3',4,6,6' (176)	2,2',3,3',4,4',6,6' (197)	[III]

The numbers in parentheses are taken from Ballschmiter and Zell [3]. Note that PCB No. 176 appears twice.

* The indicated compounds comprise nearly 25% of Clophen A 60 and are present in human milk and butter [12].

MATERIALS AND METHODS

Synthesis of the racemates and enantiomers of II and III. The racemic 2,2',3,4,4',6-hexachlorobiphenyl ((±)-II) and 2,2',3,3',4,4',6,6'-octachlorobiphenyl ((±)-III) were synthesized by the Cadogan modification of the Gomberg–Bachmann reaction [16]. The coupling of 2,4-dichloroaniline or 2,3,4,6-tetrachloroaniline with 1,2,3,5-tetra-chlorobenzene (in 10-fold molar excess) was carried out as described [15]. The structures were confirmed by ^{1}H-NMR (200 MHz) and mass spectrometry. The purity as determined by gas chromatography was in all cases greater than 98%. The enantiomers of II were resolved by microcrystalline TAC chromatography: $P_{(+)} > 0.8$; $P_{(-)} > 0.95$ [13]. (+)- and (−)-III were prepared via the nitration of 2,2',4,4',6,6'-hexachlorobiphenyl and subsequent reduction of the nitro function which produced the racemic 3,3'-diamino-2,2',4,4',6,6'-hexachlorobiphenyl. The latter was converted to a 1:1 mixture of diastereomers by (−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPA-Cl). HPLC separation of the diastereomers, cleavage of the MTPA groups, and Sandmeyer replacement of the amino groups with chlorines yielded the (+)- and (−)-III in practically 100% enantiomeric purity [13].

Biochemicals. PB, MC, 4-dimethylaminoantipyrine (aminopyrine), CO and aldrin were obtained as previously described [5]. EIC was synthesized by Dr. Karl Platt, Mainz, FRG.

Animal treatment and isolation of microsomes. Immature male Sprague–Dawley rats (average weight 80 g), purchased from Süddeutsche Versuchstierfarm (Tuttlingen, FRG), were placed in plastic cages and allowed free access to food and water. PCBs were dissolved in corn oil and were injected i.p. on days 1 and 3 (total dose is presented in Tables 2 and 3). MC (100 μmol/kg single dose, dissolved in corn oil) and PB (400 μmol/kg single dose, in isotonic saline) were administered on days 3, 4 and 5. The volume of each injection was 0.5 ml/100 g body weight. Rats were killed on day 6 by cervical dislocation after having been fasted for 24 hr. Each liver was perfused *in situ* with ice-cold 0.25 M sucrose containing 0.1 mM EDTA (pH 7.4), excised, weighed and homogenized as described [17]. Microsomes were prepared by differential centrifugation, adjusted to a protein concentration of 6 mg protein/ml, frozen in liquid nitrogen, and stored at −80° until needed.

Assays of drug-metabolizing enzymes activities. The quantity of protein in the liver microsomal suspensions was measured by the method of Lowry *et al.* [18], while total cytochrome P-450 content was determined spectrophotometrically [19] from the CO-binding difference spectra of dithionite-reduced microsomes with the molar absorption coefficient $\varepsilon = 91\ cm^{-1}\ mM^{-1}$ and $A = A_{max(450)} - A_{490}$. EIC, also used as ligand (cuvette concentration 4.5 mM), produced two absorption maxima in the difference spectrum, around 455 and 428 nm. Aminopyrine *N*-demethylase [20] and aldrin epoxidase [21] were measured essentially as reported.

Statistics. Differences between treated and control groups or between groups treated with antipodes were tested for statistical significance by the method of Dunnett [22].

Extraction of PCB from rat liver. Two rats were treated as described above with 200 μmol/kg of (±)-II. The excised livers were mashed with a 5-fold excess (by weight) of anhydrous sodium sulfate. The resulting mass was extracted three times with 50 ml of boiling *n*-hexane. Each extraction was carried out for 1 hr; after the third hexane extraction, no additional PCB could be extracted with boiling chloroform. The combined hexane extracts were cleaned up by chromatography on Florisil and silica gel [23]. Yield ≈ 0.5 mg. The enantiomeric composition of the extracted PCB was estimated by HPLC on TAC [24] by comparison to a standard of known enantiomeric purity (see Fig. 3). An impurity which was clearly separable by gas and TAC chromatography did not interfere with the estimation of enantiomeric purity in the extract.

Analysis of PCB concentrations in rat liver microsomes. Analysis of PCB concentrations was done by Dr. Brunn, Giessen, FRG, according to the method of Beck and Mathar [25]. The microsomal preparations were mashed with 10 g sea sand and 10 g anhydrous sodium sulfate and treated with boiling *n*-hexane in a Soxhlet-extractor for 6 hr. Cleanup of the hexane extracts was performed by chromatography on basic alumina. The concentration of the purified hexachlorobiphenyl was determined by capillary gas chromatography on two columns of different polarity (30 m DB-1 and DB-5 obtained from ICT, Frankfurt/M., FRG). Peaks were confirmed by capillary gas chromatography/mass spectrometry in the EI-mode. A recovery of 93 ± 3% was found for this method.

RESULTS

Because of the difficulty of synthesis of separation of PCB atropisomers, the dose–response curves for the PCBs were first carried out with the racemates. This was done so that a dose could be picked at which the PCB atropisomers would be effective inducers, but not in the saturation area of the curve, in order to maximize differences in their induction potencies.

2,2',3,4,4',6-Hexachlorobiphenyl (II). The effects of the treatment with (±)-II, (+)-II or (−)-II on microsomal drug-metabolizing enzymes are presented in Table 2. Rats treated with PB or MC were included in each experiment, and their effects are presented in Tables 2 and 3 for comparison.

Increasing the dose of the racemate of II resulted in increases in the monooxygenase activities as well as in cytochrome P-450 content, although these effects were only statistically significant after doses of 100–200 μmol/kg. The data in Table 2 indicate clearly that II is a PB-type inducer of cytochrome P-450 with intermediate potency. Because of the small quantities of enantiomers of II that were available, we chose a dose of 100 μmol/kg (and a larger animal number, N = 8) in order to test these enantiomers as inducers.

Like the racemic mixture, the enantiomers of II (at a total dose of 100 μmol/kg) were both PB-type inducers of cytochrome P-450 although they were not

Table 2. Effects of racemic- and enantiomeric-2,2′,3,4,4′,6-hexachlorobiphenyl (II) on drug-metabolizing enzyme activities in the immature male Sprague–Dawley rat

Treatment	% Liver wt of body wt	Aminopyrine *N*-demethylase [nmol·mg^{-1}·min^{-1}]	Aldrin epoxidase [nmol·mg^{-1}·min^{-1}]	Cytochrome P-450 [nmol·mg^{-1}] (Peak maximum, nm)
Corn oil (N = 3)	3.85 ± 0.56	3.93 ± 0.37	4.25 ± 0.80	0.445 ± 0.083 (450.3 ± 0.3)
Phenobarbital (N = 3)	4.88 ± 0.29*	10.57 ± 0.99†	31.86 ± 1.86†	1.571 ± 0.040† (449.7 ± 0.11)
3-Methylcholanthrene (N = 3)	4.62 ± 0.13	5.09 ± 0.72	3.24 ± 0.57	1.105 ± 0.166† (447.9 ± 0.1)
(±)-2,2′,3,4,4′,6-Hexachlorobiphenyl				
25 μmol/kg (N = 3)	3.88 ± 0.20	3.98 ± 0.25	3.08 ± 0.15	0.468 ± 0.040 (450.3 ± 0.3)
50 μmol/kg (N = 3)	3.96 ± 0.35	5.54 ± 0.89	4.36 ± 1.91	0.566 ± 0.061 (450.5 ± 0.1)
100 μmol/kg (N = 3)	4.15 ± 0.49	8.14 ± 2.84*	6.60 ± 2.10	0.751 ± 0.192 (450.2 ± 0.2)
200 μmol/kg (N = 3)	3.98 ± 0.30	8.63 ± 1.00*	15.44 ± 2.36†	0.987 ± 0.133† (450.1 ± 0.3)
400 μmol/kg (N = 3)	4.67 ± 0.60	9.56 ± 2.24†	20.76 ± 2.70†	1.095 ± 0.242† (449.9 ± 0.1)
Corn oil (N = 6)	3.70 ± 0.06	3.40 ± 0.21	3.00 ± 0.37	0.453 ± 0.071 (450.3 ± 0.2)
Phenobarbital (N = 6)	4.93 ± 0.31†	8.98 ± 0.78†	27.95 ± 1.24†	1.412 ± 0.120† (450.1 ± 0.2)
3-Methylcholanthrene (N = 2)	5.20 ± 0.40†	5.04 ± 0.77*	4.46 ± 0.87	1.097 ± 0.220† (448.4 ± 0.1)
2,2′,3,4,4′,6-Hexachlorobiphenyl (100 μmol/kg)				
Racemate (N = 8)	3.84 ± 0.30	5.11 ± 0.75†	10.00 ± 2.10†	0.648 ± 0.129 (450.1 ± 0.3)
(+)-Enantiomer (N = 8)	4.03 ± 0.17*	5.82 ± 0.81†	10.62 ± 1.88†	0.789 ± 0.125* (450.0 ± 0.1)
(−)-Enantiomer (N = 8)	3.91 ± 0.15	4.44 ± 0.47†‡	5.62 ± 1.88*‡	0.527 ± 0.093‡ (450.1 ± 0.2)

All values are means ± SD.
*,† Significantly different from corn oil—controls at α = *0.05 or †0.01 (Dunnett).
‡ Significantly different from (+)-enantiomer at α = 0.01.

Table 3. Effects of racemic- and enantiomeric-2,2′,3,3′,4,4′,6,6′-octachlorobiphenyl (III) on drug-metabolizing enzyme activities in the immature male Sprague–Dawley rat

Treatment	% Liver wt of body wt	Aminopyrine *N*-demethylase [nmol·mg^{-1}·min^{-1}]	Aldrin epoxidase [nmol·mg^{-1}·min^{-1}]	Cytochrome P-450 [nmol·mg^{-1}] (Peak maximum, nm)
Corn oil (N = 3)	3.35 ± 0.21	3.75 ± 0.57	ND*	0.487 ± 0.058 (450.1 ± 0.3)
Phenobarbital (N = 3)	5.12 ± 0.23‡	9.70 ± 0.37‡	ND	1.473 ± 0.100‡ (449.7 ± 0.3)
3-Methylcholanthrene (N = 3)	4.48 ± 0.34‡	5.42 ± 0.28†	ND	1.397 ± 0.047‡ (447.8 ± 0.2)
(±)-2,2′,3,3′,4,4′6,6′-Octachlorobiphenyl				
25 μmol/kg (N = 3)	3.35 ± 0.42	5.34 ± 0.91†	ND	0.653 ± 0.060 (450.2 ± 0.03)
50 μmol/kg (N = 3)	3.71 ± 0.21	4.80 ± 0.45	ND	0.597 ± 0.023 (450.4 ± 0.2)
100 μmol/kg (N = 3)	3.63 ± 0.11	5.84 ± 0.51‡	ND	0.720 ± 0.101‡ (450.3 ± 0.3)
200 μmol/kg (N = 3)	3.62 ± 0.33	7.54 ± 0.58‡	ND	0.863 ± 0.015‡ (450.2 ± 0.1)
400 μmol/kg (N = 3)	3.75 ± 0.27	7.30 ± 0.57‡	ND	0.863 ± 0.127‡ (450.0 ± 0.1)
Corn oil (N = 4)	3.55 ± 0.09	3.97 ± 0.44	3.96 ± 0.46	0.390 ± 0.078 (450.0 ± 0.2)
Phenobarbital (N = 4)	4.90 ± 0.14‡	13.75 ± 0.58‡	22.6 ± 4.3†	1.313 ± 0.057‡ (450.0 ± 0.2)
3-Methylcholanthrene (N = 4)	4.33 ± 0.28†	5.21 ± 0.23	2.76 ± 0.86	0.910 ± 0.224‡ (448.4 ± 0.3)
2,2′,3,3′,4,4′,6,6′-Octachlorobiphenyl (100 μmol/kg)				
Racemate (N = 4)	3.45 ± 0.14	5.22 ± 0.97	4.57 ± 1.02	0.449 ± 0.070 (450.2 ± 0.2)
(+)-Enantiomer (N = 4)	3.22 ± 0.75	5.74 ± 1.55	7.15 ± 0.6	0.503 ± 0.076 (449.8 ± 0.2)
(−)-Enantiomer (N = 4)	3.73 ± 0.32	6.36 ± 1.08‡	7.17 ± 1.83	0.479 ± 0.039 (450.1 ± 0.4)

All values are means ± SD.
* Not done.
†,‡ Significantly different from corn oil—controls at α = †0.05 or ‡0.01.

equipotent. In fact, the (+)-II significantly increased the activities of aminopyrine *N*-demethylase (1.7×), aldrin epoxidase (3.5×) and cytochrome P-450 content (1.7×) over control. The (−)-II was less potent, and the differences between these parameters for the (+)-II and (−)-II were statistically significant ($\alpha = 0.01$). The (+)-II also increased the yield of microsomal protein more potently than did (−)-II, and this difference was also statistically significant ($\alpha = 0.05$, data not shown). The racemate, also applied at the dose of 100 μmol/kg, led to monooxygenase activities and cytochrome P-450 content that were intermediate between those of the enantiomers.

2,2′,3,3′,4,4′,6,6′-Octachlorobiphenyl (III). In Table 3 the effects of (±)-III, (+)-III and (−)-III on microsomal monooxygenases are presented. Dose–response effects of racemic III demonstrated that III (in the dose range tested) is also a PB-type inducer of cytochrome P-450 although less potent than (±)-II. Both compounds, however, elicited statistically significant increases in aminopyrine *N*-demethylase and cytochrome P-450 at all doses higher than 100 μmol/kg.

The application of (±)-, (+)- and (−)-III (100 μmol/kg) resulted in biological responses which were not as clearcut as was the case with II. Enzyme activities and cytochrome levels were not generally significantly increased over control values; only with the (−)-enantiomer was the increase of aminopyrine *N*-demethylase statistically significant. (+)- and (−)-III did not show clearly differing potencies as inducers.

Estimation of the enantiomeric purity of II after extraction from rat liver. The differing potencies of the enantiomers of II as inducers may have resulted from the individual pharmacokinetic characteristics of the compounds. To check this possibility, racemic II was injected into rats and, after a particular time period, the PCB was recovered from the whole liver, as described in Materials and Methods. Because relatively little material could be obtained (≈0.5 mg), a polarimetric analysis [15] of the extract was not possible but rather an estimation of the relative content of each enantiomer was made by HPLC on TAC [24] chromatography (with UV detection). The results of this analysis are presented in Fig. 3. The upper chromatogram was obtained from 0.5 mg of racemic II. The (−)-antipode eluted first and its peak was therefore sharper than the peak of the (+)-antipode. The middle chromatogram depicts II from the liver extract. It should be noted that the extracted PCB contained much less of the (−)-antipode. The bottom chromatogram is that of a known mixture of the two enantiomers ($P_{(+)} = 0.32$), in which the relationship (+):(−) is about 2:1. By comparing the lower two chromatograms, one can estimate that in the liver the PCB antipodes were present approximately in this ratio of 2:1.

The observation that the whole liver concentration of the (−)-antipode was reduced greatly was confirmed by measurements of the concentrations of II in the liver microsomes purified from rats treated with (±)-, (+)- and (−)-II. The relative microsomal PCB content was calculated from the microsomal

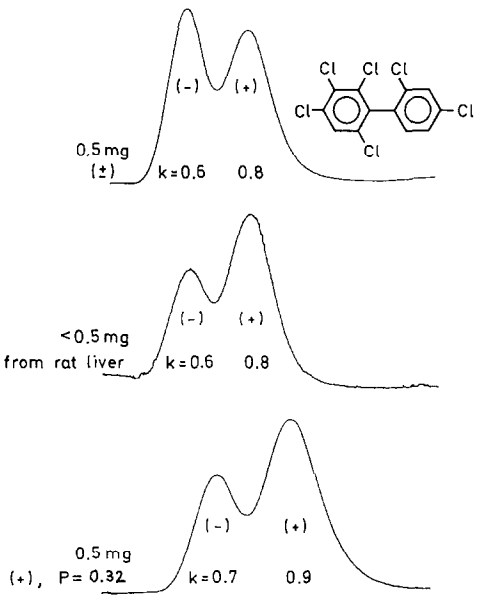


Fig. 3. HPLC of II in ethanol/H_2O, 96:4, on TAC [24] (particle size 10 μm; presssure = 80 bar) with UV detection at 278 nm. The signs of rotation stem from polarimetric detection of the (±)-II sample at 365 nm. k: capacity factor. Top: (±)-II. Middle: II extracted from rat liver; the amplitude was increased in order to facilitate comparisons. Bottom: (+)-II with known enantiomeric purity of P = 0.32.

Table 4. Calculated whole liver PCB concentration

	PCB concentration (μg PCB/g liver)
(±)-II	97 ± 26
(+)-II	114 ± 48
(−)-II	68.5 ± 7

Values are means ± SD, N = 3.

concentrations according to the following relationship:

$$\text{Relative microsomal PCB content [}\mu\text{g PCB/g liver]} = C_m \times V_m$$

where V_m = total volume of the microsomal preparation of each rat liver in [ml]/liver weight [g] and C_m = PCB concentration in the microsomal preparation of each rat liver in [μg/ml].

Table 4 shows that the relative microsomal PCB content for rats treated with the (+)-II was almost twice as high as that for rats treated with (−)-II. The PCB content of (±)-II-treated rats lay in between.

DISCUSSION

From the data presented in this paper the following trends are evident. Racemic II is a more potent PB-type inducer of cytochrome P-450 than is III. This is consistent with other animal studies which show that increasing the number of ortho substituents above 2 lowers the induction potency of the 2,2′,4,4′-skele-

ton (present in both II and III) [2]. Furthermore, PCBs with four ortho chlorines are generally weak or inactive as inducers [26]. In contrast to animal studies, recent results with hepatocytes in culture demonstrate that 3- and 4-fold ortho-substituted PCBs may be potent inducers of cytochrome P-450p. The basis for this discrepancy is unclear, but may involve pharmacokinetic influences [7]. Indeed it has been postulated that PCBs with multiple ortho chlorines, by virtue of the resulting steric effects, are less able to penetrate certain membrane barriers [27–29]. A more recent model, however, favors differences in metabolism or hydrophobicity over steric hindrance as the explanation for differential accumulation of PCBs [30].

Knowledge of the stability of the enantiomers of II and III under physiologic conditions (37° over several days) was a prerequisite to the testing of their biologic potency. Now we can predict on the basis of the energy barrier to racemization, which for both II and III lies well above 120 kJ/mol, that a measurable, non-enzymatic racemization at body temperature is not possible [13, 15]. The energy which leads to thermal racemization via coplanar transition states is determined principally by intramolecular parameters (steric hindrance of the ortho substituents) and only to a very small extent by intermolecular interactions (e.g. with solvents, membranes, and proteins). A reduction of the energy barrier to racemization by an enzyme would be theoretically possible if the central C—C bond were elongated or temporarily broken. Degradation of biphenyls, however, occurs at sites on the aromatic ring system while the central C—C bond remains untouched [29].

Another evidence against a highly active racemase is the fact that racemic II once injected was recovered as an optically-active PCB. One might argue that (−)-II was not completely extracted because of its stronger binding to the liver. We assume, however, since no additional PCB could be extracted with chloroform, that multiple extractions with boiling hexane completely removed all remaining compound. An artificial enrichment of the antipodes during the cleanup was not possible due to the exclusive use of achiral solvents and adsorption materials.

The enantiomers of the octachlorobiphenyl (III) administered at a dose of 100 μmol/kg were just as weak as the racemate. It is therefore probable that the induction elicited by the racemate is the additive effect of each contributing enantiomer. Obviously the enzyme induction caused by racemic-III is not the result of either a potent inhibition or a potentiation of one antipode by the other. The possibility remains that differential induction could be seen at higher doses or with larger experimental groups.

The differential induction seen with the hexachlorobiphenyl (II) could be explained by differences in pharmacokinetic parameters (absorption, distribution, metabolism, excretion) or in induction potency, or by a combination of the above. The relative concentrations of the enantiomers of II both in the liver extract and in the microsomes lead to the conclusion that the higher concentration of the (+)-enantiomer may be responsible for its increased induction potency. Although we cannot exclude the possibility that differences in inherent induction potency exist, enantioselective transport or metabolism is more likely to account for these observations.

The results presented here are contrary to an assumption of Safe and co-workers, that "it is unlikely that metabolic or bioconcentration-factors play a role in determining relative enzyme induction potencies" of II and other 2,4-dichlorosubstituted PCBs [2]. If differences between such similar molecules as enantiomers can lead to concentration differences in the liver as high as 2-fold, then concentration differences for PCBs with different substitution patterns (which are not so closely related) could be much higher.

In conclusion this paper demonstrates differential effects in the induction of cytochrome P-450-dependent monooxygenases by PCB atropisomers (which possess axial chirality). We have recently reported similar effects for the enantiomers of *trans*-stilbene oxide [31], molecules which possess active-center chirality. In both cases pharmacokinetic factors complicated the interpretation of these *in vivo* effects. Future investigations will be directed toward the elucidation of these effects in *in vitro* systems.

Acknowledgements—The authors wish to thank Miss Nicola Pustet for the HPLC measurements, Dr. Petra Rauch for the performance of the aldrin epoxidase assay, Dr. Karl Platt for the synthesis of the EIC and the Alexander von Humboldt Foundation (grant to L.R.) for financial support. Special thanks go to Dr. H. Brunn, Staatliches Medizinal-, Lebensmittel- und Veterinäruntersuchungsamt Mittelhessen, Giessen, FRG, for the analysis of PCB residues in liver microsomes.

REFERENCES

1. Kimbrough RD (Ed.), *Halogenated Biphenyls, Terphenyls, Naphthalenes, Dibenzodioxins and Related Products*. Elsevier, Amsterdam, 1980.
2. Denomme MA, Bandiera S, Lambert I, Copp L, Safe L and Safe S, Polychlorinated biphenyls as phenobarbitone-type inducers of microsomal enzymes: Structure–activity relationships for a series of 2,4-dichlorosubstituted congeners. *Biochem Pharmacol* **32**: 2955–2963, 1983.
3. Ballschmiter K and Zell M, Analysis of polychlorinated biphenyls (PCB) by glass capillary gas chromatography: Composition of technical Aroclor- and Clophen-PCB mixtures. *Z Anal Chem* **302**: 20–31, 1980.
4. Parkinson A, Safe SH, Robertson LW, Thomas PE, Ryan DE, Reik LM and Levin W, Immunochemical quantitation of cytochrome P-450 isozymes and epoxide hydrolase in liver microsomes from polychlorinated or polybrominated biphenyl-treated rats: A study of structure–activity relationships. *J Biol Chem* **258**: 5967–5976, 1983.
5. Parkinson A, Robertson L, Safe L and Safe S, Polychlorinated biphenyls as inducers of hepatic microsomal enzymes: Structure–activity rules. *Chem Biol Interact* **30**: 271–285, 1980.
6. McKinney JD and Singh P, Structure–activity relationships in halogenated biphenys: Unifying hypothesis for structural specifity. *Chem Biol Interact* **33**: 271–283, 1981.
7. Scheutz EG, Wrighton SA, Safe SH and Guzelian PS. Regulation of cytochrome P-450p by phenobarbital and phenobarbital-like inducers in adult rat hepatocytes in primary monolayer culture and *in vivo*. *Biochemistry* **25**: 1124–1133, 1986.

8. Poland A, Mak I, Glover E, Boatman RJ, Ebetino FH and Kende AS, 1,4-Bis[2-(3,5-dichloropyridyloxy)]-benzene, a potent phenobarbital-like inducer of microsomal monooxygenase activity. *Mol Pharmacol* **18**: 571–580, 1980.
9. Conney AH, Pharmacological implications of microsomal enzyme induction. *Pharmacol Rev* **19**: 317–366, 1967.
10. Carlson GP and Tardiff RG, Effect of chlorinated benzenes on the metabolism of foreign organic compounds. *Toxicol Appl Pharmacol* **36**: 383–394, 1976.
11. Kaiser KLE, On the optical activity of polychlorinated biphenyls. *Environ Pollut* **7**: 93–101, 1974.
12. Schulte E and Malisch R, Calculation of the real PCB content in environmental samples—gas chromatographic determination of the PCB concentration in human milk and butter. *Z Anal Chem* **319**: 54–59, 1984.
13. Püttmann M, Oesch F, Robertson LW and Mannschreck A, Characteristics of polychlorinated biphenyl (PCB) atropisomers. *Chemosphere* **15**: 2061–2064, 1986.
14. Testa B, *Principles of Organic Stereochemistry*. Marcel Dekker, New York, 1979.
15. Mannschreck A, Pustet N, Robertson LW, Oesch F and Püttmann M, Enantiomers of polychlorinated biphenyls (PCBs). *Justus Liebigs Ann Chem* 2101–2103, 1985.
16. Cadogan JIG, A convenient new method of aromatic arylation. *J Chem Soc* 4257–4258, 1962.
17. Schramm H, Robertson LW and Oesch F, Differential regulation of hepatic glutathione transferase and glutathione peroxidase activities in the rat. *Biochem Pharmacol* **34**: 3735–3739, 1985.
18. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
19. Omura T and Sato R, The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its haemoprotein nature. *J Biol Chem* **239**: 2370–2378, 1964.
20. Parkinson A and Safe S, The detection of enzyme induction by rat liver microsomes prepared by isoelectric precipitation. *J Pharm Pharmacol* **31**: 444–447, 1979.
21. Wolff T, Deml E and Wanders H, Aldrin epoxidation, a highly sensitive indicator specific for cytochrome P-450-dependent monooxygenase activities. *Drug Metab Dispos* **7**: 301–305, 1979.
22. Dunnett GW, New tables for multiple comparisons with a control. *Biometrics* **20**: 482–491, 1964.
23. Hutzinger O, Safe S and Zitko V, *The Chemistry of PCBs*, pp. 200–204. CRC Press, Cleveland, Ohio, 1974.
24. Mannschreck A, Koller H and Wernicke R, Microcrystalline cellulose triacetate, a versatile stationary phase for the separation of enantiomers. *Kontakte (Darmstadt)* 40–45 (1985); cited in *Chem Abstract* **103**: 110495 (1985).
25. Beck H and Mathar W, Analysenverfahren zur Bestimmung von ausgewählten PCB-Einzelkomponenten in Lebensmitteln. *Bundesgesundhbl* **28**: 1–12, 1985.
26. Goldstein JA, Hickman P, Bergman H, McKinney JD and Walker MP, Separation of pure polychlorinated biphenyl isomers into two types of inducers on the basis of induction of cytochrome P-450 or P-448. *Chem Biol Interact* **17**: 69–87, 1977.
27. Goldstein JA, McKinney JD, Lucier GW, Hickman P, Bergman H and Moore JA, Toxicological assessment of hexachlorobiphenyl isomers and 2,3,7,8-tetrachlorodibenzofuran in chicks. II: Effects on drug metabolism and porphyrin accumulation. *Toxicol Appl Pharmacol* **36**: 81–92, 1976.
28. Hutzinger O, Tulp MTM and Zitko V, Chemicals with pollution potential. In: *Aquatic Pollutants: Transformations and Biological Effects* (Eds. Hutzinger O, Lelyveld LH and Zoeteman BJC), pp. 13–31. Pergamon Press, Oxford, 1978.
29. Safe S, Metabolism uptake, storage and bioaccumulation. In: *Halogenated Biphenyls, Terphenyls, Naphthalenes, Dibenzodioxins and Related Products* (Ed. Kimbrough RD), pp. 81–86. Elsevier, Amsterdam, 1980.
30. Opperhuizen A, Van der Velde EW, Gobas F, Liem DA, Van der Steen JM and Hutzinger O, Relationship between bioconcentration in fish and steric factors of hydrophobic chemicals. *Chemosphere* **14**: 1871–1896, 1985.
31. Rauch P, Püttman M, Oesch F, Okamoto Y and Robertson LW, Differential induction of cytochrome P-450 and epoxide hydrolase by the enantiomers of *trans*-stilbene oxide. *Biochem Pharmacol* **36**: 4355–4359, 1987.