

Toxicity of Polychlorinated Biphenyl with Special Reference to Porphyrin Metabolism

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Oral administration of a commercial PCB mixture to chickens caused a hepatic-type porphyria characterized by hepatic accumulation and urinary excretion of uroporphyrin. To clarify the mechanism of the porphyrinogenic activity of these PCBs, we studied the structural requirement of synthetic PCB for porphyrinogenic activities by using the cultured chick embryo liver cells and examined the relationship between induction of δ -aminolevulinic acid (ALA) synthetase and inhibition of uroporphyrinogen decarboxylase. We established that the porphyrinogenic effect of PCBs exhibits a sharply defined structure-activity relationship in that only 3,4,3',4'-tetrachlorobiphenyl and 3,4,5,3',4',5'-hexachlorobiphenyl produced a marked accumulation of uroporphyrin. We also demonstrated that in ALA-supplemented cultures, these same compounds lead to accumulation of a large amount of uroporphyrin III, whereas with other PCBs, which were weak inducers of porphyrin synthesis, the accumulated porphyrin was mostly protoporphyrin.

Kinetic studies of the sequential decarboxylation of uroporphyrinogen with purified uroporphyrinogen decarboxylase were performed. The 3,4,3',4'-tetrachlorobiphenyl and 3,4,5,3',4',5'-hexachlorobiphenyl strongly inhibit uroporphyrinogen decarboxylase directly at two steps, i.e. first in the formation of hexacarboxylic porphyrinogen III from heptacarboxylic porphyrinogen III and second in the formation of heptacarboxylic porphyrinogen III from uroporphyrinogen III.

The results confirmed that porphyrinogenic PCBs primarily inhibit uroporphyrinogen decarboxylase, leading to a depletion of heme as a result of which synthesis of ALA synthetase increased.

In 1968, Yusho (polychlorinated biphenyl [PCB] poisoning) occurred in the western part of Japan (1), but disturbances of porphyrin metabolism due to PCBs were not investigated at that time. In 1970, Vos and Koeman (2) first reported that oral administration of commercial PCB to chickens caused hepatic-type porphyria, which was characterized by excretion and hepatic accumulation of uroporphyrin (3). We studied the structural requirement of synthetic PCBs for inducing porphyrin in cultured chick embryo liver cells (4) and the inhibitory effect of PCB isomers on uroporphyrinogen decarboxylase, clarifying the mechanism of porphyrinogenic activity of PCB.

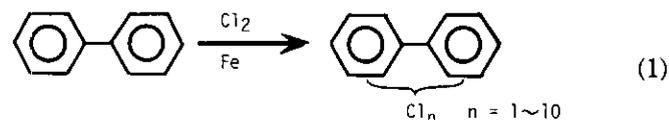
Heme Synthesis

A schematic outline of porphyrin and heme biosynthesis is presented in Figure 1 (5). The first step is the condensation of succinyl-CoA and glycine to form δ -aminolevulinic acid (ALA). This reaction is catalyzed by ALA synthetase, which is regulated by feedback repression of heme, i.e., the end product of the biosynthetic pathway. Two molecules of ALA then condense to form porphobilinogen (PBG) and then four

molecules of PBG condense to form uroporphyrinogen III. Uroporphyrinogen decarboxylase catalyzes the decarboxylation of uroporphyrinogen into coproporphyrinogen through hepta-, hexa-, and pentacarboxylic porphyrinogen intermediates. Protoporphyrinogen is formed by oxidative decarboxylation of propionate side chains of coproporphyrinogen III to vinyl groups. Protoporphyrin is formed by enzymatic oxidation of protoporphyrinogen. Finally, iron chelation to the protoporphyrin ring gives heme.

Synthesis of PCB Isomers and Their Properties

Commercial PCBs are prepared by chlorination of biphenyl in the presence of iron powder [eq. (1)].



Symmetrical PCB isomers are synthesized as shown in eq. (2). Chloroaniline was treated with sodium nitrate to give the corresponding diazonium derivative, which was replaced by iodine, followed by Ullmann condensation (4).

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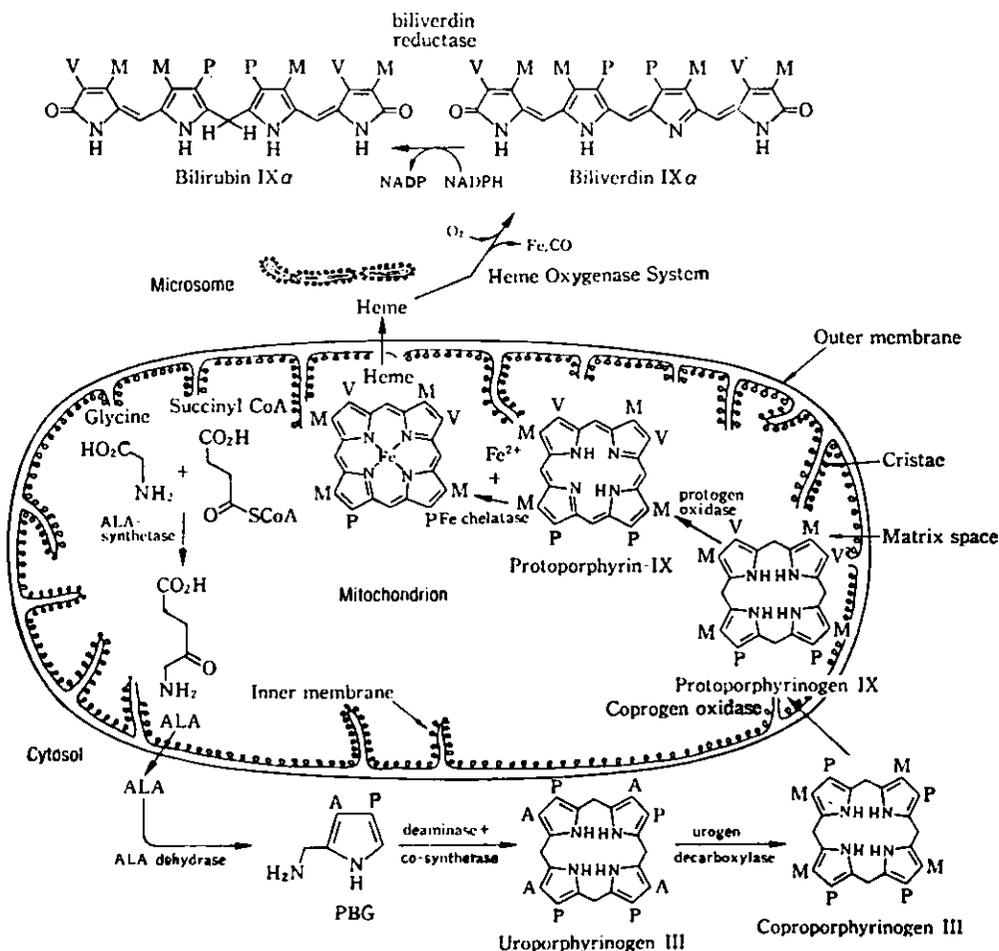
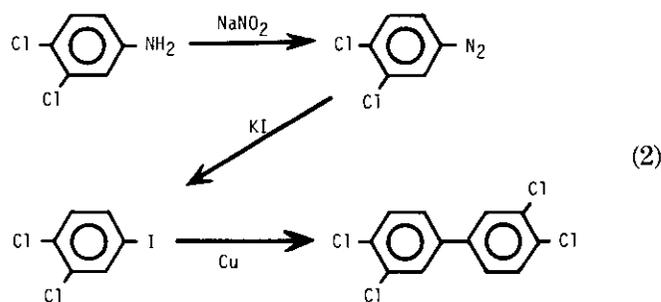


FIGURE 1. Outline of heme biosynthesis.

Table 1 presents the melting points, gas chromatographic retention times and K band of the symmetrical PCB isomers (4). The relative retention times were correlated with the lipophilicity of the synthetic com-



pounds. The 3,4,3',4'-tetra- and 3,4,5,3',4',5'-hexachlorobiphenyl isomers showed the highest retention times among the tetrachloro- and hexachlorobiphenyls investigated, respectively.

Spectroscopic observations on K bands and ϵ values were in good agreement with those reported by MacNeil

Table 1. Some physical properties of symmetrical chlorobiphenyl isomers synthesized for testing porphyrin-inducing activities.

	Melting point, °C	Rt ^a	K-band of ultraviolet spectra ^b	
			nm	(ϵ M)
Biphenyl	69-71	-	248	(16,000)
4,4'-Dichlorobiphenyl	148	28	259	(24,000)
2,3,2',3'-Tetrachlorobiphenyl	117-120	60	229 sh	(19,600)
2,4,2',4'-Tetrachlorobiphenyl	39-40	49	242 sh	(12,900)
2,5,2',5'-Tetrachlorobiphenyl	83-85	46	233 sh	(18,500)
2,6,2',6'-Tetrachlorobiphenyl	197	33	229 sh	(14,200)
3,4,3',4'-Tetrachlorobiphenyl	177	106	262	(24,000)
3,5,3',5'-Tetrachlorobiphenyl	166-168	70	254	(15,000)
2,3,4,2',3',4'-Hexachlorobiphenyl	150-151	195	231 sh	(20,300)
2,4,6,2',4',6'-Hexachlorobiphenyl	122	85	231 sh	(31,000)
3,4,5,3',4',5'-Hexachlorobiphenyl	201	300	266	(23,200)

^aRetention time relative to *p,p'*-DDE (= 100) on an OV-1 column at 190°C.

^bsh: shoulder.

et al. (6). In a chlorobiphenyl isomer with two or more chlorine atoms at the *ortho* and *ortho'* positions, considerable steric hindrance was expected. The shift of the K band toward shorter wavelengths was observed.

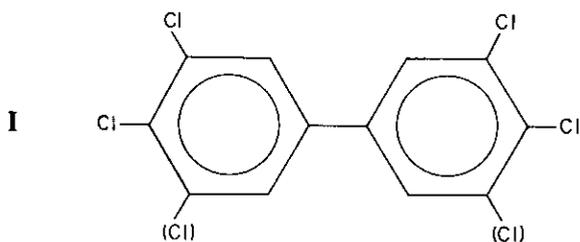
Table 2. Effects of chlorobiphenyl isomers on porphyrin formation in chick embryo liver cells.^a

Compound added	Porphyrin, pmole/mg protein
None	13 ± 1.5
Biphenyl	15 ± 2.4
4,4'-Dichlorobiphenyl	56 ± 20
2,3,2',3'-Tetrachlorobiphenyl	80 ± 20
2,4,2',4'-Tetrachlorobiphenyl	81 ± 29
2,5,2',5'-Tetrachlorobiphenyl	15 ± 3.0
2,6,2',6'-Tetrachlorobiphenyl	62 ± 16
3,4,3',4'-Tetrachlorobiphenyl	3400 ± 480
3,5,3',5'-Tetrachlorobiphenyl	420 ± 170
2,3,4,2',3',4'-Hexachlorobiphenyl	200 ± 65
2,4,6,2',4',6'-Hexachlorobiphenyl	140 ± 35
3,4,5,3',4',5'-Hexachlorobiphenyl	2600 ± 410
Decachlorobiphenyl	48 ± 24
Kanechlor-200	280 ± 75
Kanechlor-300	550 ± 50
Kanechlor-400	1600 ± 190
Kanechlor-500	250 ± 60
Kanechlor-600	50 ± 8.0
DDC	120 ± 33

^aLiver cells were prepared and incubated as described in the literature (4,7). After the medium was changed, a series of chlorobiphenyl isomers dissolved in 25 μL dimethyl sulfoxide were added. The concentrations of chemicals were in all cases 2 μg/mL. Porphyrin and protein were measured after 24-hr incubation. Values presented are the means ± SE for five experiments.

Porphyrin-Inducing Activity of Synthetic and Commercial Polychlorinated Biphenyls

Chick embryo liver cell cultures were prepared by using a slightly modified procedure of Granick (4,7), Table 2 presents the porphyrin-inducing activities of synthetic and commercial polychlorinated biphenyls. It was found that 3,4,3',4'-tetra- and 3,4,5,3',4',5'-hexachlorobiphenyl isomers were the most active inducers. 3,5,3',5'-Tetrachlorobiphenyl was a moderate inducer. The 4,4'-dichloro, 2,3,2',3'-, 2,4,2',4'- and 2,6,2',6'-tetrachloro- and 2,3,4,2',3',4'- and 2,4,6,2',4',6'-hexachlorobiphenyl isomers were weak inducers; the 2,5,2',5'-tetrachloro- and decachlorobiphenyl isomers were inactive. Kanechlor-400 was found to be the strongest inducer among the commercial polychlorinated biphenyl samples tested. On the basis of these data, we established that requirements for porphyrin-inducing activity of PCB are a structure of the type I.



First, a coplanar structure of the chlorobiphenyl is essential for porphyrinogenic activity, as shown in the most potent inducers such as 3,4,3',4'-tetra- and

Table 3. Relationship of structure to accumulation in liver of chlorobiphenyl isomers.^a

Compound administered	Number of deaths	Concentration in liver, μg/g
2,3,2',3'-Tetrachlorobiphenyl	0	< 0.5
2,4,2',4'-Tetrachlorobiphenyl	0	2.6 ± 1.6
2,5,2',5'-Tetrachlorobiphenyl	0	< 0.5
2,6,2',6'-Tetrachlorobiphenyl	0	< 0.5
3,4,3',4'-Tetrachlorobiphenyl	0	< 0.5
3,5,3',5'-Tetrachlorobiphenyl	4	2500 ± 1200
2,3,4,2',3',4'-Hexachlorobiphenyl	0	12 ± 4
2,4,6,2',4',6'-Hexachlorobiphenyl	4	1800 ± 280
3,4,5,3',4',5'-Hexachlorobiphenyl	4	650 ± 130
Kanechlor-400	1	86 ± 95
Kanechlor-500	2	480 ± 95
Kanechlor-600	2	3200 ± 800

^aThe mice were fed a diet containing 300 ppm of each of chlorobiphenyl isomers and commercial polychlorinated biphenyls. After 14 weeks the residue levels in liver were determined by gas-liquid chromatography. Values presented are the means ± SE for four mice.

3,4,5,3',4',5'-hexachlorobiphenyl isomers. There was a good correlation between coplanar structure and greater basochromic shift of the K band (see Table 1). Occupation of the 4,4'-positions by chlorine increased the double-bond character of C₍₁₎-C_(1') bond resulting in the formation of a more coplanar structure (8). It is thus reasonable to assume that 3,5,3',5'-tetrachlorobiphenyl is a less effective porphyrin inducer than 3,4,3',4'-tetrachlorobiphenyl. It was also demonstrated that the K band for *ortho-ortho'*-substituted isomers (2,3,2',3'-, 2,4,2',4'-, 2,5,2',5'-, 2,6,2',6'-, 2,3,4,2',3',4'-, and 2,4,6,2',4',6'-) showed a large hypsochromic shift, suggesting that these compounds possess very hindered structures. These distorted structures could explain their weak porphyrinogenic activity. The angles (θ) between the two planes of the benzene ring were reported to be 23° and 70° for biphenyl and 2,2'-dichlorobiphenyl, respectively (9).

Second, occupation of both *para* and *meta* positions by chlorine atoms is required for porphyrin induction, since 4,4'-dichlorobiphenyl was inactive but 3,4,3',4'-tetra- and 3,4,5,3',4',5'-hexachlorobiphenyl isomers were active. Polychlorinated biphenyl-induced porphyria did not depend simply on the percentage of chlorination. For example, 3,4,3',4'-tetrachlorobiphenyl was the most active agent among the chlorobiphenyls investigated.

Relationship of Structure to Accumulation of PCB isomers in the Liver

Is porphyrin-inducing activity due to the direct action of added chemicals or to some active metabolite formed by the cells? To answer this question, we first studied what kind of PCB isomers would accumulate in mouse liver after feeding a diet containing PCB isomers.

Table 3 shows the relationship of structure of PCB isomers to accumulation in liver. On the basis of these data, we classified PCB isomers into three types based

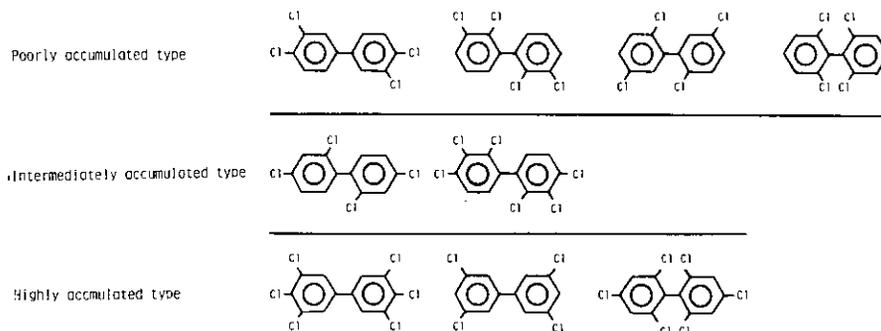


FIGURE 2. Tissue accumulation of PCB isomers.

Table 4. Effect of PCBs on porphyrin formation from exogenous ALA in cultured chick embryo liver cells.^a

PCB added	Porphyrins, pmole/mg protein		Prevalent type of porphyrin
	Cells	Medium	
None	49 ± 4	240 ± 13	Proto
2,3,2',3'-Tetrachlorobiphenyl	54 ± 4	230 ± 14	Proto
2,4,2',4'-Tetrachlorobiphenyl	53 ± 9	230 ± 10	Proto
2,5,2',5'-Tetrachlorobiphenyl	54 ± 6	230 ± 8	Proto
2,6,2',6'-Tetrachlorobiphenyl	51 ± 4	240 ± 14	Proto
3,4,3',4'-Tetrachlorobiphenyl	210 ± 9	89 ± 6	Uro
3,5,3',5'-Tetrachlorobiphenyl	130 ± 10	150 ± 16	Uro + Proto
2,3,4,2',3',4'-Hexachlorobiphenyl	65 ± 9	240 ± 17	Proto
2,4,6,2',4',6'-Hexachlorobiphenyl	58 ± 4	250 ± 9	Proto
3,4,5,3',4',5'-Hexachlorobiphenyl	190 ± 9	91 ± 4	Uro

^aChick embryo liver cells were cultured in 2 mL Falcon dishes in Eagle's minimum essential medium containing 10% fetal calf serum for 20 hr. After the original medium was replaced, various PCBs in 4 μ L DMSO (final concentration 2 μ g/mL) were added and the mixture was incubated for 1 hr. 0.2 mole ALA was then added and the incubation continued for another 3 hr. Total porphyrin content of cells and medium was quantified and the major porphyrins were characterized by HPLC. Porphyrin content of cells and medium was expressed/mg cell protein. Values represent the means \pm SD of 3-6 individual experiments.

on PCB metabolism as shown in Figure 2. The 3,4,3',4'-, 2,3,2',3'-, 2,5,2',5'-, and 2,6, 2',6'-tetrachlorobiphenyls do not accumulate appreciably in mice liver. These four compounds have unsubstituted pairs of carbon atoms in the molecule, and hence they are likely to be metabolized to the hydroxylated derivatives. Steric hindrance cannot be expected in the molecule (see Table 1). Similar results were reported by Gage and Holm (10); the rate of loss of polychlorinated biphenyl isomers from mouse fat was influenced by unsubstituted pairs of carbon atoms in the molecule. On the other hand, 3,5,3',5'-tetra-, and 2,4,6,2',4',6'- and 3,4,5,3',4',5'-hexachlorobiphenyl isomers markedly accumulated in mouse liver. These compounds contain only isolated unsubstituted carbon atoms, and this situation may account for the difficulty of metabolic conversion to hydroxylated derivatives. The 2,4,2',4'-tetrachloro- and 2,3,4,2',3',4'-hexachlorobiphenyl isomers possess unsubstituted pairs of carbon atoms; however, they are not so readily metabolized, since they have chlorine atoms at the *ortho-ortho'* positions, resulting in the formation of sterically hindered structures. In fact, 3,4,5,3',4',5'-hexachlorobiphenyl was one of the most active agents, although it was less metabolized. Therefore, it seems likely that porphyrin induction in the chick embryo liver system is due to the original chemicals and not to their metabolites.

Relationship between Induction of ALA Synthetase and Inhibition of Uroporphyrinogen Decarboxylase by PCB

The next question arises: why is porphyrin synthesis induced by PCB? In order to clarify the mechanism of the porphyrinogenic activity of these PCBs, we used cultured chick embryo liver cells supplemented with ALA and examined the relationship between induction of ALA synthetase and inhibition of uroporphyrinogen decarboxylase (11).

When cultured chick embryo liver cells were supplemented with ALA, a large amount of protoporphyrin accumulated, primarily in the medium (Table 4). A small amount of uroporphyrin, usually <10% of total porphyrins, and some coproporphyrin were also formed. Addition of a strong inducer such as 3,4,3',4'-tetrachlorobiphenyl or 3,4,5,3',4',5'-hexachlorobiphenyl resulted in the accumulation of a large amount of uroporphyrin, while protoporphyrin accumulation was greatly reduced. Moreover, much of the uroporphyrin remained intracellular (Table 4). With a moderate inducer such as 3,5,3',5'-tetrachlorobiphenyl, the increase in uroporphyrin and the decrease in protopor-

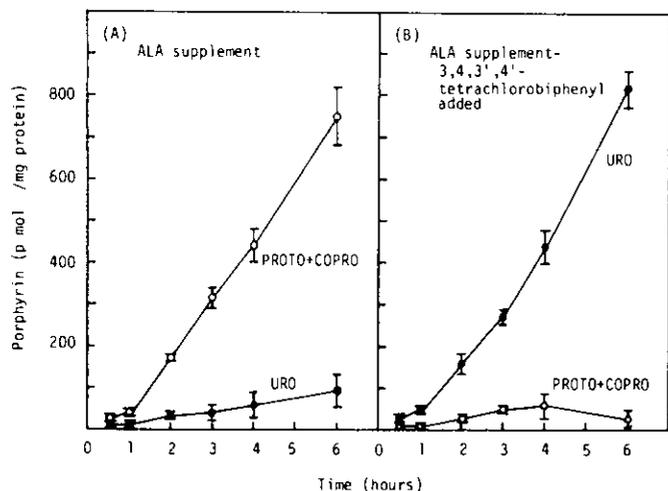


FIGURE 3. Time course of porphyrin formation from exogenous ALA by cultured chick embryo liver cells (A) in the presence of 3,4,3',4'-tetrachlorobiphenyl (B). Experimental conditions were as in Table 4, except that after addition of ALA, incubations were terminated at various times, from 0.5 to 6 hr. Values represent the means \pm SD of three individual experiments. Uro (porphyrin) reflects the sum of penta-, hepta- and octacarboxylic porphyrins.

pyrin were more moderate. Addition of a weak inducer such as 2,3,2',3'-, 2,4,2',4'-, 2,5,2',5'- or 2,6,2',6'-tetrachlorobiphenyl and 2,3,4,2',3',4'- or 2,4,6,2',4',6'-hexachlorobiphenyl had little or no effect on porphyrin accumulation after 3 hr incubation (Table 4), but extension of the incubation time led to accumulation of uroporphyrin to some extent over the control in the cells.

The time course of porphyrin accumulation with 3,4,3',4'-tetrachlorobiphenyl is shown in Figure 3. Whereas ALA supplementation alone (Figure 3A) resulted mainly in protoporphyrin accumulation, addition of 3,4,3',4'-tetrachlorobiphenyl (Figure 3B) reversed the pattern, in that mostly uroporphyrin was formed at the expense of proto- and coproporphyrin. The uroporphyrin formed was almost entirely the type III isomer. These results suggested that the same PCBs which most actively induce porphyrin synthesis also inhibit uroporphyrinogen decarboxylase.

Inhibitory Effect of PCB Isomers on Purified Uroporphyrinogen Decarboxylase

In order to confirm the inhibition of uroporphyrinogen decarboxylase by PCB, we purified the enzyme from chicken erythrocytes (12). Purification was accomplished by chromatography on DEAE-cellulose, ammonium sulfate fractionation, chromatography on Sephadex G-100, and chromatofocusing. The most purified preparation was homogeneous on polyacrylamide gel electrophoresis and had a specific activity of 1420 units/mg of protein, the highest value so far reported.

Table 5. Effect of PCBs on inhibition of uroporphyrinogen decarboxylase.^a

PCB added	Coproporphyrinogen formed, pmole	Inhibition, %
None	1480	
2,3,2',3'-Tetrachlorobiphenyl	820	45
2,4,2',4'-Tetrachlorobiphenyl	490	67
2,5,2',5'-Tetrachlorobiphenyl	960	35
2,6,2',6'-Tetrachlorobiphenyl	790	47
3,4,3',4'-Tetrachlorobiphenyl	290	80
3,5,3',5'-Tetrachlorobiphenyl	340	77
2,3,4,2',3',4'-Hexachlorobiphenyl	980	34
2,4,6,2',4',6'-Hexachlorobiphenyl	460	69
3,4,5,3',4',5'-Hexachlorobiphenyl	87	94

^aThe assay system contained 2 nmole of uroporphyrinogen III (200 nm), 1 mM GSH, 0.1 mM EDTA, 57 mM potassium phosphate, pH 6.8, 1.4 μ g of uroporphyrinogen decarboxylase (1.75 nM) and 25 μ g of PCB (tetrachlorobiphenyl, 8.6 μ M; hexachlorobiphenyl, 6.9 μ M) dissolved in 100 μ L of dimethyl sulfoxide in a total volume of 10 mL. After preincubation for 15 min, the substrate was added and the assay was allowed to proceed at 37°C for 75 min in the dark. PCBs in either *n*-hexane or the reaction mixture show no turbidity and have almost the same molar extinction coefficients and absorption maxima, suggesting that PCBs were soluble under conditions used.

The molecular weight, as determined by Sephadex G-150 gel chromatography, was 79,000. The subunit molecular weight, as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, was 39,700, suggesting that uroporphyrinogen decarboxylase is dimeric. The purified enzyme had an isoelectric point of 6.2 and a pH optimum of 6.8. Sulfhydryl reagents inhibited the enzyme activity, but neither metal ions nor cofactor requirements could be demonstrated.

The inhibitory effects of synthetic PCBs on the purified enzyme are summarized in Table 5. All the PCBs tested inhibited uroporphyrinogen decarboxylase. The strongest porphyrin inducers such as 3,4,3',4'-tetrachlorobiphenyl and 3,4,5,3',4',5'-hexachlorobiphenyl strongly inhibited the enzyme (80 and 94%, respectively). The moderate inducer, 3,5,3',5'-tetrachlorobiphenyl, moderately inhibited the enzyme (77%). Addition of weak inducers such as 2,3,2',3'-, 2,4,2',4'-, 2,5,2',5'-, and 2,6,2',6'-tetrachlorobiphenyl and 2,3,4,2',3',4'- and 2,4,6,2',4',6'-hexachlorobiphenyl, however, resulted in a partial inhibition of the enzyme (34–69%). These results indicate that there is a good relationship between the inhibition of uroporphyrinogen decarboxylase in cultured liver cells (Table 4) and inhibition of purified uroporphyrinogen decarboxylase. However, the inhibition of the enzyme by weak inducers seems to be slightly stronger than that observed in cultured liver cells. This may be due to low permeability of the weak porphyrinogenic PCB isomers, which have low lipophilicity, through the membrane of liver cells. Their low lipophilicity was inferred from the observation that they showed lower retention times on gas-liquid chromatography than the strong inducers (Table 1).

In order to clarify which steps of the decarboxylation from uroporphyrinogen to coproporphyrinogen are inhibited by PCB isomers, we studied the kinetics of the sequential decarboxylation using purified enzyme. We developed a simple and sensitive enzyme assay based on the separation and quantification of free porphyrins with 8- to 4-carboxylic acids using HPLC equipped with a spectrofluorometric detector.

The typical inhibitory effect of PCB on the enzyme was studied with the use of 3,4,5,3',4',5'-hexachlorobiphenyl at two different concentrations. As shown in Figure 4, in the control experiment (A), uroporphyrinogen III was almost quantitatively converted into coproporphyrinogen III. With a low concentration of PCB (5.5 μM), a large amount of heptacarboxylic porphyrinogen III accumulated and a small amount of coproporphyrinogen III was formed (B), whereas the addition of a higher concentration of PCB (8.3 μM) resulted in the complete inhibition of the decarboxyla-

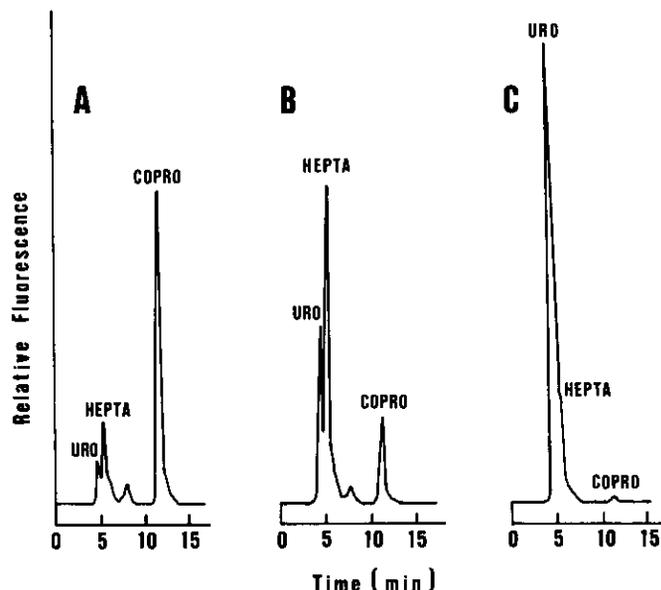


FIGURE 4. Inhibitory effect of 3,4,5,3',4',5'-hexachlorobiphenyl on uroporphyrinogen decarboxylase: (A) control; (B) addition of 3,4,5,3',4',5'-hexachlorobiphenyl, 20 μg (5.5 μM); (C) 30 μg (8.3 μM). URO, uroporphyrinogen III; HEPTA, heptacarboxylic porphyrinogen III; COPRO, coproporphyrinogen III. The assay conditions: column (300 \times 3.9 mm); packing: $\mu\text{Bondapak C}_{18}$; mobile phase: acetonitrile, 10 mM KH_2PO_4 , pH 3.0 (1:1 v/v); flow rate: 0.8 mL/min; sample size: 30 μL ; excitation: 404 nm; emission: 620 nm.

tion (C). These results show that 3,4,3',4'-tetrachlorobiphenyl and 3,4,5,3',4',5'-hexachlorobiphenyl which specifically induce ALA synthetase also strongly inhibit uroporphyrinogen decarboxylase directly at two steps, i.e., first in the formation of hexacarboxylic porphyrinogen III from heptacarboxylic porphyrinogen III and second in the formation of heptacarboxylicporphyrinogen from uroporphyrinogen III.

Acquired Porphyria in C57BL/6 Mice Due to PCB Isomer Intoxication

Finally we report the results of our recent genetic studies on mice. In order to investigate the influence of genetic factors on porphyrin induction by PCB, we used C57BL/6 mice. Livers of these mice treated with 3,4,5,3',4',5'-hexachlorobiphenyl were larger than those of ddY mice. Under ultraviolet light, we observed a pronounced red fluorescence in livers of C57BL/6 mice treated with the PCB isomer. Table 6 summarizes the porphyrin content in the livers of mice treated with 3,4,5,3',4',5'-hexachlorobiphenyl. There is a clear difference in the response of the two strains to porphyrin induction by PCB. A large amount of uroporphyrin fraction accumulated in the livers of C57BL/6 mice treated with PCB but not in the livers of ddY mice.

It is well known that C57BL/6 mice carry the *Ah^b* allele, giving a responsive phenotype to the induction of aromatic hydrocarbon hydroxylase (13). This suggests

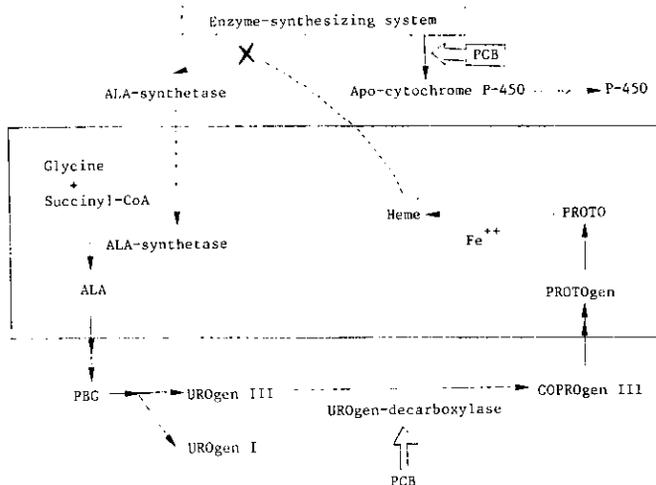


FIGURE 5. A possible mechanism of porphyrin induction by PCB.

Table 6. Porphyrin content in the liver of mice treated with PCB.^a

Strain	Treatment	Porphyrin, $\mu\text{g/g}$ liver ^b		
		Uro	Copro	Proto
ddY	Control	0.12 \pm 0.06	0.06 \pm 0.02	0.17 \pm 0.07
	3,4,5,3',4',5'-Hexachlorobiphenyl	0.26 \pm 0.05	0.06 \pm 0.02	0.24 \pm 0.13
C57BL/6	Control	0.11 \pm 0.02	0.08 \pm 0.02	0.26 \pm 0.03
	3,4,5,3',4',5'-Hexachlorobiphenyl	11.30 \pm 3.44	2.01 \pm 0.53	0.99 \pm 0.17

^aMale mice (7 weeks old) were treated with a diet containing 300 ppm 3,4,5,3',4',5'-hexachlorobiphenyl. After 3 weeks the animals were killed and the porphyrins in liver were determined.

^bMean \pm SD of four mice.

that induction of apocytochrome P-450 may take part in inducing porphyrin synthesis.

Mechanism of Porphyrinogenic Activity of PCB

On the basis of these results, we propose a possible mechanism of porphyrin accumulation caused by PCB as shown in Figure 5. Porphyrinogenic PCB's primarily inhibited uroporphyrinogen decarboxylase, leading to a depletion of heme. In addition, induction of apocytochrome P-450 by PCB may contribute to a decrease of heme. As a result, synthesis of ALA synthetase increased leading to an accumulation of uroporphyrin in liver.

REFERENCES

1. Kuratsune, M., Morikawa, Y., Hirohata, T., Nishizumi, M., Kohechi, S., Yoshimura, T., Matsuzaka, J., Yamaguchi, A., Saruta, N., Ishinishi, N., Kunitake, E., Shimono, O., Takigawa, K., Oki, K., Sonoda, M., Ueda, T., and Ogawa, M. An epidemiologic study on "Yusho" on chlorobiphenyls poisoning. *Fukuoka Acta Med.* 60: 513-532 (1960).
2. Vos, J. G., and Koeman, J. H. Comparative toxicologic study with polychlorinated biphenyls in chickens with special reference to porphyria, edema formation, liver necrosis, and tissue residues. *Toxicol. Appl. Pharmacol.* 17: 656-668 (1970).
3. Goldstein, J. A., Hickman, P., and Jue, D. L. Experimental hepatic porphyria induced by polychlorinated biphenyls. *Toxicol. Appl. Pharmacol.* 27: 437-448 (1974).
4. Kawanishi, S., Mizutani, T., and Sano, S. Induction of porphyrin synthesis in chick embryo liver cell culture by synthetic polychlorobiphenyl isomers. *Biochim. Biophys. Acta* 540: 83-92 (1978).
5. Granick, S., and Sassa, S. δ -Aminolevulinic acid synthetase and the control of heme and chlorophyll synthesis. In: *Metabolic Pathway* (H. J. Vogel, Ed.), Vol. 5, Academic Press, New York, 1971, pp. 77-141.
6. MacNeil, J. D., Safe, S., and Hutzinger, O. The ultraviolet absorption spectra of some chlorinated biphenyls. *Bull. Environ. Contam. Toxicol.* 15: 66-77 (1976).
7. Granick, S., Sinclair, P., Sassa, S., and Grieneringer, G. Effects of heme, insulin and serum albumin on heme and protein synthesis in chick embryo liver cells cultured in a chemically defined medium and a spectrofluorometric assay for porphyrin composition. *J. Biol. Chem.* 250: 9215-9225 (1975).
8. Ruzo, L. O., Zabik, M., and Schuetz, R. D. Photochemistry of bioactive compounds. Photochemical processes of polychlorinated biphenyls. *J. Am. Chem. Soc.* 96: 3809-3813 (1974).
9. Suzuki, H. Relations between electronic absorption spectra and spatial configurations of conjugated systems. I. Biphenyl. *Bull. Chem. Soc. Japan* 32: 1340-1350 (1959).
10. Gage, J. C., and Holm, S. The influence of molecular structure on the retention and excretion of polychlorinated biphenyls by the mouse. *Toxicol. Appl. Pharmacol.* 36: 555-560 (1976).
11. Kawanishi, S., Seki, Y., and Sano, S. Polychlorobiphenyls that induce δ -aminolevulinic acid synthetase inhibit uroporphyrinogen decarboxylase in cultured chick embryo liver cells. *FEBS Letters* 129: 93-96 (1981).
12. Kawanishi, S., Seki, Y., and Sano, S. Uroporphyrinogen decarboxylase. Purification, properties, and inhibition by polychlorinated biphenyl isomers. *J. Biol. Chem.* 258: 4285-4292 (1983).
13. Gielen, J. E., Goujon, F. M., and Nebert, D. W. Genetic regulation of aryl hydrocarbon hydroxylase induction. II. Simple Mendelian expression in mouse tissues *in vivo*. *J. Biol. Chem.* 247: 1125-1137 (1972).