Metabolism *in Vivo* of 3, 4, 5, 3',4'-Pentachlorobiphenyl and Toxicological Assessment of the Metabolite in Rats

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Metabolism *in vivo* of 3,4,5,3',4'-pentachlorobiphenyl (PenCB) and toxicological assessment of the metabolite were investigated using male Wistar rats. Only one metabolite was isolated from the feces of rats administered 3,4,5,3',4'-PenCB. By gas chromatography-mass spectrometry, the methylated metabolite was identified with the synthesized authentic sample, 4'-methoxy-3,4,5,3',5'-PenCB. This indicated that the metabolite was 4'-hydroxy-3,4,5,3',5'-PenCB, which was produced *via* a 4',5'-epoxide formation and subsequent NIH-shift of the 4'-chlorine to the 5'-position. Administration of the metabolite at either single i.p. dose of 3 or 10 mg/kg to rats did not cause any toxic and biological effects such as body weight loss, atrophy of thymus and spleen, liver hypertrophy, increase of liver lipids, or 3-methylcholanthrene-type induction of liver enzymes. These changes were observed in rats administered with 3,4,5,3',4'-PenCB at a single dose of 3 mg/kg. In addition, a trace amount of 4'-hydroxy-3,4,5,3',5'-PenCB could be detected in rat liver 5 d after treatment with 3,4,5,3',4'-PenCB or 4'-hydroxy-3,4,5,3',5'-PenCB. The amount of this metabolite excreted in feces during 5 d after treatment with 3,4,5,3',4'-PenCB accounted for only 1.3% of dose. In 4'-hydroxy-3,4,5,3',5'-PenCB-treated rats, about 60% of dose was excreted as unchanged in feces for 5 d. These results suggest that this metabolite is a detoxified product and has no longer the high affinity for the liver, being excreted rapidly into the feces.

Keywords — 3,4,5,3',4'-pentachlorobiphenyl; enzyme induction; polychlorinated biphenyl; 4'-hydroxy-3,4,5,3',5'-pentachlorobiphenyl; acute toxicity; fecal excretion; distribution

Introduction

Polychlorinated biphenyls (PCB) are widespread environmental contaminants which elicit numerous toxic effects including immunosuppression with thymic atrophy, hyperkeratosis, hepatotoxicity, edema and lethality. Previously, we demonstrated that 3,4,5,3',4'-pentachlorobiphenyl (PenCB), one of the coplanar PCB, showed the most potent toxicity and 3-methylcholanthrene (MC)-type inducing ability of drug-metabolizing enzymes among all the PCB congeners in rats.¹⁾ Moreover, Bradlaw and Casterline screened several PCB congeners for their ability to induce benzo[*a*]pyrene (BP) 3-hydroxylase activity in the liver cells in culture, and found also that 3,4,5,3',4'-PenCB was most potent.²⁾

Recently, Kannan *et al.*³⁾ detected small amounts of 3,4,5,3',4'-PenCB in industrial PCB preparations such as Aroclor and Kanechlor. They reported that Kanechlor 400, the causal agent of Yusho which occurred in the southwestern part of Japan in 1968, contained 68 ppm

of this congener. Kashimoto *et al.* also found this congener in the liver and small intestine of Yusho patients at much higher level than in those of normal persons.⁴⁾ Because of its persistence in the liver and the potent toxicity, 3,4,5,3',4'-PenCB continues to attract much attention. Although we have studied the toxicity and biological effects of 3,4,5,3',4'-PenCB in rats,^{1,5,6)} guinea pigs,⁷⁾ mice⁸⁾ and chicken,⁹⁾ the question whether 3,4,5,3',4'-PenCB is metabolized in these animals in connection with its mechanism for toxicity has not yet been clarified. In this paper, we describe the *in vivo* metabolism of 3,4,5,3',4'-PenCB in rats and the toxicological assessment of its metabolite isolated from the rat feces.

Materials and Methods

Chemicals — 3,4,5,3',4'-PenCB was synthesized by the method of Saeki *et al.*¹⁰⁾ 3,4,5-Trichloroaniline was purchased from Aldrich Co. (St. Louis, U.S.A.). 1,2,3-Trichlorobenzene, 2,6-dichloro-4-aminoanisole and

2,6-dichlorophenol were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Kieselgel 60 and Wakogel S-I for silica gel column chromatography were obtained from Merck AG (Darmstadt, FR Germany) and Wako Pure Chemical Industries, Ltd. (Osaka, Japan), respectively. All other chemicals used were of highest quality commercially available and obtained from the sources described previously.¹¹⁾

Thin-Layer Chromatography (TLC) — Thin layer plates (0.25 mm thick) of silica gel were prepared by coating glass plates with a mixture of Kieselgel 60G (Merck AG) and Wakogel B5-FM (Wako Pure Chemical Co.), 1:1 (w/w). For identification of M-1, *n*-hexane-CHCl₃ (3:1, v/v) was used as a developing solvent (*R_f* value of methylated M-1, 0.51). Spots were visualized under ultraviolet (UV) light (254 nm).

Syntheses of Monomethoxy-PenCBs — 4'-Hydroxy-3,4,5,3',5'-PenCB was synthesized as reported elsewhere,¹¹⁾ except that 3,4,5-trichloroaniline and 2,6-dichlorophenol were used as starting materials. In brief, 3,4,5-trichloroaniline (1.4 g) dissolved in 5.2 ml of conc. HCl was diazotized by addition of 0.5 g of saturated aq. NaNO₂ on an ice bath. The solution was allowed to react with 2,6-dichlorophenol (3 g) dissolved in 10 ml of acetonitrile under reflux at 110 °C for 2 h. The reaction mixture was added to 30 ml of water and extracted three times with 50 ml of CHCl₃. The organic phase was pooled, washed once with an equal volume of water and back-extracted three times with 50 ml of 2N NaOH. The alkaline solution was neutralized with 2.7N HCl and again extracted with 70 ml of CHCl₃. The brownish residue obtained after evaporation of the solvent was dissolved in a small volume of *n*-hexane and placed on a silica gel column (Kieselgel 60, 100 g) equilibrated with *n*-hexane. After washing the column with *n*-hexane, 4'-hydroxy-3,4,5,3',5'-PenCB was eluted with 1500 ml of *n*-hexane-CHCl₃ (1:1, v/v). The fractions were pooled and applied onto a second silica gel column (20 g), which was washed with 200 ml of *n*-hexane-CHCl₃ (3:1, v/v). 4'-Hydroxy-3,4,5,3',5'-PenCB was then eluted with 300 ml of *n*-hexane-CHCl₃ (3:2, v/v). It was recrystallized from water-acetic acid (1:1, v/v) to yield 9 mg of colorless needles, mp

221—222 °C. MS *m/z*: 340 (M⁺, 100%), 342 (155%), 344 (102%), 346 (34%). The methylated derivative (S-1) of this compound was obtained by refluxing with 920 mg of dimethyl sulfate at 80 °C in the presence of 1.0 g of K₂CO₃ as described previously,¹²⁾ mp 159—160 °C. MS *m/z*: 354 (M⁺, 100%), 356 (154%), 358 (100%), 360 (34%). ¹H-NMR (in CDCl₃) δ: 3.95 (3H, s, -OCH₃), 7.46 (2H, s, H-2' and H-6'), 7.53 (2H, s, H-2 and H-6). *t_R* in GC-MS, 9.23 min.

3'-Hydroxy-3,4,5,2',4'-PenCB was obtained as a by-product in the coupling reaction as described above and its methylated derivative was designated as S-3 showing the *t_R* at 6.30 min in gaschromatography-mass spectrometry (GC-MS). 4'-Methoxy-2,3,4,3',5'-PenCB (S-2) was obtained by the condensation of 1,2,3-trichlorobenzene with 4-amino-2,6-dichloroanisole and showed the *t_R* at 7.83 min in GC-MS. Although S-1 was also produced in this reaction, S-2 was major rather than S-1. The structures of S-2 and S-3 were assumed from their synthetic routes and mass spectra (Fig. 2), because the amounts of S-2 and S-3 were insufficient to be analyzed by nuclear magnetic resonance (NMR).

Isolation of the Metabolite — 3,4,5,3',4'-PenCB dissolved in corn oil was administered orally to ten male Wistar rats (body wt. 160—200 g) at a single dose of 3 mg/kg. The feces were collected for 5 d following injection, dried at 80 °C overnight and then extracted continuously with CHCl₃ for 10 h. The CHCl₃ phase was evaporated to dryness and the residue was dissolved in 10 ml of acetone. It was methylated by heating with 920 mg of dimethyl sulfate at 80 °C for 2 h in the presence of K₂CO₃ (1.0 g). The methylated extract was applied to a silica gel column (Kieselgel 60, 200 g) equilibrated with *n*-hexane. Following elution of unchanged 3,4,5,3',4'-PenCB with 900 ml of *n*-hexane, the methylated M-1 was eluted with 500 ml of *n*-hexane-CHCl₃ (3:1, v/v). After examination of each fraction for the metabolite by GC, fractions containing the metabolite were combined, concentrated to a smaller volume and applied to a second silica gel column (20 g) for further purification.

Quantification of 3,4,5,3',4'-PenCB and M-1 in Feces and Liver — 3,4,5,3',4'-PenCB and 4'-hydroxy-3,4,5,3',5'-PenCB in the feces and the liver of young male Wistar rats (90–100 g) were quantified after administration of 3,4,5,3',4'-PenCB at a single i.p. dose of 3 mg/kg. Feces were collected everyday for 5 d after dosing. A portion (1 g) of powdered dry feces was extracted continuously with CHCl_3 for 10 h and the extract was adjusted to 40 ml with CHCl_3 . An aliquot (1–4 ml) was evaporated to dryness, dissolved in 2–3 ml of *n*-hexane and applied to a silica gel column (Wakogel S-I, 0.5 g) on which anhydrous Na_2SO_4 was placed. 3,4,5,3',4'-PenCB and 4'-hydroxy-3,4,5,3',5'-PenCB were eluted with 40 ml of *n*-hexane and with subsequent 40 ml of *n*-hexane– CHCl_3 (7:3, v/v), respectively. On the other hand, liver homogenate (2 ml) was extracted once with 30 ml of CHCl_3 –methanol (2:1, v/v) according to the method of Folch *et al.*¹³⁾ The organic phase was evaporated to dryness and the residue was suspended in 2 ml of *n*-hexane, and applied to a silica gel column (Wakogel S-I, 0.5 g) in a similar manner as given above. The fraction containing 4'-hydroxy-3,4,5,3',5'-PenCB was evaporated *in vacuo*. The residue was then dissolved in 0.2 ml of *n*-hexane and trimethylsilylated as reported previously.¹¹⁾ The reaction mixture diluted with *n*-hexane was injected into a gas chromatograph. Calibration curves showed good linearity ranging from 40 to 160 pg/injection for 3,4,5,3',4'-PenCB and from 80 to 320 pg/injection for 4'-hydroxy-3,4,5,3',5'-PenCB. The recovery of 3,4,5,3',4'-PenCB from liver homogenate was 77.3% (mean of three determinations), by which the values obtained from the livers were corrected.

Analytical Methods — GC was carried out on a Hitachi 263-70 gas chromatograph equipped with a ^{63}Ni electron-capture detector under the conditions as follows: column, DB-1301 wide-bore capillary column (30 m $\times$ 0.45 mm); carrier gas, N_2 (10 ml/min); injection port temp., 270 °C; detector temp., 270 °C; column temp., 235 °C. GC-MS was performed with a JEOL JMS-DX-300 mass spectrometer equipped with a JEOL JMA-3500 data system in electron-impact mode. The gas chromatograph fitted with

an Ultra #1 fused-silica capillary column (25 m $\times$ 0.2 mm, Hewlett Packard) was coupled directly to the MS source. The carrier gas used was helium at a flow rate of 1 ml/min. The other conditions were: column temp., 200 °C; injection port temp., 290 °C. 270 MHz ^1H -NMR spectrum was obtained with a JEOL JNM FX-270 spectrometer in CDCl_3 with tetramethylsilane as an internal standard.

Toxicological Assessment of the Metabolite — In this experiment, male Wistar rats (body wt. 90–100 g) were injected i.p. with 3,4,5,3',4'-PenCB (3 mg/kg) or 4'-hydroxy-3,4,5,3',5'-PenCB (3 and 10 mg/kg) and their body weights were measured daily. On day 5 after the injection, rats were killed following starvation for 12 h and the tissues including liver, thymus and spleen were immediately removed and weighed. Livers were further perfused with ice-cold saline and homogenized with a Teflon-homogenizer in 0.25M sucrose–0.1 mM EDTA–10 mM Tris-HCl buffer (pH 7.4). For measurement of liver enzyme activities, the 9000 $\times$ g supernatant was prepared as described elsewhere.¹⁾ Cytochrome P-450 (P-450) contents were determined by the method of Matsubara *et al.*¹⁴⁾ The activities of BP 3-hydroxylase, benzphetamine (BZ) *N*-demethylase and DT-diaphorase were measured by the methods of Nebert and Gelboin,¹⁵⁾ Yoshimura *et al.*¹⁾ and Ernster *et al.*,¹⁶⁾ respectively. Liver total lipids were assayed according to the method of Folch *et al.*¹³⁾ Protein was determined by the method of Lowry *et al.*¹⁷⁾ using bovine serum albumin as a standard.

Results

Detection of the Metabolite from Feces

To isolate the metabolite of 3,4,5,3',4'-PenCB, feces for 5 d from ten rats treated with 3,4,5,3',4'-PenCB at a single *p.o.* dose of 3 mg/kg were collected and extracted with CHCl_3 for 10 h. The CHCl_3 extract was methylated by treatment with dimethyl sulfate and applied to a silica gel column. The gas chromatograms of the fractions eluted successively with *n*-hexane (A) and *n*-hexane– CHCl_3 (3:1, v/v)(B) are shown in Fig. 1. After the elution of unchanged

Fig. 1. GC of Methylated Extracts of Feces from 3,4,5,3',4'-PenCB-Treated Rats

The extracts of feces with CHCl_3 were methylated and chromatographed on a silica gel column as described in Materials and Methods. Each fraction was examined for the metabolite by GC.

(A) fraction of *n*-hexane, (B) fraction of *n*-hexane- CHCl_3 (3:1, v/v).

compound in the *n*-hexane fraction, a peak at t_R 5.6 min, attributed to the metabolite, was detected in the fraction of *n*-hexane- CHCl_3 (3:1, v/v). The fraction containing the metabolite was further purified by a second chromatography on a silica gel column for identification by GC-MS.

Identification of M-1

The structure of the metabolite was analyzed by GC-MS. As shown in Fig. 2, the mass spectrum of the metabolite gave a molecular ion peak at m/z 354 and diagnostic isotope peaks at m/z 356, 358, 360 due to five chlorine substituents. Since the major PCB metabolites are monophenols,^{11,18-22)} this spectrum suggests that the metabolite should be the methylated derivative of monohydroxy-PenCB (designated as M-1). Furthermore, the above spectrum of the methylated M-1 showed high intensity of fragment ion peak at m/z 339 corresponding to the fragment ion $[\text{M}^+ - \text{CH}_3]$. Such a fragmentation pattern in mass spectrum has been known to be characteristic for the PCB congeners possessing a methoxy group at 4- or 4'-position.^{12,23-25)} Thus, M-1 appears to have a hydroxy group at 4 (4')-position. We then synthesized this expect-

ed compound to compare with M-1. The synthetic 4'-methoxy-3,4,5,3',5'-PenCB was identical to the methylated derivative of M-1 with respect to t_R (9.23 min) and the pattern of mass fragmentation in GC-MS. The R_f value (0.51) of the methylated derivative of M-1 in TLC using *n*-hexane- CHCl_3 (3:1, v/v) as a solvent, also agreed with that of the synthetic sample (data not shown).

Toxicological Assessment of M-1

To clarify whether the metabolite M-1 shows any toxic and biological effects, two doses (3 and 10 mg/kg) of 4'-hydroxy-3,4,5,3',5'-PenCB were administered i.p. to rats and the effects were compared with those in rats treated with 3,4,5,3',4'-PenCB at an i.p. dose of 3 mg/kg. Growth curves of rats for 4 d following injection were shown in Fig. 3. As reported previously,¹⁾ administration of 3,4,5,3',4'-PenCB to rats resulted in significant suppression of growth which began 2 d after injection, whereas in 4'-hydroxy-3,4,5,3',5'-PenCB-treated rats no effect on body weight gain was observed at both doses.

Table I shows the effects of 4'-hydroxy-

Fig. 2. GC-MS of the Methylated M-1 and Three Synthetic Monomethoxy-PenCB

The metabolite and the synthetic samples were analyzed by GC-MS as described in Materials and Methods. The t_R of each compound was as follows; M-1 (as methylated form), 9.23 min; S-1, 9.23 min; S-2, 7.83 min; S-3, 6.30 min.

3,4,5,3',5'-PenCB and 3,4,5,3',4'-PenCB on the tissue weights and liver lipid contents. 3,4,5,3',4'-PenCB caused significant atrophies of thymus and spleen, and also hypertrophy of liver accompanied by the marked increase of liver lipids, similarly to our previous study.¹⁾ In contrast, 4'-hydroxy-3,4,5,3',5'-PenCB showed no such toxicological changes even at a dose of 10 mg/kg.

Next, the inductive effects of 4'-hydroxy-3,4,5,3',5'-PenCB on liver enzymes were examined using liver 9000 $\times$ g supernatant. As shown in Table II, 4'-hydroxy-3,4,5,3',5'-PenCB did

not significantly induce P-450, BP 3-hydroxylase and DT-diaphorase, all of which was markedly elevated by 3,4,5,3',4'-PenCB treatment.¹⁾ In addition, 4'-hydroxy-3,4,5,3',5'-PenCB did not suppress the activity of BZ *N*-demethylase, different from 3,4,5,3',4'-PenCB. These results indicate that M-1 is devoid of the potent toxicity and MC-type inducing ability of liver enzymes which the parent compound possesses.

Distribution of M-1 to the Liver

The distribution of 4'-hydroxy-3,4,5,3',5'-PenCB (M-1) to the liver 5 d after injection was examined in both 3,4,5,3',4'-PenCB-treated and

Fig. 3. Growth Curves of Rats Administered with 4'-Hydroxy-3,4,5,3',5'-PenCB and 3,4,5,3',4'-PenCB. Male Wistar rats (body wt. 90–100 g) were administered i.p. with 3,4,5,3',4'-PenCB (●) at a single dose of 3 mg/kg and with 4'-hydroxy-3,4,5,3',5'-PenCB at a single dose of 3 mg/kg (△) or 10 mg/kg (□). Control rats (○) were injected corn oil (1 ml/kg). Each values represents the means $\pm$ S.D. of four rats.

a) Significantly different from the control, $p < 0.05$.

4'-hydroxy-3,4,5,3',5'-PenCB-treated rats. The amount of M-1 in the livers of 3,4,5,3',4'-PenCB-treated rats was below the determination limit ($< 0.03\%$ of dose). On the contrary, about 59% of the dose was accumulated in the liver as an unchanged compound. In the livers of rats injected with 4'-hydroxy-3,4,5,3',5'-PenCB, the unchanged compound was detected but the

amount was below the determination limit ($< 0.03\%$ of dose). Thus, it is evident that M-1 has no binding affinity to the liver components.

Fecal Excretion of M-1

Table III shows the fecal excretion of unchanged 3,4,5,3',4'-PenCB and M-1 in rats. When 3,4,5,3',4'-PenCB (3 mg/kg) was injected i.p. to rats, fecal excretion of M-1 was maximum (0.84% of dose) during the first 24 h after the injection and the total amount of M-1 excreted in the feces for 5 d accounted for only 1.33% of dose. In addition, unchanged 3,4,5,3',4'-PenCB found in the feces accounted for about 2.8% of dose during 5 d. Neither the unchanged compound nor the metabolite was excreted in the urine. Unchanged 4'-hydroxy-3,4,5,3',5'-PenCB was quickly excreted in the feces and the amount excreted during the first 24 h was about 50% of dose (Table IV). Cumulative excretion in the feces for 5 d accounted for about 60% of dose. These facts indicated that 3,4,5,3',4'-PenCB is slowly metabolized to M-1 in rats, but once formed, M-1 was readily eliminated from the body to the feces.

Discussion

In the present study, we were able to isolate one metabolite from the feces of rats treated with 3,4,5,3',4'-PenCB and to identify the metabolite M-1 as 4'-hydroxy-3,4,5,3',5'-PenCB. The

TABLE I. Effect of 3,4,5,3',4'-PenCB and 4'-Hydroxy-3,4,5,3',5'-PenCB on the Tissue Weights and Liver Lipid Contents of Rats

Treatment	Tissue weight (g/100 g body weight)			Total lipid (mg/g liver)
	Liver	Thymus	Spleen	
Control	3.49 ± 0.16 (100)	0.277 ± 0.032 (100)	0.491 ± 0.096 (100)	43.63 ± 8.58 (100)
3,4,5,3',4'-PenCB (3 mg/kg)	$5.68 \pm 0.73^{a)}$ (163)	$0.125 \pm 0.035^{a)}$ (45)	$0.278 \pm 0.049^{a)}$ (57)	$63.84 \pm 8.77^{a)}$ (146)
4'-Hydroxy- 3,4,5,3',5'-PenCB (3 mg/kg)	3.52 ± 0.12 (101)	0.289 ± 0.027 (104)	0.397 ± 0.099 (81)	49.24 ± 5.71 (113)
4'-Hydroxy- 3,4,5,3',5'-PenCB (10 mg/kg)	3.39 ± 0.18 (97)	0.316 ± 0.069 (114)	0.476 ± 0.071 (97)	48.59 ± 7.60 (111)

Each value represents the mean $\pm$ S.D. of four rats, and those in parentheses are the relative ratio to the control.
a) Significantly different from the control, $p < 0.05$.

TABLE II. Effect of 3,4,5,3',4'-PenCB and 4'-Hydroxy-3,4,5,3',5'-PenCB on Liver Enzyme Activities of Rats

Treatment	P-450 content ^{a)}	BP 3- hydroxylase ^{b)}	BZ N- demethylase ^{c)}	DT- diaphorase ^{d)}
Control	0.123 ± 0.025 (100)	3.78 ± 0.53 (100)	1.474 ± 0.136 (100)	0.161 ± 0.078 (100)
3,4,5,3',4'-PenCB (3 mg/kg)	0.248 ± 0.128 ^{e)} (202)	52.51 ± 33.35 ^{e)} (1389)	0.461 ± 0.233 ^{e)} (31)	3.173 ± 1.741 ^{e)} (1969)
4'-Hydroxy- 3,4,5,3',5'-PenCB (3 mg/kg)	0.126 ± 0.017 (102)	6.78 ± 4.11 (179)	1.719 ± 0.509 (117)	0.107 ± 0.011 (66)
4'-Hydroxy- 3,4,5,3',5'-PenCB (10 mg/kg)	0.124 ± 0.038 (101)	7.17 ± 5.72 (190)	1.573 ± 0.390 (107)	0.111 ± 0.025 (69)

Each value represents the mean ± S.D. of three or four rats, and those in parentheses are the relative ratio to the control.
a) nmol/mg protein, *b)* pmol/min/mg protein, *c)* nmol/min/mg protein, *d)* μmol/min/mg protein.
e) Significantly different from the control, *p* < 0.05.

TABLE III. Fecal Excretion of Unchanged 3,4,5,3',4'-PenCB and Its Metabolite M-1 in Rats Injected i.p. with 3,4,5,3',4'-PenCB

Compound	% excretion					
	Days after administration					Total
	1	2	3	4	5	
3,4,5,3',4'- PenCB	2.01 ± 1.81	0.23 ± 0.13	0.17 ± 0.08	0.16 ± 0.03	0.23 ± 0.04	2.80 ± 1.62
M-1	0.84 ± 0.41	0.34 ± 0.22	0.12 ± 0.10	0.04 ± 0.03	BD ^{a)}	1.33 ± 0.62

Each value represents the mean ± S.D. of three rats.
a) BD, detected but below determination limit (0.03% of dose/whole liver).

TABLE IV. Fecal Excretion of 4'-Hydroxy-3,4,5,3',5'-PenCB in Rats after the i.p. Injection

Compound	% excretion					
	Days after administration					Total
	1	2	3	4	5	
4'-Hydroxy- 3,4,5,3',5'- PenCB	49.85 ± 8.69	5.59 ± 1.81	2.75 ± 0.68	1.20 ± 0.60	0.58 ± 0.34	59.96 ± 5.59

Each value represents the mean ± S.D. of three rats.

Fig. 4. The Postulated Metabolic Pathways of 3,4,5,3',4'-PenCB in Rats

structure of the metabolite in which a chlorine substituent at the 4'-position is shifted to the 5'-position strongly suggests that metabolism of 3,4,5,3',4'-PenCB proceeds *via* a 4',5'-epoxide formation. The postulated metabolic pathways of 3,4,5,3',4'-PenCB is shown in Fig. 4. Some investigators including the authors have reported such a NIH shift of the chlorine involved in the hydroxylation of certain PCB congeners. These are, for example, 4,4'-dichlorobiphenyl (DCB), 3,4,3',4'-tetrachlorobiphenyl (TCB) and 2,4,5,2',4',5'-hexachlorobiphenyl.^{11,20,22)}

The MS data of the methoxy- and dimethoxy-PCB gave us some valuable information in determining the position of a methoxy group on a benzene ring.^{12,23,24)} The MS of M-1 showed the high intensity of the fragment ion peak [$M^+ - CH_3$], which indicates the substitution of a methoxy group at the 4- or 4'-position (Fig. 2). If it has a methoxy group at the 5' (*meta*)- or the 6' (*ortho*)-position, the spectrum would not show the fragment ion peak of [$M^+ - CH_3$], but that of [$M^+ - COCH_3$] or [$M^+ - CH_3Cl$] with higher intensity.^{12,25)}

The metabolite of 3,4,5,3',4'-PenCB was a detoxified product (Fig. 3, Table I and II). This conclusion was in agreement with that of toxicological evaluation on the metabolites of 3,4,3',4'-TCB, a prototype of coplanar PCB. Namely, neither 5-hydroxy- nor 4-hydroxy-metabolite of 3,4,3',4'-TCB showed the toxic and biological effects.¹¹⁾ Both biologic (induction of BP 3-hydroxylase) and toxic responses (thymus atrophy) caused by polychlorinated aromatic hydrocarbons with MC-type inducing ability such as the coplanar PCB, polychlorinated dibenzofurans (PCDF) and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin have been recognized to be mediated by Ah-receptor which initially binds inducing chemicals in the cytoplasm and then translocates into the nucleus.²⁶⁻²⁸⁾ Bandiera *et al.* reported that 3,4,5,3',4'-PenCB binds to Ah-receptor with high affinity.²⁹⁾ Safe *et al.* have provided the evidence using 4'-substituted-2,3,4,5-TCB as probes that the binding affinity of 4'-hydroxy-2,3,4,5-TCB to the receptor is much less than that of 2,3,4,5,4'-PenCB.³⁰⁾ Based on these findings, it seems likely that the introduction of a hydroxyl group into

3,4,5,3',4'-PenCB results in a marked decrease of affinity for Ah-receptor.

In contrast to the fact that 3,4,5,3',4'-PenCB accumulated mostly to rat liver, 4'-hydroxy-3,4,5,3',5'-PenCB (M-1) was detectable only in a small amount in the livers of both 3,4,5,3',4'-PenCB-treated and 4'-hydroxy-3,4,5,3',5'-PenCB-treated rats. Previously, we demonstrated that 2,3,4,7,8-pentachlorodibenzofuran (PenCDF), which is closely related to 3,4,5,3',4'-PenCB structurally and toxicologically,³¹⁾ induced three forms of P-450 (P-448H, P-448L, and P-452) in rat liver microsomes and bound noncovalently but tightly to P-448H (P-450d), a high-spin form of P-450, at a molar ratio of about 1:1.³²⁾ Thus, we postulated that P-448H induced by PenCDF treatment might function as a storage site of PenCDF in the liver. 3,4,5,3',4'-PenCB treatment to rats is also reported to elevate P-448H (P-450d) at a greater extent than the other forms of P-450 and the amount comprises about 50–60% of total P-450 contents in liver microsomes.^{6,33)} Considering these observations, it appears that lack of the binding affinity to P-448H is one of the reasons for quick elimination of 4'-hydroxy-3,4,5,3',5'-PenCB (M-1) from the liver.

Interestingly, when 3,4,5,3',4'-PenCB was administered i.p. to rats, about 2.8% of dose for 5 d following injection was excreted as unchanged in the feces (Table III). We previously detected a small amount of unchanged compound in the feces of rats treated i.v. with 2,4,3',4'-TCB for relatively long periods³⁴⁾ and further demonstrated the excretion of unchanged 2,4,3',4'-TCB from the intestinal wall to the lumen.³⁵⁾ Generally in rats, the phenolic metabolites of PCB are known to be excreted to the bile, but not the unchanged compounds.^{19,34)} Therefore, the observation in the present study suggests the similar non-biliary excretion route of unchanged 3,4,5,3',4'-PenCB. Such excretion from small intestinal wall, so-called "exsorption" has also been reported in studies on the metabolism of drugs³⁶⁾ and chlorinated compounds such as hexachlorobenzene,³⁶⁾ decachlorobiphenyl,³⁶⁾ chlordecone³⁷⁾ and PenCDF.³⁸⁾ Particularly, for the highly chlorinated PCB and PCDF congeners with high persistence in the

body, this exsorption would be only a significant excretion route to the feces and would become important from a toxicological point of view.

The *in vitro* studies on PCB metabolism have demonstrated that different forms of microsomal P-450 participate in a hydroxylation of PCB. For example, Kaminsky *et al.*³⁹⁾ have shown with several DCB congeners that *ortho* (2,2',6 or 6')-substituted DCB such as 2,2'-DCB and 2,6-DCB were mainly metabolized by P-450PB-B (P-450b), a major form in liver microsomes of phenobarbital (PB)-treated rats, whereas non-*ortho* (3,3',4,4',5 or 5')-substituted DCB such as 3,3'-DCB, 3,5-DCB, 3,4-DCB and 4,4'-DCB were metabolized by P-450BNF-B (P-450c), a major form in liver microsomes of β -naphthoflavone- or MC-treated rats. In addition, Shimada and Sawabe⁴⁰⁾ have reported that 2,4,2',4'-TCB and 2,4,2',5'-TCB were activated to bind macromolecule by P-450PB (P-450b) isolated from PB-treated rats, whereas 3,4,3',4'-TCB was activated by P-448MC (P-450c) isolated from MC-treated rats. From these findings, it is probable that 3,4,5,3',4'-PenCB could induce and be metabolized by P-448L (P-450c).

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