Studies of the influence of chloro-substituent sites and conformational energy in polychlorinated biphenyls on uroporphyrin formation in chick-embryo liver cell cultures

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Treatment of cultured chick-embryo liver cells with polychlorinated biphenyls (PCB) results in decreased uroporphyrinogen decarboxylase activity and increased uroporphyrin accumulation. In the present study we examined the effect of the chloro- or bromo-substituent sites in biphenyls (BP) on uroporphyrin accumulation in cultured hepatocytes and the three-dimensional structure of these congeners determined by molecular orbital calculations using a MNDO ('modified neglect of diatomic overlap') method. Among 20 congeners examined, those which were effective in stimulating porphyrin accumulation contained at least two Cl or Br atoms at the lateral adjacent positions in each phenyl ring, e.g. 3,4,3',4'-tetrachloro-, 2,4,3',4'-tetrachloro-, 3,4,5,3',4',5'-hexachloro- and 3,4,5,3',4',5'-hexabromo-biphenyl, whereas those which contained less than two halogen atoms or more than three halogen atoms in each phenyl ring or those which contained halogen atoms at 2,2'-positions were not effective. On the basis of the conformational energy (ΔE , difference from the most stable conformational energy), which is calculated as a function of the dihedral angle (θ) between the two phenyl rings, biphenyl congeners can be classified into four groups with different conformations. The conformation of active PCB was relatively flexible, whereas inactive species had a rigidly angulated conformation. Furthermore, the calculated probability of the conformation distribution for each congener indicated that the probability of co-planarity was higher for active biphenyls than for inactive congeners. These structural characteristics suggest the significance of both the chloro-substituent sites and the conformational energy reflecting the phenyl-ring twist angles in determining the inhibitory effect of PCB on uroporphyrinogen decarboxylase activity.

INTRODUCTION

Polyhalogenated aromatic hydrocarbons (PAH), such as polyhalogenated biphenyls, dibenzo-*p*-dioxins, dibenzofurans, azo- and azoxy-benzenes and naphthalenes, are known to cause similar toxic responses in man and animals (Kuratsune *et al.*, 1972; McConnell & Moore, 1979). Exposure of experimental animals to polychlorinated biphenyls (PCB) or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), both widely distributed environmental pollutants, produces a characteristic pattern of toxicity, involving a prolonged wasting syndrome before death. Lymphoid involution accompanied by immune suppression, embryotoxicity and teratogenicity have been widely observed in many species examined. Hyperkeratosis, chloracne, oedema, hyperplasia of the epithelium of the stomach, intestine and urinary bladder and hepatocellular damage are also observed in certain species. In rats and mice, treatment of animals with PCB or TCDD leads to the development of hepatic porphyria after several weeks (Elder, 1978).

PCB are potent inducers of cytochrome *P*-450 (Alvares *et al.*, 1973; Alvares & Kappas, 1977) and its associated hydroxylases, δ -aminolaevulinic acid (ALA) synthase, DT-diaphorase, glucuronyltransferase, aldehyde de-

hydrogenase and ornithine decarboxylase (Poland & Kende, 1977). The most extensively studied biochemical response of these compounds is the induction of aryl hydrocarbon hydroxylase (AHH). This response is thought to be mediated by the reversible stereospecific binding of the inducer chemical to a cytosol receptor protein. The expression of this receptor function in mice is determined by the Ah locus, which appears to be involved not only in the binding of the chemical but also in the pleiotropic induction of a series of enzymic activities. A study of the induction of AHH by PCB and TCDD revealed a distinct structure-activity relationship (Poland & Glover, 1973; Poland *et al.*, 1979). The most active compounds appeared to be molecules containing two halogen atoms at outer lateral positions in each phenyl ring. No direct study of the three-dimensional structure of PCB with respect to their capacity to stimulate synthesis of porphyrins, however, has been reported. Since PCB occur in the environment in the form of as many as 209 possible congeners, it would be important to establish the three-dimensional structure-activity relationship that might allow a prediction of the biological toxicity of PCB.

Cell cultures *in vitro* are ideal for the study of these extremely toxic compounds, since cells in culture are

Abbreviations used: PAH, polyhalogenated aromatic hydrocarbons; PCB, polychlorinated biphenyls; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; ALA, δ -aminolaevulinic acid; AHH, aryl-hydrocarbon hydroxylase; HCB, 3,4,5,3',4',5'-hexachlorobiphenyl; MNDO, 'modified neglect of diatomic overlap'; TCB, 2,4,3',4'-tetrachlorobiphenyl; HCB, 3,4,5,3',4',5'-hexachlorobiphenyl; HBB, 3,4,5,3',4',5'-hexabromobiphenyl; DCB, 3,4-dichlorinated biphenyl; PCB, 2,3,4,6,2'-pentachlorobiphenyl; OCB, 2,3,4,5,2',3',4',5'-Octachlorobiphenyl; DecaCB, 2,3,4,5,6,2',3',4',5',6'-decachlorobiphenyl.

isolated, amounts of chemicals used are minimal and individual variation can be minimized. By using percentage viability or the growth rate, however, Knutson & Poland (1980) could not find any effect of TCDD in 23 cell types examined in culture. Nonetheless, it would be of significant value if an assay could be developed with a cell culture that is highly sensitive and reliably reflects the toxic effects of PAH.

Isolated chick-embryo liver cultures are useful for this purpose in that the cells quickly respond to the addition of PAH and show inhibition of uroporphyrinogen decarboxylase, accumulation of uroporphyrin and induction of ALA synthase (Kawanishi *et al.*, 1978; de Verneuil *et al.*, 1983). Since there are certain similarities between PCB that induce AHH (Poland *et al.*, 1979) and those which induce uroporphyrin (Sassa *et al.*, 1985), we have investigated both the effects of PCB on uroporphyrin formation and the three-dimensional structure of PCB that affects uroporphyrin formation. The objective of the study was to attempt to define the quantitative aspects of the molecular properties of PCB such that the results of these findings would be sufficiently accurate and reliable to predict the structural requirement of PCB for the formation of uroporphyrin.

MATERIALS AND METHODS

Materials

All halogenated biphenyls were purchased from Ultra Scientific, Hope, RI, U.S.A. A highly purified preparation of 3,4,5,3',4',5'-hexachlorobiphenyl (HCB) was also kindly provided by Dr. Arleen B. Rifkind, Cornell University Medical College, New York, NY, U.S.A., which had been originally prepared by Dr. James McKinney, National Institute Environmental Health Sciences, Research Triangle Park, NC, U.S.A. Each of the PCB was at least 99% pure with respect to congener content as determined by g.l.c.

Cell cultures

Monolayer cultures of hepatocytes were prepared from 17-day-old chick embryos and incubated in a 24-well plate in a humidified CO₂ incubator at 37 °C with CO₂/air (1:19) as described previously (Sassa & Kappas, 1977). A serum-free culture medium, i.e., a modified F12 medium, contained 1 µg of bovine insulin 50 ng of cortisol and 1 µg of tri-iodothyronine/ml (Sassa & Kappas, 1977). After 24 h of incubation, the medium was replaced and addition of chemicals was made. Cells were then incubated for another 24 h with the chemical.

Fluorimetry of porphyrins

Porphyrins were extracted into a mixture of 1 M-HClO₄/methanol (1:1, v/v) and their concentrations were determined spectrofluorometrically in a Hitachi/Perkin-Elmer MPF4 fluorescence spectrophotometer equipped with an R928 photomultiplier. The percentage of uroporphyrin, coproporphyrin and protoporphyrin was differentially determined by using the method of Grandchamp *et al.* (1980). In some experiments, the porphyrins synthesized in hepatocytes were identified by using h.p.l.c. (de Verneuil *et al.*, 1983). The protein content of the cells was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

Molecular orbital calculations

Molecular orbital calculations of PCB were performed using the semi-empirical molecular orbital method MNDO ('Modified Neglect of Diatomic Overlap') (Dewar & Thiel, 1977) with a program obtained from the Quantum Chemistry Program Exchange (QCPE) (Department of Chemistry, Indiana University, Bloomington, IN 47405, U.S.A.). Calculations were performed on a VAX 11/750 computer at the Suntory Institute.

Calculations on PCB were performed at an idealized geometry with the following bond lengths: single C-C, 0.152 nm (1.52 Å); aromatic C-C 0.140 nm (1.40 Å); C-H, 0.109 nm (1.09 Å); C-Cl, 0.170 nm (1.70 Å). The conformational energy of PCB congeners was calculated as a function of its dihedral angle, θ , at every 15 ° starting from 0° up to 180°.

RESULTS

Chick-embryo liver cell cultures treated with 2,4,3',4'-tetrachlorobiphenyl (TCB), 3,4,3',4'-TCB, 3,4,5,3',4',5'-hexachlorobiphenyl (HCB) and 3,4,5,3',4',5'-hexabromobiphenyl (HBB) were found to cause accumulation of porphyrins in the cells by 8 h and porphyrin content of the cultures increased over 24 h during incubation. Differential fluorometric analysis for uro-, copro- and proto-porphyrin demonstrated that uroporphyrin constituted essentially all the porphyrins which had accumulated. H.p.l.c. analysis of the cultures also indicated that, among the total porphyrins accumulated, uroporphyrin and heptacarboxylate porphyrin accounted for approx. 85% and 15% respectively of porphyrins formed (Fig. 1).

Fig. 1. H.p.l.c. profile of porphyrins extracted from chick-embryo liver culture

Chick-embryo liver cells were prepared and incubated as described in the Materials and methods section. After incubation of cultures for 24 h with or without PCB, porphyrins were extracted from the liver cells, esterified in methanol/H₂SO₄ (9:1, by vol), and analysed by h.p.l.c. (de Verneuil *et al.*, 1983). (a) Untreated cultures; (b) cultures treated with 0.5 µg of 3,4,3',4'-TCB/ml. Injection of samples into the h.p.l.c. apparatus was made at zero time (indicated as 'BGN'). The porphyrin with a retention time of 6.86 min is heptacarboxylate porphyrin and that with a retention time of 7.95 min is uroporphyrin.

Table 1. Effect of PCB on uroporphyrin accumulation in cultured chick-embryo liver cells

Chick embryo liver cells were prepared and incubated in a serum-free modified F12 medium supplemented with insulin (1 $\mu\text{g}/\text{ml}$), cortisol (0.05 $\mu\text{g}/\text{ml}$) and tri-iodothyronine (1 $\mu\text{g}/\text{ml}$) as described in the Materials and methods section. After 24 h of incubation, the medium was replaced with fresh medium containing 0.5 μg of PCB or PBB congeners/ml. Cultures were then incubated for 24 h and porphyrins were quantified. Untreated control cultures contained undetectable levels of uroporphyrin (< 10 pmol/mg of protein). Results are the means $\pm$ S.E.M. of quadruplicate determinations.

No.	Compound	Uroporphyrin formation (pmol/mg of protein)
1	Biphenyl	< 10
2	4-MCB	< 10
3	2,2'-DCB	< 10
4	3,4-DCB	< 10
5	3,3'-DCB	< 10
6	4,4'-DCB	< 10
7	2,6,2',6'-TCB	< 10
8	2,4,3',4'-TCB	1025 $\pm$ 79
9	2,4,2',5'-TCB	13.1 $\pm$ 0.3
10	2,3,2',3'-TCB	< 10
11	3,4,3',4'-TCB	2227 $\pm$ 56
12	2,3,4,6,2'-PCB	< 10
13	3,4,5,3',4',5'-HCB	1450 $\pm$ 59
14	3,4,5,3',4',5'-HBB	1508 $\pm$ 132
15	2,4,5,2',4',5'-HCB	14.8 $\pm$ 2.0
16	2,4,5,2',4',5'-HBB	36.6 $\pm$ 7.7
17	2,3,6,2',3',6'-HCB	< 10
18	2,4,6,2',4',6'-HCB	< 10
19	2,3,4,5,2',3',4',5'-OCB	< 10
20	2,3,4,5,6,2',3',4',6'-DecaCB	< 10

Since the differential fluorometric method for the three porphyrins identifies both 8-carboxylate porphyrin and heptacarboxylate porphyrin as uroporphyrin, the above data, obtained by the two methods, can be considered to be equivalent.

In contrast with these halogenated biphenyls, other biphenyls, such as non-halogenated biphenyl, 4'-monochlorinated biphenyl, 3,4-dichlorinated biphenyl (DCB), 2,2'-DCB, 3,3'-DCB, 4,4'-DCB, 2,6,2',6'-TCB, 2,4,2',5'-TCB, 2,3,2',3'-TCB, 2,3,4,6,2'-pentachlorobiphenyl (PCB), 2,4,5,2',4',5'-HCB, 2,4,5,2',4',5'-HBB, 2,3,6,2',3',6'-HCB, 2,4,6,2',4',6'-HCB, 2,3,4,5,2',3',4',5'-octachlorobiphenyl (OCB) and 2,3,4,5,6,2',3',4',5',6'-decachlorobiphenyl (DecaCB) did not show accumulation of porphyrins (Table 1). Except for 2,4,3',4'-TCB, all three porphyrinogenic PCB had at least two halogen atoms at lateral adjacent (3,4,3',4' or 3,4,5,3',4',5') positions in each phenyl ring and lacked halogen substitutions at the innermost (2,2',6,6') position in the phenyl ring. Other chemicals did not contain halogen atoms in the lateral adjacent positions or contained halogen atoms at the innermost positions. Substitution of chlorine by bromine did not alter the uroporphyrin-accumulating activity. These structural requirements are very similar to those reported for the induction of AHH and for competition for the cytosolic binding species in chick embryos (Poland & Glover, 1973; Poland *et al.*, 1979).

The conformational energy of 13 of the 20 representative PCB congeners was calculated, using MNDO, as a function of their dihedral angles (θ) occurring between the two phenyl rings. All congeners showed the lowest conformational energy at a dihedral angle of 90° ($0^\circ \leq \theta \leq 180^\circ$) (results not shown). Thus it is difficult to

delineate the structure-activity relationship only on the basis of the conformational energy associated with the most stable form of the congeners. In order to depict detailed structure-activity relationships, we then examined the possible range of the dihedral angle of these congeners within the range of energy fluctuation which does not overcome the barrier state between different states of a molecule, i.e., approx. 83.7 kJ (20 kcal)/mol at 25 °C (Table 2). On the basis of the value of the possible range for θ , PCB congeners can be classified into four different groups. Type I PCB can assume all possible dihedral angles ($0^\circ \leq \theta \leq 180^\circ$) around the axis between the two phenyl rings, and the biphenyl, 4'-MCB, 3,4-DCB, 4,4'-DCB and 3,4,5,3',4',5'-HCB, belong to this group. Type II PCB, represented by 2,4,3',4'-TCB, had a smaller θ (28°–152°) than Type I PCB; however, the two phenyl rings in this congener can still rotate significantly. In contrast with Type I and Type II PCB, Type III PCB has a much smaller range for θ , i.e., 52°–142° and all of them (2,3,2',3'-TCB, 2,4,5,2',4',5'-HCB and 2,3,4,5,2',3',4',5'-OCB) contained chlorine atoms at the 2,2' positions. Type IV congeners displayed the narrowest range of θ , i.e., 58°–122°, or only $\pm 32^\circ$ from 90°, and all congeners of this group (2,6,2',6'-TCBB, 2,4,6,2',4',6'-HCB and 2,3,4,5,6,2',3',4',5',6'-DecaCB) contained chlorine atoms at the 2,2' and 6,6' positions. The last congener, i.e., the DecaCB, was so angulated that it could not take a conformation at $\theta = 0^\circ$. According to this classification, uroporphyrin-forming activity was associated with Type I and Type II PCB, which have a greater possibility than Type III and IV PCB of assuming a co-planar structure with θ as low as 28° (e.g., Type II PCB in Table 2). Because Type I congeners having less than two chlorine atoms per phenyl ring were not active,

Table 2. Classification of PCB based on the possible range of the dihedral angle between the two phenyl rings

Type	Congener	Uroporphyrin formation (pmol/mg of protein)	Possible range of the dihedral angle (θ) ($^{\circ}$)
I	BP	< 10	0 ~ 180
	4-MCB	< 10	0 ~ 180
	3,4-DCB	< 10	0 ~ 180
	4,4'-DCB	< 10	0 ~ 180
	3,4,3',4'-TCB	2200	0 ~ 180
	3,4,5,3',4',5'-HCB	1500	0 ~ 180
II	2,4,3',4'-TCB	1000	28 ~ 152
III	2,3,2',3'-TCB	< 10	52 ~ 142
	2,4,5,2',4',5'-HCB	< 10	52 ~ 142
	2,3,4,5,2',3',4',5'-OCB	< 10	52 ~ 142
IV	2,6,2',6'-TCB	< 10	58 ~ 122
	2,4,6,2',4',6'-HCB	< 10	58 ~ 122
	2,3,4,5,6,2',3',4',5',6'-DecaCB	< 10	58 ~ 122

Fig. 2. Probability of congener conformation as a function of dihedral angle

The probability of each congener conformation was calculated as a function of the dihedral angle at every 15° .
 ●, 3,4,3',4'-TCB; ○, 2,4,3',4'-TCB; ▲, 2,3,2',3'-TCB; △, 2,6,2',6'-TCB.

it is clear that not only the co-planar structure, but also two (or three) chlorine atoms are necessary at laterally adjacent sites in each phenyl ring.

The probability of the conformation of each congener at 25°C was then calculated as a function of the dihedral angle, assuming that the probability follows the Boltzman distribution. Fig. 2 shows the relationship between the probability of congener conformation and the dihedral angle of the four congeners which are representative of each specific type. All congeners had a maximum probability at $\theta = 90^{\circ}$. Type I PCB, i.e., congeners having readily rotating phenyl rings ($0^{\circ} \leq \theta \leq 180^{\circ}$), showed an approx. 32% probability at $\theta = 90^{\circ}$ and ~8% at $\theta = 60^{\circ}$ and 120° . Type II and III congeners, which had narrower ranges of θ , had a much higher probability at $\theta = 90^{\circ}$ than the Type I congeners. Type IV PCB, which had the narrowest range for θ ($\theta \leq 32^{\circ}$) existed essentially all at $\theta = 90^{\circ}$ (~96%).

In order to clarify the relationship between the probability of the congener conformation and the dihedral angle, the results in Fig. 2 were expressed in the form of cumulative probability of conformation as a

Fig. 3. Cumulative probability index for congener conformation as a function of the dihedral angle

The data in Fig. 2 are expressed as a cumulative index of probability as a function of the dihedral angle. ●—● 3,4,3',4'-TCB; ○—○ 2,4,3',4'-TCB; ▲—▲ 2,3,2',3'-TCB; △—△ 2,6,2',6'-TCB.

function of the dihedral angle (Fig. 3). Although all PCB had the greatest probability for the most angulated structure ($\theta = 90^{\circ}$), the probability for a planar structure differed significantly, depending on the type of PCB. For example, the cumulative probability of the congener at each given angle was approx. 30%, 15%, 1% and < 0.1% for 3,4,3',4'-TCB (Type I), 2,4,3',4'-TCB (Type II), 2,3,2',3'-TCB (Type III) and 2,6,2',6'-TCB (Type IV) respectively. In other words the ratio of the probable structure for these congeners at $0^{\circ} \leq \theta \leq 70^{\circ}$ is 300:150:10:< 1 in the above order. This relationship is

Fig. 4. Three-dimensional structure of two representative tetrachlorobiphenyls (*a,b*) and of 3,4,3',4'-TCB and 2,3,7,8-TCDD (*c*)

Key to colours of elements: C, white; H, green; Cl, purple; O, red. (*a*) 2,6,2',6'-TCB at $\theta = 90^\circ$; (*b*) 3,4,3',4'-TCB at $\theta = 0^\circ$. Each dot represents the van der Waals surface and the dot density is equal to five dots/ 0.01 nm^2 ($\text{\AA}^2$). The total dimensions of 3,4,3',4'-TCB are $0.34 \text{ nm} \times 1 \text{ nm} \times 0.34 \text{ nm}$ ($3.4 \text{ \AA} \times 10 \text{ \AA} \times 3.4 \text{ \AA}$). (*c*) The three-dimensional structure of 3,4,3',4'-TCB (at $\theta = 0^\circ$) is compared with that of 2,3,7,8-TCDD. These two compounds have a very similar three-dimensional structure as well as the laterally adjacent localization of the chlorine atom.

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in good agreement with their ability to stimulate uroporphyrin accumulation (Table 1).

Figs 4(a) and 4(b) show the three-dimensional structures of a porphyrinogenic PCB (Type I) and a non-porphyrinogenic PCB (Type IV) that were constructed by using the computer program 'GRAMPS' (O'Donnell & Olson, 1981). GRAMPS ('GRAphics for Multi-Picture Systems') is a software program designed as a user-oriented, interactive, general-purpose graphics system. The van der Waals surface of each atom is expressed with a specific colour, namely: C, white; H, green, and Cl, purple. The dot density is five dots/ 0.01 nm^2 ($1 \text{ \AA}^2$). For Type IV PCB, the most probable conformation for 2,6,2',6'-TCB is shown in Fig. 4(a) ($\theta = 90^\circ$), and for Type I PCB, a conformation for 3,4,3',4'-TCB at $\theta = 0^\circ$ is shown for comparison, since the latter could take this configuration whereas the former could not.

DISCUSSION

The results of the present study demonstrate several important structural characteristics of PCB congeners with respect to their ability to cause uroporphyrin accumulation in chick-embryo liver cells grown in primary cultures. Namely, certain substituent sites for halogens in PCB play a key role in determining uroporphyrin accumulation; thus (i) at least two halogen atoms, but less than four halogen atoms, must be present in each phenyl ring in the biphenyl molecule, (ii) halogen atoms must be present at the 3,4,5,3',4',5'-positions, or 2,4,3',4'-positions in the phenyl ring, and (iii) halogen atoms must not occupy the 2,2'-positions. Associated with these specific substituent sites the possible range of dihedral angles for porphyrinogenic congeners (Type I and II) was always large, e.g. 0° – 180° for the most potent Type I and 28° – 152° for the moderately potent Type II. By contrast, the range of dihedral angles of non-porphyrinogenic congeners were small, e.g. 52° – 142° for Type III and 58° – 122° for Type IV PCB.

The cumulative probability index of the congener conformation most clearly illustrated the structure-activity relationship as a function of the dihedral angle. For example, Type I, II, III and IV PCB existed with a probability of approx. 300:150:10: < 1 ($0^\circ \leq \theta \leq 70^\circ$). Although these calculations were made assuming a temperature of 25°C , it is reasonable to expect that the trend would remain under physiological conditions at 37°C as in the case of cells in culture.

The information obtained by molecular orbital calculations on PCB molecules thus indicates that the uroporphyrin-forming activity is associated with a planar structure with two or three chlorine atoms at the lateral adjacent positions in each phenyl ring. 2,2'-Chloro-substituents are contra-indicated for porphyrinogenic activity, since it is clear from MNDO calculations that the molecule becomes highly angulated and rigid.

Although all porphyrinogenic PCB belonged to Types I or II, several non-porphyrinogenic PCB were also found to belong to Type I. These non-porphyrinogenic PCB in Type I had less than four chlorine atoms in the molecule; thus it is also clear that at least two chlorine (halogen) atoms in each phenyl ring are necessary for porphyrinogenic activity.

A comparable calculation was also performed for TCDD, which is known to cause similar uroporphyrin

accumulation, inhibition of uroporphyrinogen decarboxylase and induction of ALA synthase in chick-embryo liver cell culture (de Verneuil *et al.*, 1983). Data on TCDD also indicated that not only the two phenyl rings are in the same plane, but also that its three-dimensional structure is very close to that of 3,4,3',4'-TCB (Fig. 4c). In the case of TCDD, free rotation of the two phenyl rings is entirely prohibited by the two oxygen atoms between the rings. Together with the similar laterally adjacent chlorine atoms, as in porphyrinogenic PCB, the rigid coplanar structure of TCDD may explain why this chemical is a more potent inducer of porphyrinogenesis as well as of cytochrome P-448 and its associated mixed-function oxidase activities than PCB (Poland & Knutson, 1982).

Several structure-activity relationship studies have been reported with respect to the induction of hepatic microsomal enzymes. Depending on the specific PCB congener types, cytochrome P-450 isoenzyme increases can be of the type induced by phenobarbital or by PAH (Goldstein *et al.*, 1977). PCB congeners chlorinated symmetrically in 3,3',4,4'-positions were originally reported to increase PAH-type cytochrome P-450 isoenzymes and chloro-substituents at 2,4,2',4'-positions leads to induction of phenobarbital-like cytochrome P-450 isoenzymes (Goldstein *et al.*, 1977). Some PCB, chlorinated at one *ortho* position and at *meta* and *para* positions of both phenyl rings induce both classes of cytochrome P-450 species, and some HCBs with 2,2'-chloro-substituents exhibit a similar mixed induction (Parkinson *et al.*, 1980a,b). On the other hand, a more recent study from the same group reported that, because of the considerable structural diversity, rules for predicting which PCB are phenobarbital-like inducers cannot be clearly defined (Parkinson *et al.*, 1980c).

Kaminsky *et al.* (1981) made molecular orbital calculations on DCB using MNDO for the structure-activity relationship of their metabolism by purified rat liver cytochrome P-450 species. By using reconstituted purified cytochrome P-450 isoenzymes PB-B and BNF-B, they reported that isoenzyme PB-B preferentially metabolized non-co-planar DCB and BNF-B co-planar DCB. The cytochrome isoenzymes also exhibited differing regioselectiveness for DCB metabolism; isoenzyme PB-B hydroxylated monochlorinated phenyl rings, whereas BNF-B hydroxylated chlorinated rings. Their findings demonstrate the usefulness of molecular orbital calculations in predicting the chloro-substituent sites necessary in the regioselectivity of metabolism which is affected by the phenyl ring-twist angles.

The present results on the molecular orbital calculations of PCB congeners represent the first description of the three-dimensional structure-activity relationship of these congeners in relation to uroporphyrin formation and also provide strong support for the existence of the specific structure-activity relationships for this response to occur. On the basis of the molecular orbital calculations of porphyrinogenic PCB, e.g., 3,4,3',4'-TCB and 2,3,7,8-TCDD, it can be assumed that the cytosolic binding species for these PAH may recognize a molecule having a dimension of $0.34 \text{ nm} \times 1 \text{ nm} \times 0.34 \text{ nm}$ ($3.4 \text{ \AA} \times 10 \text{ \AA} \times 3.4 \text{ \AA}$). On the other hand, it is known that stimulation of drug metabolism potentiates uroporphyrin formation and/or enzyme induction responses by PCB and TCDD (de Verneuil *et al.*, 1983). Perhaps, therefore, the binding of the PCB or TCDD to the

receptor and stimulation of enzyme induction responses by increased drug metabolism are not mutually exclusive phenomena. For example, it is conceivable that the binding of the chemical to the receptor may be determined by a planar structure with lateral adjacent halogens, whereas the magnitude of the biological response may be augmented by the stimulation of drug metabolism. In support of this concept, it has been shown that accumulation of uroporphyrin occurs in cultured chick-embryo liver cells by treatment with 3,4,3',4'-TCB or TCDD alone, but that the magnitude of response is greatly increased by simultaneous treatment of the cultures with phenobarbital or β -naphthoflavone (de Verneuil *et al.*, 1983; Sassa *et al.*, 1985).

This study was supported in part by grants from the U.S. Public Health Service (nos. ES-01055 and AM-32850). We thank Dr. A. Olson, Scripps Clinic and Research Foundation, for providing us with the GRAMPS program, and Ms. Jill A. Brighton for the preparation of the manuscript.

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Received 30 July 1985/24 October 1985; accepted 6 December 1985