Effect of Cytochrome P450 Inducers on Liver Microsomal Metabolism of Tetrachlorobiphenyls in Rats, Guinea Pigs and Hamsters

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Effect of cytochrome P450 (P450) inducers on liver microsomal metabolism of 3,4,3',4'-, 3,5,3',5'- and 2,5,2',5'-tetrachlorobiphenyl (TCB) was studied using male Wistar rats, male Hartley guinea pigs and male Golden syrian hamsters. In metabolism of 3,4,3',4'- and 3,5,3',5'-TCB, liver microsomes from 3-methylcholanthrene (MC)- or 3,4,5,3',4'-pentachlorobiphenyl (PenCB)-treated hamsters showed hydroxylase activities for both TCB isomers, although the activities were much less than those of rats. In contrast, liver microsomes from untreated and phenobarbital (PB)-, MC- or PenCB-treated guinea pigs showed no hydroxylase activity. In 2,5,2',5'-TCB metabolism, 3-hydroxylase activity was observed in untreated guinea pigs and hamsters, but not in untreated rats. The activity was induced by PB treatment in all three species, at rates of 324, 19 and 20 pmol/min/mg protein in rats, guinea pigs and hamsters, respectively. This activity was not enhanced by treatment with either MC or PenCB. Only in hamsters was 4-hydroxylated metabolite formed in all microsomes used in addition to the 3-hydroxylated one, and the formation was accelerated 2.0-, 2.7- and 4.8-fold by treatment with PB, MC and PenCB, respectively. These results suggest that different P450 isoforms in hamster liver microsomes are involved in 3- and 4-hydroxylation of 2,5,2',5'-TCB. Thus, there are species differences in the basal ability to hydroxylate TCB isomers, and in the extent of effect of P450 inducers on the metabolism of these isomers among the three species.

Key words tetrachlorobiphenyl; metabolism; species difference; guinea pig; hamster; rat

Polychlorinated biphenyls (PCBs) have been known as contaminants in the environment since the late 1960s¹⁾ and also as causal agents of Yusho (PCB poisoning) which occurred in Japan in 1968.²⁾ PCBs are metabolized mainly through hydroxylation by liver microsomal monooxygenase systems containing cytochrome P450 (P450).³⁻⁶⁾

Tetrachlorobiphenyl (TCB) isomers are the major components of PCB preparations such as Kanechlor 400, Aroclor 1248 and Clophen A40.¹⁾ Among TCB isomers, 3,4,3',4'-TCB is a prototype of highly toxic coplanar PCBs having the 3-methylcholanthrene (MC)-type inducing ability of liver enzymes.^{7,8)} and 2,5,2',5'-TCB shows lower toxicity and the phenobarbital (PB)-type inducibility of liver enzymes.⁸⁾ Our recent studies using male Wistar rats showed that 3,4,3',4'-TCB was metabolized to 4- and 5-hydroxylated metabolites by CYP1A1, a major form induced by MC treatment, and that 2,5,2',5'-TCB was converted to 3-hydroxy-2,5,2',5'-TCB by CYP2B1 and CYP2B2, major forms inducible by PB treatment.⁹⁾ More recently, 3,5,3',5'-TCB, which is present in low to non-detectable levels in PCB preparations and in the environment, was hydroxylated to 4-hydroxy-3,5,3',5'-TCB by liver microsomes from MC-treated rats and by CYP1A1.¹⁰⁾

In general, different species have different basal ability to metabolize PCBs. For example, the dog can metabolize 2,4,5,2',4',5'-hexachlorobiphenyl much more rapidly than the rat or the monkey.¹¹⁾ Moreover, different species show different induction profile of liver enzymes. In particular, benzo[a]pyrene 3-hydroxylase activity is induced by MC-type P450 inducers in the guinea pig much less effectively than in the rat.¹²⁾

It is known that the guinea pig is highly sensitive to PCBs and related compounds such as polychlorinated dibenzofurans (PCDFs) and dibenzo-p-dioxins (PCDDs), whereas the hamster is the most insensitive.^{12,13)} Although

a number of studies have been reported concerning the species differences in the toxicity of these compounds, little information is yet available about the metabolism of PCBs in guinea pigs and hamsters. Therefore, we chose three symmetric TCB isomers, 3,4,3',4'-, 3,5,3',5'- and 2,5,2',5'-TCB (Fig. 1) and examined their metabolism using liver microsomes of rats, guinea pigs and hamsters.

MATERIALS AND METHODS

Chemicals 3,4,3',4'-TCB was synthesized by the method of Nakatsu *et al.*¹⁴⁾ 2,5,2',5'- and 3,5,3',5'-TCB were synthesized by the Ullman reaction using 2,5-iodobenzene and 3,5-iodobenzene, respectively, as starting material.¹⁵⁾ 3,4,5,3',4'-Pentachlorobiphenyl (PenCB) was synthesized by the method of Saeki *et al.*¹⁶⁾ Two hydroxylated metabolites of 3,4,3',4'-TCB were synthesized as described previously.¹⁷⁾ 3-Hydroxy-2,5,2',5'-TCB was isolated from rat feces according to the method of Hanioka *et al.*¹⁸⁾ 4-Hydroxy-3,5,3',5'-TCB was synthesized as described elsewhere.¹⁰⁾ Benzphetamine hydro-

Fig. 1. Chemical Structures of Three Symmetric TCB Isomers Used in This Study

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chloride (BZ) was generously donated by the Upjohn Co. (Kalamazoo, U.S.A.). 7-Ethoxyresorufin (7-ER), glucose-6-phosphate (G-6-P) dehydrogenase and bovine serum albumin (BSA) were purchased from Sigma Chemical Co. (St. Louis, U.S.A.). PB (Na salt) and MC were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Nicotine adenine dinucleotide phosphate (NADP⁺) and G-6-P were purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan). All other chemicals used were of the highest purity commercially available.

Synthesis of 4-Hydroxy-2,5,2',5'-TCB 4-Hydroxy-2,5,2',5'-TCB was synthesized as described previously.¹⁹⁾ Briefly, the crude products obtained from the reaction mixture of 2,5-dichloroaniline (1.3 g) and 2,5-dichlorophenol (2.6 g) were purified by a chromatography on a silica gel column with *n*-hexane and *n*-hexane-chloroform (3:1, v/v) as mobile phase. Three possible monohydroxy-TCBs, 6-hydroxy-, 3-hydroxy- and 4-hydroxy-2,5,2',5'-TCB were eluted as a mixture with the latter solvent. Each hydroxy-TCB was then separated by high-performance liquid chromatography (HPLC) under the following conditions: column, Wakosil 5C18 (10 × 300 mm); mobile phase, methanol-water (9:1, v/v); flow rate, 2 ml/min; detector, 254 nm. The retention times of 6-hydroxy-, 3-hydroxy- and 4-hydroxy-2,5,2',5'-TCB in HPLC were 19.2, 21.8 and 25.3 min, respectively, and the ratio of formation was 1:3:10. The mass spectral data of the methylated derivative of these compounds are as follows:

4-Hydroxy-2,5,2',5'-TCB, MS *m/z*: 326 (*M*⁺ + 6, 21%), 324 (*M*⁺ + 4, 76%), 322 (*M*⁺ + 2, 132%), 320 (*M*⁺, 100%), 305 (*M*⁺ - 15, 16%), 277 (*M*⁺ - 43, 44%), 207 (41%).

6-Hydroxy-2,5,2',5'-TCB, MS *m/z*: 326 (*M*⁺ + 6, 23%), 324 (*M*⁺ + 4, 68%), 322 (*M*⁺ + 2, 120%), 320 (*M*⁺, 100%), 270 (*M*⁺ - 50, 124%), 207 (43%).

3-Hydroxy-2,5,2',5'-TCB, MS *m/z*: 326 (*M*⁺ + 6, 27%), 324 (*M*⁺ + 4, 79%), 322 (*M*⁺ + 2, 159%), 320 (*M*⁺, 100%), 277 (*M*⁺ - 43, 32%), 207 (31%).

The methylated derivative of 4-hydroxy- and 6-hydroxy-2,5,2',5'-TCB showed a typical fragment ion [*M*⁺ - 15] for 4-methoxy-PCB and [*M*⁺ - 50] for 2- or 6-methoxy-PCB in the mass spectrum, respectively, as described in previous studies.^{20,21)} The yield of 4-hydroxy-2,5,2',5'-TCB was 23 mg.

Animal Treatment and Preparation of Liver Microsomes PB and MC were dissolved in a saline and corn oil, respectively, and injected intraperitoneally at a dose of 80 and 20 mg/kg/d for 3 d, respectively, to male Wistar rats (body wt. 150 g), male Hartley guinea pigs (body wt. 250 g) and male Golden syrian hamsters (body wt. 90 g). These animals were killed 24 h after the last injection of each inducer. PenCB was injected intraperitoneally to rats and hamsters at a single dose of 5 mg/kg, and to guinea pigs at a single dose of 0.1 mg/kg. Animals were killed 5 d after the injection. Liver microsomes were prepared as described previously.²²⁾

Microsomal Metabolism of TCB The analysis of TCB metabolites was conducted as reported elsewhere.^{9,10)} The assay system used contained 40 μM TCB dissolved in 0.1 ml dimethylsulfoxide, 6 mM MgCl₂, 0.8 mg/ml BSA, 100 mM *N*-hydroxyethylpiperazine-*N'*-2-ethanesulfate

(HEPES) buffer (pH 7.4), NADPH-generating system (0.33 mM NADP, 8 mM G-6-P, 5 units of G-6-P dehydrogenase) and 5 mg protein of microsomes in a final volume of 5 ml. The incubation was initiated by adding the NADPH-generating system and was run for 20 min at 37 °C. After incubation, the metabolite(s) and unchanged substrate were extracted twice with a mixture of 7 ml of chloroform-methanol (2:1) and 14 ml of *n*-hexane and once with 20 ml of *n*-hexane. The organic phase was pooled and evaporated to dryness. The residue was dissolved in *n*-hexane and an aliquot of the solution was then trimethylsilylated with 40 μl of *N,O*-bis(trimethylsilyl)acetamide and applied to gas chromatography (GC).

Analytical Methods P450 content was measured by the method of Omura and Sato.²³⁾ Activities of BZ *N*-demethylase and 7-ER *O*-deethylase were determined according to methods of Yoshimura *et al.*⁸⁾ and Prough *et al.*,²⁴⁾ respectively. Protein measurement was made by the method of Lowry *et al.*²⁵⁾ using BSA as a standard.

GC was done using a gas chromatograph HP 5890 Series II equipped with an electron capture detector under the following conditions: column, a DB-1 capillary column (15 m × 0.25 mm i.d. × 0.33 mm thickness); carrier gas, N₂ (1 ml/min); column temp., 210 °C (for 3,4,3',4'- and 3,5,3',5'-TCB) or 200 °C (for 2,5,2',5'-TCB); injection port temp., 250 °C; detector temp., 250 °C. The retention times of trimethylsilylated derivatives of authentic compounds are: 4-hydroxy-3,5,3',4'-TCB, 12.26 min; 5-hydroxy-3,4,3',4'-TCB, 11.37 min; 4-hydroxy-3,5,3',5'-TCB, 10.29 min; 3-hydroxy-2,5,2',5'-TCB, 8.27 min; 4-hydroxy-2,5,2',5'-TCB, 8.67 min.

RESULTS

Microsomal Monooxygenase Activity The P450 contents and the activities of BZ *N*-demethylase and 7-ER *O*-deethylase in liver microsomes of untreated and P450 inducer-treated rats, guinea pigs and hamsters are presented in Table 1. On the basis of the results in rats, activities of BZ *N*-demethylase and 7-ER *O*-deethylase were selected as typical activities markedly inducible by PB-type and MC-type inducers, respectively. PenCB

Table 1. Drug Metabolizing Activities of Liver Microsomes from Untreated and Inducer-Treated Rats, Guinea Pigs and Hamsters

Animal	Treatment	P-450 content ^{a)}	Benzphetamine ^{b)}	
			<i>N</i> -demethylase	7-Ethoxyresorufin ^{b)} <i>O</i> -deethylase
Rat	None	0.80 (1.0)	8.54 (1.0)	0.012 (1.0)
	PB	1.68 (2.1)	15.63 (1.8)	0.013 (1.1)
	MC	1.54 (1.9)	6.00 (0.7)	6.03 (507.0)
	PenCB	1.74 (2.2)	2.34 (0.3)	5.52 (464.0)
Guinea pig	None	1.08 (1.0)	5.13 (1.0)	0.427 (1.0)
	PB	1.62 (1.5)	17.12 (3.3)	0.415 (1.0)
	MC	1.61 (1.5)	4.71 (0.9)	0.453 (1.1)
	PenCB	1.87 (1.7)	6.02 (1.2)	0.492 (1.2)
Hamster	None	1.03 (1.0)	5.54 (1.0)	0.179 (1.0)
	PB	1.50 (1.5)	12.02 (2.2)	0.114 (0.6)
	MC	1.92 (1.9)	5.03 (0.9)	2.87 (16.0)
	PenCB	2.85 (2.8)	5.71 (1.0)	1.72 (9.6)

a) nmol/mg protein, b) nmol/min/mg protein. Each value represents the mean of duplicate determinations using pooled microsomes from 4 rats, 3 guinea pigs and 10 hamsters. Figure in parenthesis is the ratio relative to the result of untreated microsomes.

Table 2. Metabolism of 3,4,3',4'-TCB by Liver Microsomes from Untreated and Inducer-Treated Rats, Guinea Pigs and Hamsters

Animal	Treatment	Metabolite formed (pmol/min/mg protein)	
		5-OH	4-OH
Rat	None	N.D.	N.D.
	PB	N.D.	N.D.
	MC	7.0 ± 1.3	34.5 ± 1.7
	PenCB	5.8 ± 0.7	32.2 ± 1.3
Guinea pig	None	N.D.	N.D.
	PB	N.D.	N.D.
	MC	N.D.	N.D.
	PenCB	N.D.	N.D.
Hamster	None	N.D.	N.D.
	PB	0.9 ± 0.7	N.D.
	MC	0.4 ± 0.1	3.6 ± 0.7
	PenCB	2.6 ± 1.1	2.9 ± 0.5

N.D., not detected. Each value represents the mean ± S.D. of three determinations.

Table 3. Metabolism of 3,5,3',5'-TCB by Liver Microsomes from Untreated and Inducer-Treated Rats, Guinea Pigs and Hamsters

Animal	Treatment	4-Hydroxy-3,5,3',5'-TCB formed (pmol/min/mg protein)
Rat ^{a)}	None	N.D.
	PB	N.D.
	MC	45.1 ± 1.6
	PenCB	27.6 ± 0.9
Guinea pig	None	N.D.
	PB	N.D.
	MC	N.D.
	PenCB	N.D.
Hamster	None	N.D.
	PB	N.D.
	MC	9.1 ± 0.8
	PenCB	10.1 ± 1.4

a) Data were taken from reference 10. Each value represents the mean ± S.D. of three determinations. N.D., not detected.

was used as another MC-type inducer, because it induces CYP1A2 much more than CYP1A1, in contrast to MC.^{26,27)} In all three species, P450 content was increased by treatment of PB, MC or PenCB. Among them, PenCB treatment on hamster resulted in the highest induction (2.8-fold) of total P450 content. BZ *N*-demethylase activity was about 2 or 3 times that of the corresponding controls after PB treatment in the three species. In rats, this activity was decreased to 70% and 30% of the corresponding controls by treatments of MC and PenCB, respectively, in agreement with our earlier report,⁸⁾ but was little affected in guinea pigs and hamsters. 7-ER *O*-deethylase activity was markedly induced by treatment of MC or PenCB in rats and hamsters, whereas the activity was only slightly increased in guinea pigs.

3,4,3',4'-TCB Metabolism Liver microsomes from MC- and PenCB-treated rats metabolized 3,4,3',4'-TCB effectively to 4-hydroxy-3,5,3',4'-TCB and 5-hydroxy-3,4,3',4'-TCB at a ratio of 5:1 (Table 2). In hamsters, both hydroxylation activities were also observed in liver microsomes by MC- or PenCB-treatment, but were much

lower than those of rats. In contrast, none of the microsomes from guinea pigs showed hydroxylase activity for 3,4,3',4'-TCB.

3,5,3',5'-TCB Metabolism 3,5,3',5'-TCB is metabolized to 4-hydroxy-3,5,3',5'-TCB in liver microsomes only from MC- and PenCB-treated rats.¹⁰⁾ The 4-hydroxylation activity of 3,5,3',5'-TCB in liver microsomes from the three species is shown in Table 3. Similarly to rats, the activity was observed in MC- and PenCB-treated hamsters but was much less than that in rats. In untreated and PB-treated microsomes in rats and hamsters, this TCB isomer was not metabolized. On the other hand, no guinea pig microsomes had hydroxylating ability for 3,5,3',5'-TCB. These results indicate that the effect of P450 inducers on 3,5,3',5'-TCB metabolism is similar to that on 3,4,3',4'-TCB metabolism among the three species.

2,5,2',5'-TCB Metabolism The 3-hydroxylation is the main pathway of 2,5,2',5'-TCB metabolism in rats.^{9,28)} Figure 2 shows gas chromatograms of the trimethylsilylated metabolites of 2,5,2',5'-TCB formed by incubation with liver microsomes of untreated or inducer-treated hamsters. In contrast to rats and guinea pigs, incubation with hamster liver microsomes resulted in the formation of 3-hydroxy-2,5,2',5'-TCB and also a metabolite having a retention time at 8.67 min in GC. The latter metabolite was identified as a trimethylsilylated derivative of 4-hydroxy-2,5,2',5'-TCB, in agreement with the mass spectrum and the retention time of the synthetic sample. Moreover, two unidentified peaks, a and b, following the peak of 4-hydroxy-2,5,2',5'-TCB were also observed at retention times of 9.05 min and 9.46 min in all hamster liver microsomes. The area of peak a in GC was increased 2.4-, 3.4- and 3.7-fold that of the corresponding controls by treatment of PB, MC and PenCB, respectively. These results indicate that peak a should be a metabolite depending upon the induction of a certain P450 isoform(s). Peak b was not affected by treatment with any of the inducers. The precise chemical structures of these metabolites remain to be determined, although a large-scale study using PenCB-treated hamster microsomes showed that another monohydroxy-TCB other than 3- and 4-hydroxylated metabolites was formed (data not shown).

The formation rates of 3- and 4-hydroxylated metabolites of 2,5,2',5'-TCB with liver microsomes from untreated, PB-treated, MC-treated and PenCB-treated animals are shown in Table 4. The 3-hydroxylation of 2,5,2',5'-TCB was enhanced by PB treatment in all the three species and the rate in PB-microsomes was 324, 19 and 20 pmol/min/mg protein in rats, guinea pigs and hamsters, respectively. Treatment of hamster with MC or PenCB decreased the 3-hydroxylation activity to 57% and 66% that of the controls, respectively. It is of interest to note that the observed activities in liver microsomes from untreated guinea pigs and hamsters were at quite a high level.

In untreated hamster, on the other hand, 4-hydroxy-2,5,2',5'-TCB was formed at a similar rate to that of 3-hydroxy-2,5,2',5'-TCB and the formation was accelerated 2.0-, 2.7- and 4.8-fold by treatment of PB, MC or PenCB, respectively. These results suggest that different

Fig. 2. Gas Chromatograms of the Trimethylsilylated Metabolites of 2,5,2',5'-TCB Formed by Liver Microsomes from Untreated(A), PB-Treated(B), MC-Treated(C) and PenCB-Treated(D) Hamsters

Table 4. Metabolism of 2,5,2',5'-TCB by Liver Microsomes from Untreated and Inducer-Treated Rats, Guinea Pigs and Hamsters

Animal	Treatment	Metabolite formed (pmol/min/mg protein)	
		3-OH	4-OH
Rat	None	N.D.	N.D.
	PB	324.0 ± 15.4	N.D.
	MC	N.D.	N.D.
	PenCB	N.D.	N.D.
Guinea pig	None	8.4 ± 0.9 (1.0)	N.D.
	PB	19.3 ± 2.2 (2.3)	1.1 ± 0.1
	MC	8.4 ± 0.0 (1.0)	N.D.
	PenCB	7.7 ± 0.3 (0.9)	N.D.
Hamster	None	6.3 ± 0.5 (1.0)	5.6 ± 0.3 (1.0)
	PB	20.1 ± 0.2 (3.2)	11.1 ± 0.3 (2.0)
	MC	3.6 ± 0.4 (0.6)	15.2 ± 0.6 (2.7)
	PenCB	4.2 ± 0.4 (0.7)	26.9 ± 0.4 (4.8)

N.D., not detected. Each value represents the mean ± S.D. of three determinations and those in parentheses are the ratio relative of untreated animals.

forms of P450 are involved in the 3- and 4-hydroxylations of 2,5,2',5'-TCB in hamster liver. The 4-hydroxylation activity was also detected in PB-treated guinea pigs but the activity was very low.

DISCUSSION

Here, we demonstrated species differences in the microsomal metabolism of three symmetrical TCB isomers among rats, guinea pigs and hamsters. In rats, our recent studies showed that both 3,4,3',4'- and 3,5,3',5'-TCB which have no *ortho*-chlorine substituent are hydroxylated by rat CYP1A1.^{9,10} In guinea pigs, 3,4,3',4'-TCB and 3,5,3',5'-TCB were not metabolized by untreated or P450 inducer-treated microsomes. The 7-ER *O*-deethylase activ-

ity was not also affected by treatment with MC or PenCB, although untreated microsomes had a much higher level of the activity than that in rats. Cheng *et al.* purified CYP1A1 from liver microsomes of MC-treated guinea pigs using monoclonal antibody prepared to rat CYP1A1,²⁹ which is a typical catalyst for 7-ER *O*-deethylation.³⁰ Thus, guinea pig CYP1A1 appears distinct from rat CYP1A1 with regard to the substrate specificity and the induction profile by MC-type inducers.

It is known that hamster CYP1A1 is induced by MC-type inducers in the lung but not in the liver.³¹ In this study, we observed a marked increase of 7-ER *O*-deethylase activity and hydroxylating activities for 3,4,3',4'- and 3,5,3',5'-TCB in MC- and PenCB-treated hamster microsomes. These results indicate the possibility that a MC- or PenCB-inducible form of P450 other than CYP1A1 is involved in the 7-ER *O*-deethylation and in the hydroxylations of 3,4,3',4'- and 3,5,3',5'-TCB in hamster liver. Moreover, a great difference was found in the ratio of 4- and 5-hydroxylation of 3,4,3',4'-TCB between MC- and PenCB-treated microsomes, which probably means the involvement of at least two different P450 isoforms in the hydroxylations of 3,4,3',4'-TCB in hamsters. Several investigators have reported a few forms of P450 (CYP1A2, CYP2A8 or another form) from liver microsomes of MC-type inducer-treated hamsters.³²⁻³⁵ Which isoform of P450 is responsible for the metabolism of 7-ER and coplanar TCB isomers is unknown at present.

The 3-hydroxylation of 2,5,2',5'-TCB was enhanced by PB treatment in the three animal species and this induction profile was correlated with that of BZ *N*-demethylation. In rats, CYP2B1 and CYP2B2 are active in the 3-hydroxylation of 2,5,2',5'-TCB and BZ *N*-demethylation.^{9,30} The results obtained in this study suggest that an isoform of P450 belonging to the 2B subfamily

may participate in this hydroxylation. In guinea pigs, P450GP-1, which is constitutive and PB-inducible,^{36,37)} is considered the most important P450 isoform for the 3-hydroxylation of 2,5,2',5'-TCB. In contrast, a P450 isoform classified in the 2B subfamily in hamster liver remains to be purified and characterized.³⁸⁾

It should be noted that, unlike rats, untreated and induced hamster microsomes exhibited the 4-hydroxylation activity of 2,5,2',5'-TCB at a relatively high level, and this activity was accelerated by treatment with MC or PenCB. These results suggest that different P450 isoforms are involved in 3- and 4-hydroxylation of 2,5,2',5'-TCB in hamsters. Considering the induction profile of the 4-hydroxylase activity, it appears that CYP1A2, CYP2A8 or another form, which is induced by MC, plays an important role in 4-hydroxylation of 2,5,2',5'-TCB as well as hydroxylations of 3,4,3',4'- and 3,5,3',5'-TCB. Recently, Borlakoglu *et al.* reported on 2,5,2',5'-TCB metabolism that a much greater amount of 4-hydroxylated than 3-hydroxylated metabolite was formed by liver microsomes of both untreated and Aroclor 1254-treated rats.³⁹⁾ Our data in this study did not agree with their data.

Although PenCB treatment resulted in the most potent inductive effect of P450 content in the three species, the 7-ER *O*-deethylase activity in rats and hamsters was induced by PenCB less effectively than by MC. Similar results were observed in 4-hydroxylation of 3,4,3',4'-TCB in rats and hamsters and also in 4-hydroxylation of 3,5,3',5'-TCB in rats. Yoshihara *et al.* reported that addition of PenCB to the incubation mixture containing liver microsomes from MC-treated rats partially inhibited the P450-dependent hydroxylations of testosterone and progesterone.²²⁾ Our previous data⁴⁰⁾ and data in this study showed that a high concentration of PenCB was detected in liver microsomes 5 d after treatment of rats and hamsters with this isomer. These findings suggest that the residual PenCB in microsomes may also affect the metabolism of 7-ER and TCB isomers to some degree.

The toxic effects of coplanar PCBs, PCDFs and PCDDs are mediated through the initial binding to the cytosolic aryl hydrocarbon (Ah) receptor⁴¹⁾ and the degree varied with the congener involved.⁴²⁾ Our previous studies^{17,43)} using 3,4,3',4'-TCB and PenCB suggested that their hydroxylated metabolites possibly have much less affinity to Ah-receptor than the parent PCBs because they showed no toxic or biological effects such as body weight loss, thymus atrophy or induction of liver enzymes. The order of sensitivity for the toxicity of coplanar PCBs, PCDFs and PCDDs is guinea pig > rat >> hamster.¹³⁾ From these facts, if TCB is converted to the hydroxylated metabolite more rapidly, its sensitivity to the toxicity would be lower. However, the ability to metabolize TCB isomers apparently varied from species to species and was not always parallel to the sensitivity. For example, the fact that guinea pigs hardly metabolize the toxic coplanar TCB, 3,4,3',4'-TCB, may explain its highest sensitivity among all species reported so far. In contrast, hamsters which resist most strongly the toxicity of coplanar PCBs and related compounds, showed lower hydroxylating activity of 3,4,3',4'-TCB than rats. These results indicate that species difference in the sensitivity to toxicity of the coplanar

PCBs cannot be explained only by the animal's ability to metabolize PCB. More recently, it has been reported that the 4-hydroxylated metabolites of PCBs including 3,4,3',4'-TCB are distributed in the blood at high concentration and affect the metabolism of vitamin A and thyroid hormone in mammals.⁴⁴⁾ This finding is not in accord with the fact that the guinea pig, the species most sensitive to PCB toxicity, does not have the ability to form this toxic 4-hydroxylated metabolite.

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