recently, Craft *et al.* (2002) have reported that TCDD-like and phenobarbital-like PCB congeners induce hypothyroxinemia in both rats and mice through induction of hepatic UDP-GTs and that there is a species difference in magnitude of decrease in serum thyroid hormone level, and the difference is attributed to that in the increase of hepatic UDP-GTs. To date, however, only limited data are available on the species difference in the altered level of serum thyroid hormone by PCBs.

In the present study, we examined the species differences between rats and mice in altered levels of drug-metabolizing enzyme and serum thyroid hormone by Kanechlor-500, a commercial PCB mixture.

MATERIALS AND METHODS

Chemicals. Individual methylsulfonyl-PCBs ($MeSO_2$-PCBs) used as analytical standards were synthesized according to the methods of Haraguchi *et al.* (1987). 4-Methyl-3-$MeSO_2$-2′,3′,4′,5,5′-pentaCB was used as an internal standard in the analysis of $MeSO_2$-PCBs. Panacete 810 (medium-chain triglycerides) was purchased from Nippon Oils and Fats Co. Ltd. (Tokyo, Japan).

Animal treatments. Male Wistar rats, weighing 160–200 g, and male ddy mice, weighing 28–36 g, were housed, three or four per cage, with free access to commercial chow and tap water, and maintained on a 12-h dark/light cycle (8:00 A.M.-8:00 P.M. light) in an air-controlled room (temperature: 24.5 ± 1°C, humidity: 55 ± 5%). Rats and mice received a single ip injection of KC500 (100 mg/kg body weight) dissolved in Panacete 810 (5 ml/kg). Control animals were treated with vehicle alone (5 ml/kg).

Analysis of serum hormones. All animals used were killed by decapitation on day 4 after the dosing, and the thyroid gland and liver were removed and weighed. Blood was collected from each animal between 10:30 and 11:30 A.M. After clotting at room temperature, serum was separated by centrifugation and stored at −50°C until used. Levels of total T_4, total triiodothyronine (T_3), and thyroid-stimulating hormone (TSH) were measured by radioimmunoassays using Amerlex-MT4, Amerlex-MT3 (Ortho-Clinical Diagnostics Co.; Amersham, UK), and Biotrak rTSH [^{125}I] assay system (Amersham Life Science, Ltd.; Little Chalfont, UK), respectively.

Hepatic microsomal enzyme assays. Hepatic microsomes were prepared according to the method as described previously (Kato *et al.*, 1995a). The protein content was determined by the method of Lowry *et al.* (1951) with bovine serum albumin as a standard. Amount of microsomal cytochrome P450 (P450) was estimated according to the method of Omura and Sato (1964). Microsomal *O*-dealkylase activities of 7-benzyloxy-, 7-ethoxy-, and 7-pentoxy-resorufins were determined by the method of Burke *et al.* (1985). The activities of microsomal UDP-GT toward T_4, 4-nitrophenol, and chloramphenicol were determined by the methods of Barter and Klaassen (1992), Isselbacher *et al.* (1962) and Ishii *et al.* (1994), respectively. In addition, all UGP-GT activities were measured after activation of UDP-GT by 0.05% Brij 58.

RT-PCR analysis for gene expression of UGT1A1 and UGT1A6. Total hepatic RNAs were prepared with ISOGEN (NipponGene, Japan) and used for the determination of the gene expression of UDP-GT isoforms, UGT1A1 and UGT1A6, and ribosomal protein L27a (RPL27), an internal control. A portion (4 μg) of total RNA was converted to cDNA by use of poly d(N)6 primer (Pharmacia Biotech) and Moloney murine leukemia virus reverse transcriptase (GIBCO, BRL) in an RT-reaction mixture (20 μl). PCR was performed in a total reaction mixture (25 μl) containing 0.8 μl of the RT-reaction mixture, 0.5 μl of each primer set and AmpliTaq Gold DNA polymerase (Perkin Elmer). The primer sets used were as follows: rat UGT1A1 (Kasahara *et al.*, 2002), 5′-TGGTGTGCCGGAGCTCATGTTCG-3′ (forward) and 5′-ACTCCGCCCAAGTTCCACAAAAGCA-3′ (reverse); rat UGT1A6 (Kasahara *et al.*, 2002), 5′-TGCTCGACTTCCTGCAGGTTTC-3′ (forward) and 5′-TTCCTGTACTCTCTTAGAGGAGCCA-3′ (reverse); mouse UGT1A1 (Bernard *et al.*, 1999), 5′-CAGGTTTCTCCTCGTGTGTC-3′ (forward) and 5′-CATACTGGAATCCCTTTTGA-3′ (reverse); mouse UTG1A6, 5′-TCAGACACTTCCTGCAGGGTTTC-3′ (forward) and 5′-TTCCTGTACTCTCTTAGAGGACCCA-3′ (reverse). Amplifications of all cDNA examined were performed with GeneAmp PCR System 9700 (PE Applied Biosystems, Foster, Calif., USA). The PCR program used for the analyses of UGT1A1 and UGT1A6 in rats and mice was as follows: pretreatment, at 94°C for 2 min; denaturation at 94°C for 20 s; annealing, at 57°C for 45 s; extension, at 72°C for 45 s; and chase reaction, at 72°C for 10 min. Each PCR product was separated by electrophoresis on a 2% agarose gel and the separated PCR product was visualized by ethidium bromide staining under ultraviolet light. The predicted size of each PCR product is as follows: rat UGT1A1 and UGT1A6 (Kasahara *et al.*, 2002), 290 and 300 base pairs (bp), respectively; mouse UGT1A1 (Bernard *et al.*, 1999) and UGT1A6, 442 and 318 bp, respectively. In addition, PCR for the RPL27 in rats and mice (Wool *et al.*, 1990), an internal standard, was performed with a primer set: 5′-ATCGGTAAGCACCGCAAGCA-3′ (forward) and 5′-GGGAGCAACTCCATTATTGT-3′ (reverse) for both rats and mice, and the predicted product size is 234 bp.

Determination of $MeSO_2$ metabolites from KC500 in the liver. Amounts of $MeSO_2$-PCBs in the liver were determined with GC/MS (Mimura *et al.*, 1999). M^+ and M^{+2} for $MeSO_2$-tetraCB (m/z 368 and 370, respectively), $MeSO_2$-pentaCB (m/z 402 and 404, respectively), and $MeSO_2$-hexaCB (m/z 436 and 438, respectively) were monitored as selected ions.

Statistics. The data obtained were statistically analyzed according to Student's *t*-test.

TABLE 1
Relative Tissue Weights after the Administration of KC500 to Rats and Mice

	Rats		Mice	
Tissues	Control	KC500	Control	KC500
Thyroid gland	0.006 ± 0.001	0.006 ± 0.0003	0.009 ± 0.0003	0.010 ± 0.0008
Liver	3.02 ± 0.02	4.19 ± 0.06*	3.77 ± 0.05	4.19 ± 0.10*

Note. Values are given as % body weight. Data represent the mean ± SE for four to five rats and ten to twelve mice.

*$p < 0.05$, significantly different from the species-matched controls.

RESULTS

Hepatic drug-metabolizing enzymes. Maximal induction of hepatic drug-metabolizing enzymes by KC500 (100 mg/kg) in rats were reported to be observed at 4 days after the administration (Shimada *et al.*, 1976). Therefore, in the present experiments, relative liver weight, microsomal P450 content and microsomal *O*-dealkylase activities of 7-pentoxy-, 7-benzyroxy-, and 7-ethoxy-resorufins in rats and mice were measured 4 days after the administration of KC500 at a dose of 100 mg/kg.

In both species of rats and mice, KC500-treatment resulted in significant increase in the liver weight: 1.4- and 1.1-fold, respectively (Table 1). Likewise, the treatment resulted in significant increases in hepatic microsomal enzymes in rats and mice; P450 content: 3.2- and 1.2-fold, respectively; benzyl-

TABLE 2
Effects of KC500 on Hepatic Microsomal P450 and Alkoxyresorufin *O*-Dealkylases in Rats and Mice

	Rats		Mice	
	Control	KC500	Control	KC500
Total P450[a]	1.74 ± 0.02	2.33 ± 0.11*	0.96 ± 0.02	1.13 ± 0.04*
O-Dealkylase of alkoxyresorufin				
7-Benzyloxy-	0.08 ± 0.01	6.72 ± 0.64*	0.08 ± 0.004	0.85 ± 0.12*
7-Pentoxy-	0.03 ± 0.003	0.82 ± 0.03*	0.02 ± 0.001	0.15 ± 0.02*
7-Ethoxy-	0.16 ± 0.01	7.31 ± 0.92*	0.22 ± 0.01	0.43 ± 0.03*

Note. Total P450 is given in nmol/mg protein; *O*-Dealkylase of alkoxyresorufin is given in nmol/mg protein/min. Data represent the mean ± SE for 4–5 rats and 6–7 mice.

*$p < 0.05$, significantly different from the species-matched controls.

oxyresorufin *O*-dealkylase activity (CYP2B and CYP3A): 86- and 10.2-fold, respectively; pentoxyresorufin *O*-dealkylase activity (CYP2B): 25- and 7.3-fold, respectively; ethoxyresorufin *O*-dealkylase activity (CYP1A): 46- and 1.9-fold, respectively (Table 2). Furthermore, KC500-treatment led to significant increases in UDP-GT activities toward T_4 and 4-nitrophenol in rats but not in mice (Table 3). UDP-GT activity toward chloramphenicol was extensively increased in rats and slightly in mice.

Changes in hepatic gene expression of UGT1A1 and UGT1A6. Since the species difference between rats and mice in alteration of UDP-GT activity by KC500 was observed (Table 3), we further examined the altered gene expression of the UDP-GTs, UGT1A1 and UGT1A6, responsible for glucuronidation of T_4 and/or 4-nitrophenol, in KC500-treated rats and mice. In rats, levels of UGT1A1 and UGT1A6 increased in time-dependent manner at least up to 3 days after KC500-treatment, and the increases were observed even 4 days later, whereas in mice, no increase in the UGT1As was observed at any period examined (Fig. 1).

Methyl sulfone metabolites of PCBs. KC500 (100 mg/kg) was administered to rats and mice, and 4 days after the administration, the $MeSO_2$ metabolites in each liver were analyzed (Table 4). 3-$MeSO_2$ or 4-$MeSO_2$ derivatives of 2,3′,4′,5-tetrachlorobiphenyl (2,3′,4′,5-tetraCB), 2,2′,3′,4′,5-pentaCB, 2,2′,4′,5′,5-pentaCB, 2,2′,3′,4′,5,6-hexaCB, and 2,2′,4′,5,5′,6-hexaCB were detected as main metabolites in rats and mice. The amount of each $MeSO_2$ metabolite produced was 2–9 times higher in mice than in rats, and the total amount of $MeSO_2$ metabolites detected in liver was 5-fold greater in mice as compared with that in rats.

Serum hormone levels. Effects of KC500 on the levels of serum thyroid hormones, T_4 and T_3, in rats and mice were examined. Treatments of rats and mice with KC500 decreased total T_4 levels to 17 and 27%, respectively, of the corresponding controls, whereas serum total T_3 level was slightly decreased in mice (71% of control) but not in rats (Fig. 2). In addition, no significant change in the level of serum TSH (Fig. 3) or in thyroid weight (Table 1) was observed in either species of animals used.

DISCUSSION

In the present study, we found for the first time that KC500 treatment resulted in a significant decrease in serum T_4 levels in both rats and mice, whereas a significant increase in activity

TABLE 3
Effects of KC500 on Hepatic Microsomal UDP-Glucuronosyltransferase Activities in Rats and Mice

	Glucuronide formed, rats		Glucuronide formed, mice	
Substrate	Control	KC500	Control	KC500
Thyroxine	9.1 ± 1.3	35.7 ± 2.4*	21.2 ± 3.0	26.2 ± 3.7
4-Nitrophenol	35.3 ± 0.9	178.2 ± 9.0*	39.5 ± 0.6	38.7 ± 0.2
Chloramphenicol	0.74 ± 0.13	3.68 ± 0.38*	1.01 ± 0.03	1.22 ± 0.05*

Note. Values are given in nmol/mg protein/min. Data represent the mean ± SE for 4–5 rats and 6–7 mice.

*$p < 0.05$, significantly different from species-matched controls.

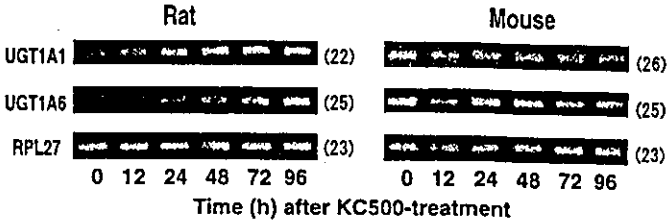

FIG. 1. Representative profile of the agarose gel-electrophoresis of the RT-PCR product for UGT1A1 and UGT1A6 in rats and mice after KC500-treatment. Total RNAs were prepared from the pooled livers of three rats or five mice in each experiment group and used for the RT-PCR analysis, as described in Materials and Methods. Time 0 means the animals were treated with vehicle alone (control). Numbers shown in parentheses represent the numbers of PCR cycles used.

TABLE 4
Concentrations of Hepatic $MeSO_2$-PCB Metabolites after the Administration of KC500 to Rats and Mice

$MeSO_2$ metabolite	Rats	Mice
3-$MeSO_2$-2,3',4',5-tetraCB	28.2 ± 6.6	78.4 ± 8.7
4-$MeSO_2$-2,3',4',5-tetraCB	22.0 ± 6.4	91.1 ± 9.7
3-$MeSO_2$-2,2',3',4',5-pentaCB	6.0 ± 1.5	10.7 ± 2.9
4-$MeSO_2$-2,2',3',4',5-pentaCB	4.1 ± 1.0	33.3 ± 9.3
3-$MeSO_2$-2,2',4',5,5'-pentaCB	12.5 ± 3.9	59.4 ± 10.7
4-$MeSO_2$-2,2',4',5,5'-pentaCB	11.0 ± 3.1	74.3 ± 10.6
3-$MeSO_2$-2,2',3',4',5,6-hexaCB	3.3 ± 1.0	17.5 ± 5.1
4-$MeSO_2$-2,2',3',4',5,6-hexaCB	3.2 ± 0.9	17.5 ± 4.7
3-$MeSO_2$-2,2',4',5,5',6-hexaCB	2.8 ± 0.7	6.1 ± 1.5
4-$MeSO_2$-2,2',4',5,5',6-hexaCB	3.6 ± 1.2	32.9 ± 7.3
Total $MeSO_2$-PCBs	96.5 ± 24.4	483.4 ± 71.0

Note. Concentrations are given in ng/g liver. Data represent the mean ± SE for 5 rats and 6–7 mice.

of the UDP-GT responsible for glucuronidation of T_4 (T_4-UDP-GT) was observed in rats but not in mice. Such species difference in increase of T_4-UDP-GT activity by xenobiotics has been reported: clofibrate, phenobarbital, pregnenolone-16α-carbonitrile, and β-naphthoflavone increase hepatic T_4-UDP-GT activity in rats but not in mice (Viollon-Abadie *et al.*, 1999). The previous report and the present findings suggest that in rats, a reduction of serum total T_4 level by KC500 would occur at least in part by an increase in T_4 glucuronidation through the induction of hepatic T_4-UDP-GTs, especially UGT1A1 and UGT1A6 (Schuur *et al.*, 1997; Van Birgelen *et al.*, 1995; Visser, 1996), while in mice, it may occur through alternate mechanisms. This is further supported by the our findings that after treatment with KC500, gene expression of hepatic UGT1A1 and UGT1A6 in the rat liver was enhanced prior to decrease in serum T_4 levels, whereas in the mouse liver, such enhancement did not occur.

In general, the decrease in serum T_4 level by PCB congeners such as 3,3',4,4'-tetraCB, 2,3',4,4',5'-pentaCB, 2,3,3',4,4',5- and 2,2',4,4',5,5'-hexaCBs and Aroclor 1254 have been reported to decrease serum thyroid hormone levels in rats (Barter and Klaassen, 1994; Liu *et al.*, 1995; Ness *et al.*, 1993; Van Birgelen *et al.*, 1995). This has been thought to occur through induction of T_4-UDP-GTs (Schuur *et al.*, 1997; Van Birgelen *et al.*, 1995; Visser, 1996). Our preliminary study (Kato *et al.*, 2002), however, indicated that the activity of T_4-UDP-GT was significantly increased in Wistar rats, but not in UGT1A-deficient Gunn rats, by KC500 treatment, although serum total T_4 levels in both strains of rat were significantly reduced by the treatment. This suggests strongly that the decrease in serum total T_4 levels in not only mice but also rats by PCBs would not be induced only through increase in hepatic T_4 glucuronidation. PCB congeners (Chauhan *et al.*, 2000) and their hydroxylated metabolites (Brouwer *et al.*, 1998; Lans *et al.*, 1993) show *in vitro* binding to transthyretin (TTR), a major thyroid hormone transporting protein, which plays an essential role in the homeostasis of T_4 (Schreiber *et al.*, 1995), suggesting that decrease in the level of serum T_4 in KC500-treated mice and rats might occur, at least in part, through a TTR-associated pathway.

To determine the mechanism for decrease in the serum T_3 level in KC500-treated mice, we examined KC500-altered levels of hepatic microsomal UGT2B2 enzyme by Western blotting with antirat UGT2B polyclonal antibody, because T_3 glucuronidation is efficiently mediated by UGT2B2 enzyme (van Raaij *et al.*, 1993; Visser *et al.*, 1993). UGT2B2, however, was not detected in any experimental group of rats or mice, although other UGT2B subfamily enzymes (UGT2B3, UGT2B6, and UGT2B12) were detected in all the experimental groups examined (data not shown). Accordingly, the decrease in serum T_3 levels in mice by KC500 seems to occur without increase in the UGT2B2.

As a possible mechanism for the decrease in the levels of serum T_4 and T_3, increase in estrogen sulfotransferase, which

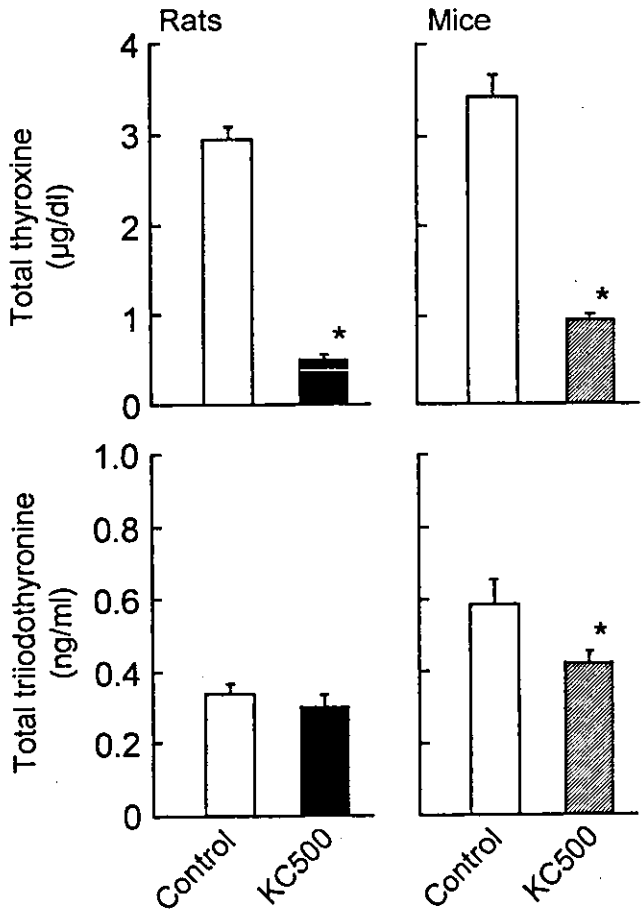

FIG. 2. Effects of KC500 on levels of serum total thyroxine and triiodothyronine in rats and mice. Animals were killed 4 days after the administration of KC500 (100 mg/kg, ip), and levels of serum thyroid hormones were measured as described in Materials and Methods. Each column represents the mean ± SE (vertical bars) for four to five rats or six to eight mice; $*p < 0.05$, significantly different from the species-matched controls.

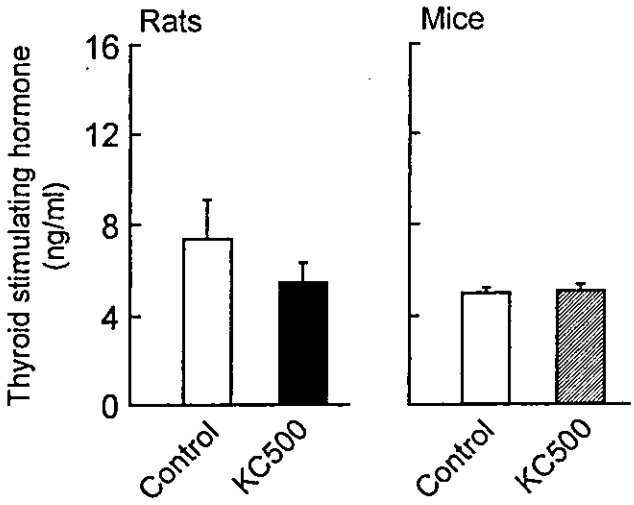

FIG. 3. Effects of KC500 on the level of serum thyroid stimulating hormone in rats and mice. Animals were killed 4 days after the administration of KC500 (100 mg/kg, ip), and levels of serum thyroid stimulating hormone were measured as described in Materials and Methods. Each column represents the mean ± SE (vertical bars) for four to five rats or seven to eight mice.

efficiently catalyzes the sulfation of iodothyronines T_4 and T_3 (Kester *et al.*, 1999), might be considered. However, since Aroclor 1254 has been reported to show only a minimal impact on overall outer ring deiodination activity (Hood and Klaassen, 2000), KC500 seems to hardly induce deiodinases converting T_4 to T_3. Furthermore, we showed in the present study that serum TSH levels in either rats or mice was not significantly changed by KC500, indicating that TSH is not attributed to a decrease in serum T_4 and/or T_3 levels by KC500. In addition, it had been reported that serum TSH level was little affected by PCBs (Hallgren *et al.*, 2001; Hood *et al.*, 1999; Liu *et al.*, 1995).

We have previously reported that 3-$MeSO_2$ metabolites of nonplanar PCBs have definite activities for inducing hepatic microsomal drug-metabolizing enzymes, and their activities were much greater than parent PCBs (Kato *et al.*, 1995a,b, 1999b). We further demonstrated that 3-$MeSO_2$-2,3′,4′,5-tetraCB, 3-$MeSO_2$-2,2′,3′,4′,5-pentaCB, 3-$MeSO_2$-2,2′,4′,5,5′-pentaCB, 4-$MeSO_2$-2,2′,4′,5,5′-pentaCB, 3-$MeSO_2$-2,2′,3′,4′,5,6-hexaCB, 3-$MeSO_2$-2,2′,4′,5,5′,6-hexaCB and 4-$MeSO_2$-2,2′,4′,5,5′,6-hexaCB could reduce serum T_4 level (Kato *et al.*, 1998, 1999a). KC500, used in the present study, includes many nonplanar PCBs which were biotransformed to $MeSO_2$ metabolites, thus posing the possibility that $MeSO_2$-PCB metabolites formed are attributed to decrease in the level of serum thyroid hormones in KC500-treated rats and mice. However, species difference between rats and mice in induced levels of hepatic drug-metabolizing enzymes was not necessarily correlated with that in the decreased level of serum thyroid hormones. Although hepatic concentrations of $MeSO_2$-PCB metabolites in KC500-treated mice were much higher than those in KC500-treated rats, the decreased levels in serum T_4 in rats and mice were almost the same. Furthermore, the magnitude of the induction of T_4-UDP-GT and cytochrome P450 enzymes was greater in rats than in mice, despite hepatic levels of $MeSO_2$ metabolites being greater in mice than in rats. Namely, the difference between rats and mice in the level of $MeSO_2$ metabolites was not correlated with the difference in induction of microsomal drug-metabolizing enzymes or in reduction of serum thyroid hormone.

In conclusion, we demonstrate for the first time that in mice, the decrease in serum T_4 level by KC500 occurs without increase in the T_4-UDP-GTs, UGT1A1, and UGT1A6 responsible for glucuronidation of T_4, and further suggest that the decrease in rats would not be produced only through induction of the T_4-UDP-GTs. However, exact mechanisms for KC500-mediated decrease in serum thyroid hormone levels in rats and mice remain unclear, and further studies are necessary for understanding the susceptibility to PCBs in animals including humans.

ACKNOWLEDGMENTS

We express our appreciation to Dr. Shinichi Ikushiro (Himeji Institute of Technology) for the generous gift of antibodies against rat UGT2B isoforms. This work was supported in part by a Grant-in-Aid for Scientific Research (C) (no. 12680549, Y.K.; 12672180, K.H.; 14572119, N.K.) from the Japan Society for the Promotion of Science, and by a Health Sciences Research Grant for Research on Environmental Health (H11-Seikatsu-024, M.D., H13-Seikatsu-013, Y.K.) from the Ministry of Health and Welfare of Japan.

REFERENCES

Barter, R. A., and Klaassen, C. D. (1992). Rat liver microsomal UDP-glucuronosyltransferase activity toward thyroxine: Characterization, induction, and form specificity. *Toxicol. Appl. Pharmacol.* **115**, 261–267.

Barter, R. A., and Klaassen, C. D. (1994). Reduction of thyroid hormone levels and alteration of thyroid function by four representative UDP-glucuronosyltransferase inducers in rats. *Toxicol. Appl. Pharmacol.* **128**, 9–17.

Bernard, P., Goudonnet, H., Artur, Y., Desvergne, B., and Wahli, W. (1999). Activation of the mouse TATA-less and human TATA-containing UDP-glucuronosyltransferase 1A1 promoters by hepatocyte nuclear factor 1. *Mol. Pharmacol.* **56**, 526–536.

Brouwer, A., Longnecker, M. P., Birnbaum, L. S., Cogliano, J., Kostyniak, P., Moore, J., Schantz, S., and Winneke, G. (1999). Characterization of potential endocrine-related health effects at low-dose levels of exposure to PCBs. *Environ. Health Perspect.* **107**, 639–649.

Brouwer, A., Morse, D. C., Lans, M. C., Schuur, A. G., Murk, A. J., Klasson-Wehler, E., Bergman, Å., and Visser, T. J. (1998). Interactions of persistent environmental organohalogens with the thyroid hormone system: Mechanisms and possible consequences for animal and human health. *Toxicol. Ind. Health* **14**, 59–84.

Burke, M. D., Thompson, S., Elcombe, C. R., Halpert, J., Haaparanta, T., and Mayer, R. T. (1985). Ethoxy-, pentoxy-, and benzyloxyphenoxazones and homologues: A series of substrates to distinguish between different induced cytochromes P-450. *Biochem. Pharmacol.* **34**, 3337–3345.

Chauhan, K. R., Kodavanti, P. R. S., and McKinney, J. D. (2000). Assessing the role of *ortho*-substitution on polychlorinated biphenyl binding to transthyretin, a thyroxine transport protein. *Toxicol. Appl. Pharmacol.* **162**, 10–21.

Craft, E. S., DeVito, M. J., and Crofton, K. M. (2002). Comparative responsiveness of hypothyroxinemia and hepatic enzyme induction in Long-Evans

rats versus C57BL/6J mice exposed to TCDD-like and phenobarbital-like polychlorinated biphenyl congeners. *Toxicol. Sci.* **68,** 372–380.

Duignan, D. B., Sipes, I. G., Ciaccio, P. J., and Halpert, J. R. (1988). The metabolism of xenobiotics and endogenous compounds by the constitutive dog liver cytochrome P450 PBD-2. *Arch. Biochem. Biophys.* **267,** 294–304.

Duignan, D. B., Sipes, I. G., Leonard, T. B., and Halpert, J. R. (1987). Purification and characterization of the dog hepatic cytochrome P450 isozyme responsible for the metabolism of 2,2′,4,4′,5,5′-hexachlorobiphenyl. *Arch. Biochem. Biophys.* **255,** 290–303.

Hallgren, S., Sinjari, T., Håkansson, H., and Darnerud, P. O. (2001). Effects of polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) on thyroid hormone and vitamin A levels in rats and mice. *Arch. Toxicol.* **75,** 200–208.

Haraguchi, K., Kuroki, H., and Masuda, Y. (1987). Synthesis and characterization of tissue-retainable methylsulfonyl polychlorinated biphenyl isomers. *J. Agric. Food Chem.* **35,** 178–182.

Hileman, B. (1993). Concerns broaden over chlorine and chlorinated hydrocarbons. *Chem. Eng. News* April 19, 11–20.

Hood, A., Hashmi, R., and Klaassen, C. D. (1999). Effects of microsomal enzyme inducers on thyroid-follicular cell proliferation, hyperplasia, and hypertrophy. *Toxicol. Appl. Pharmacol.* **160,** 163–170.

Hood, A., and Klaassen, C. D. (2000). Effects of microsomal enzyme inducers on outer-ring deiodinase activity toward thyroid hormones in various rat tissues. *Toxicol. Appl. Pharmacol.* **163,** 240–248.

Ishii, Y., Tsuruda, K., Tanaka, M., and Oguri, K. (1994). Purification of a phenobarbital-inducible morphine UDP-glucuronyltransferase isoform, absent from Gunn rat liver. *Arch. Biochem. Biophys.* **315,** 345–351.

Isselbacher, K. J., Chrabas, M. F., and Quinn, R. C. (1962). The solubilization and partial purification of a glucuronyl transferase from rabbit liver microsomes. *J. Biol. Chem.* **237,** 3033–3036.

Kasahara, T., Hashiba, M., Harada, T., and Degawa, M. (2002). Change in the gene expression of hepatic tamoxifen-metabolizing enzymes during the process of tamoxifen-induced hepatocarcinogenesis in female rats. *Carcinogenesis* **23,** 491–498.

Kato, Y., Haraguchi, K., Kawashima, M., Yamada, S., Masuda, Y., and Kimura, R. (1995a). Induction of hepatic microsomal drug-metabolizing enzymes by methylsulphonyl metabolites of polychlorinated biphenyl congeners in rats. *Chem. Biol. Interact.* **95,** 257–268.

Kato, Y., Haraguchi, K., Kawashima, M., Yamada, S., Isogai, M., Masuda, Y., and Kimura, R. (1995b). Characterization of hepatic microsomal cytochrome P-450 from rats treated with methylsulphonyl metabolites of polychlorinated biphenyl congeners. *Chem. Biol. Interact.* **95,** 269–278.

Kato, Y., Haraguchi, K., Shibahara, T., Masuda, Y., and Kimura, R. (1998). Reduction of thyroid hormone levels by methylsulfonyl metabolites of polychlorinated biphenyl congeners in rats. *Arch. Toxicol.* **72,** 541–544.

Kato, Y., Haraguchi, K., Shibahara, T., Yumoto, S., Masuda, Y., and Kimura, R. (1999a). Reduction of thyroid hormone levels by methylsulfonyl metabolites of tetra- and pentachlorinated biphenyls in male Sprague-Dawley rats. *Toxicol. Sci.* **48,** 51–54.

Kato, Y., Haraguchi, K., Tomiyasu, K., Saito, H., Shibahara, T., Masuda, Y., and Kimura, R. (1999b) The role of 3-methylsulfonyl-2,2′,4′,5,5′-pentachlorobiphenyl, a metabolite of 2,2′,4,5,5′-pentachlorobiphenyl, in the induction of hepatic microsomal drug-metabolizing enzymes by 2,2′,4,5,5′-pentachlorobiphenyl in rats. *Environ. Toxicol. Pharmacol.* **8,** 39–47.

Kato, Y., Yamazaki, T., Haraguchi, K., Ito, Y., Nemoto, K., Masuda, Y., Degawa, M., and Kimura, R. (2001). Effects of 2,2′,4,5,5′-pentachlorobiphenyl and 2,2′,3,3′,4,6′-hexachlorobiphenyl on serum hormone levels in rats and mice. *Organohalogen Compd.* **53,** 44–46.

Kato, Y., Yamazaki, T., Ikushiro, S., Ito, Y., Haraguchi, K., Iyanagi, T., Kimura R., and Degawa, M. (2002). Relation between the increase in hepatic UDP-glucuronosyltransferase and the decrease in serum thyroxine level in Kanechlor-500-treated rats. *Organohalogen Compd.* **56,** 85–87.

Kester, M. H. A., Van Dijk, C. H., Tibboel, D., Hood, A. M., Rose, N. J. M., Meinl, W., Pabel, U., Glatt, H., Falany, C. N., Coughtrie, M. W. H. and Visser, T. J. (1999). Sulfation of thyroid hormone by estrogen sulfotransferase. *J. Clin. Endocrinol. Metab.* **84,** 2577–2580.

Kuratsune, M., Yoshimura, H., Matsuzaka, J., and Yamaguchi, A. (1972). Epidemiologic study on Yusho, a poisoning caused by ingestion of rice oil contaminated with a commercial brand of polychlorinated biphenyls. *Environ. Health Perspect.* **46,** 119–128.

Lans, M. C., Klasson-Wehler, E., Willemsen, M., Meussen, E., Safe, S., and Brouwer, A. (1993). Structure-dependent, competitive interaction of hydroxy-polychlorobiphenyls, -dibenzo-*p*-dioxins, and -dibenzofurans with human transthyretin. *Chem. Biol. Interact.* **88,** 7–21.

Li, M.-H., and Hansen, L. G. (1997). Consideration of enzyme and endocrine interactions in the risk assessment of PCBs. *Rev. Toxicol.* **1,** 71–156.

Liu, J., Liu, Y., Barter, R. A., and Klaassen, C. D. (1995). Alteration of thyroid homeostasis by UDP-glucuronosyltransferase inducers in rats: A dose-response study. *J. Pharmacol. Exp. Ther.* **273,** 977–985.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193,** 265–275.

McConnell, E. E. (1989). In halogenated biphenyls, terphenyls, naphthalenes, dibenzodioxins, and related products (R.D. Kimbrough and A.A. Jensen, Eds.), pp. 161–193. Elsevier, Amsterdam.

Mimura, K., Tamura, M., Haraguchi, K., and Masuda, Y. (1999). Analysis of 209 PCB congeners by high separation gas chromatography/low resolution mass spectrometer. *Fukuoka Acta Med.* **90,** 192–201.

Ness, D. K., Schantz, S. L., Moshtaghian, J., and Hansen, L. G. (1993). Effects of perinatal exposure to specific PCB congeners on thyroid hormone concentrations and thyroid histology in the rat. *Toxicol. Lett.* **68,** 311–323.

Newsome, H. W., Davies, D., and Doucet, J. (1995). PCB and organochlorine pesticides in Canadian human milk - 1992. *Chemosphere* **30,** 2143–2153.

Omura, T., and Sato, R. (1964). The carbon monoxide-binding pigment of liver microsomes: I. Evidence for its hemoprotein nature. *J. Biol. Chem.* **239,** 2370–2378.

Phaneuf, D., DesGranges, J. L., Plante, N., and Rodrigue, J. (1995). Contamination of local wildlife following a fire at a polychlorinated biphenyl warehouse in St. Basile le Grand, Quebec, Canada. *Arch. Environ. Contam. Toxicol.* **28,** 145–153.

Safe, S. H. (1990). Polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and related compounds: Environmental and mechanistic considerations that support the development of toxic equivalency factors (TEFs). *Crit. Rev. Toxicol.* **21,** 51–88.

Safe, S. H. (1994). Polychlorinated biphenyls (PCBs): Environmental impact, biochemical and toxic responses, and implications for risk assessment. *Crit. Rev. Toxicol.* **24,** 87–149.

Schuur, A. G., Boekhorst, F. M., Brouwer, A., and Visser, T. J. (1997). Extrathyroidal effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on thyroid hormone turnover in male Sprague-Dawley rats. *Endocrinology* **138,** 3727–3734.

Schreiber, G., Southwell, B. R., and Richardson, S. J. (1995). Hormone delivery systems to the brain-transthyretin. *Exp. Clin. Endocrinol.* **103,** 75–80.

Shimada, T., Nunoura, Y., Kitanaka, E., Iwagami, S., and Mizuta, Y. (1976). Induction of mouse and rat liver microsomal enzymes by polychlorinated biphenyls in relation to the PCB levels in tissue. *Folia Pharmacol. Japon.* **72,** 955–967.

van Birgelen, A. P. J. M., Smit, E. A., Kampen, I. M., Groeneveld, C. N., Fase, K. M., van der Kolk, J., Poiger, H., van den Berg, M., Koeman, J. H., and Brouwer, A. (1995). Subchronic effects of 2,3,7,8-TCDD or PCBs on thyroid hormone metabolism: Use in risk assessment. *Eur. J. Pharmacol.* **293,** 77–85.

van Raaij, J. A., Kaptein, E., Visser, T. J., and van den Berg, K. J. (1993). Increased glucuronidation of thyroid hormone in hexachlorobenzene-treated rats. *Biochem. Pharmacol.* **45**, 627–631.

Visser, T. J. (1996). Pathways of thyroid hormone metabolism. *Acta Med. Austriaca Hefi* **1/2**, 10–16.

Visser, T. J., Kaptein, E., van Raaij, J. A., Joe, C. T., Ebner, T., and Burchell, B. (1993). Multiple UDP-glucuronyltransferases for the glucuronidation of thyroid hormone with preference for 3,3',5'-triiodothyronine (reverse T3). *FEBS Lett.* **315**, 65–68.

Viollon-Abadie, C., Lassere, D., Debruyne, E., Nicod, L., Carmichael, N., and Richert, L. (1999). Phenobarbital, β-naphthoflavone, clofibrate, and pregnenolone-16α-carbonitrile do not affect hepatic thyroid hormone UDP-glucuronosyl transferase activity, and thyroid gland function in mice. *Toxicol. Appl. Pharmacol.* **155**, 1–12.

Wool, I. G., Chan, Y. L., Paz, V., and Olvera, J. (1990). The primary structure of rat ribosomal proteins: The amino acid sequences of L27a and L28 and corrections in the sequence of S4 and S12. *Biochim. Biophys. Acta* **1050**, 69–73.

福岡医誌 94(5):174−182, 2003

2,3,3',4,4'-五塩素化ビフェニル（CB105）のハムスターにおける代謝

中村学園大学栄養科学部栄養科学科
古賀信幸，金丸知代
第一薬科大学環境化学教室
黒木広明
第一薬科大学健康化学教室
原口浩一
静岡県立大学薬学部薬剤学教室
加藤善久，木村良平

Metabolism of 2,3,3',4,4'-Pentachlorobiphenyl in Hamsters

Nobuyuki KOGA[1], Hiroaki KUROKI[2], Koichi HARAGUCHI[2], Tomoyo KANAMARU[1], Yoshihisa KATO[3] and Ryohei KIMURA[3]

[1] *Department of Nutritional Sciences, Nakamura Gakuen University, 5-7-1, Befu, Johnan-ku, Fukuoka 814-0198*
[2] *Daiichi College of Pharmaceutical Sciences, 22-1, Tamagawa-cho, Minami-ku, Fukuoka, 815-8511*
[3] *School of Pharmaceutical Sciences, University of Shizuoka, 52-1, Yada, Shizuoka 422-8526*

Abstract The in vivo metabolism of 2,3,3',4,4'-pentachlorobiphenyl (CB105) was studied in hamsters and the effect of cytochrome P450 inducers, phenobarbital (PB) and 3-methylcholanthrene (MC) on its metabolism was compared to rats. After administration of CB105 intraperitoneally at a dose of 3 mg/body, four metabolites, named M-1, M-2, M-3 and M-4, were detected in 5 days-feces of all groups and the formation ratio of the metabolites M-1 ～ M-4 was 1 : 39 : 84 : 0.2 in untreated hamsters and 1 : 19 : 6.7 : 0.7 in untreated rats. On the basis of the mass spectra of four synthetic authentic compounds and the retention times on DB-1 and MPS50 columns, M-1, M-2, M-3 and M-4 were identified as 4'-hydroxy-2,3,3',4,5'-PenCB, 5'-hydroxy-CB105, 5-hydroxy-CB105 and 4-hydroxy-2,3,3',4',5-PenCB, respectively. The pretreatment of PB and MC resulted in about 2-fold fecal excretion of four metabolites in hamsters and in about 3-fold in rats. Of four metabolites, only M-4 were detected in the serum at 5 days after CB105 administration and the concentration was 0.39 μg/ml of hamster serum and 0.28 μg/ml of rat serum. In hamsters, the concentration of M-4 was increased to 1.8-fold of untreated animals by PB treatment and 2.6-fold by MC treatment. On the other hand, the treatment of rats with PB and MC did not show such an increase of serum M-4. These results suggested that the hamster oxidized 2,3,4-trichloro-substituted benzene ring predominantly rather than 3',4'-dichloro-substituted benzene ring differently from the rat and that M-4 formed in hamster liver distributed to the blood and retained there to a considerable extent in comparison with that formed in rat liver.

はじめに

2,3,3',4,4'-五塩素化ビフェニル（PenCB）（CB 105）はカネミ油症の原因物質であるPCB製品（Kanechlor 400）の成分の1つであるとともに，油症患者の血清中PCB成分のうちで，健常者よりも濃度が低い成分として知られている．すなわち，黒木と増田は，血清中PCB異性体のうち，2,3',4,4',5-PenCB（CB 118）及びCB 105が，健常人に比べ油症患者で少ないかあるいは消失していることを報告した[18]．この事実は，油症患者において多量摂取されたPCBによってチトクロム

P 450 (P 450) が誘導され，さらに誘導された P 450 によって上記 PenCB の代謝が促進されたものと考えられる．

PCB は肝 P 450 を中心とした肝薬物代謝酵素に対し強い誘導作用を有することが報告されている[19)22)]．また，その誘導様式の違いから，phenobarbital (PB) 型，3-methylcholanthrene (MC) 型および混合型に分類されている．PB 型には，2(2') 位あるいは 6 (6') 位に 1～2 個塩素が置換された PCB 異性体 (mono-ortho-および di-ortho-PCB) が含まれ，また，MC 型には 3,3',4,4'-四塩素化ビフェニル(TCB)，3,3',4,4',5-PenCB および 3,3',4,4',5,5'-六塩素化ビフェニルなどの coplanar な PCB 異性体が含まれる．なお，前出の CB 118 と CB 105 は mono-ortho-PCB であるが coplanar PCB の性質も有することから混合型に分類されている[19)]．

一方，CB 105 の代謝に関する研究は報告がほとんどない．山本らはラットに CB 105 を経口投与し，投与後 8 日間の糞，尿及び組織を調べた結果，全く代謝物は検出されなかったと報告した[20)]．その後，Klasson-Wehler らは，CB 105 を経口投与したマウスとミンクにおける in vivo 代謝を調べ，その結果，いずれの動物においても代謝速度は非常に遅いものの 5 種類の代謝物へと変換されることを明らかにした[8)]．それらの構造は 5-水酸化 (OH)体，5'-OH 体及び 2'-OH 体と，さらに NIH 転位した代謝物の 4-OH-2,3,3',4',5-PenCB 及び 4'-OH-2,3,3',4,5'-PenCB であった．興味あることに，4'-OH 体とともに 4-OH 体が比較的多く生成されており，塩素が 3 個置換している芳香環，すなわち 2,3,4-三塩素置換ベンゼン環の方が 3',4'-二塩素置換ベンゼン環より代謝されやすいという結果であった．最近，原口らは CB 105 を腹腔内に投与したラットの糞中から合計 9 種類の代謝物を検出した[5)]．上記 5 種類のうち 2'-OH 体を除く 4 種類の水酸化体，さらに塩素が脱離した代謝物 4-OH-2,3,3',4'-TCB および 4 種類のメチルチオ (MeS)体，すなわち 5-MeS-，5'-MeS-，6-MeS-および 6'-MeS-CB 105 を新たに報告した．

ハムスターはダイオキシン類などの毒性に対して，最も感受性の低い動物として報告されていること[9)]から，代謝能との関連は興味深い．当研究室では，ハムスター肝ミクロゾームを用いて 2,2',5,5'-および 2,3',4',5-TCB の代謝を調べ，PB 誘導性の HPB-1 (CYP 2 B) および MC 誘導性の CYP1A2 と CYP2A8 が関与することを明らかにした[11)-14)]．そこで本研究では，塩素が 1 つ多い CB 105 の in vivo 代謝を調べ，ラットと比較した．また，代謝に関与する P 450 分子種を明らかにするため，代表的な P 450 誘導剤として知られている PB および MC で前処理した動物でも同様に検討した．

実　験　方　法

1．実験材料

(1) CB105 の合成

CB105 は Cadogan による方法[3)]で合成した．まず，3,4-dichloroaniline および 1,2,3-trichlorobenzene をテトラクロロエチレンで溶解し，さらに亜硝酸イソペンチルを加えて，110°C で 24 時間反応させた．反応物はアルミナカラム (Merck，ドイツ)で精製したのち，異性体を高速液体クロマトグラフィー (HPLC) により分離した．HPLC 条件は次の通りである．カラム，ODS (250×10 mm i.d.，5 μm，草野科学)；移動相，アセトニトリル-H_2O (9：1)；流速，2 ml/min；検出波長，254 nm．CB 105 の純度は最終的に 99% 以上であった．

CB 105：MS (EI) m/z (relative intensity) 324 (100) [M^+]，326 (163) [M^++2]，328 (101) [M^++4]，330 (33) [M^++6]，252 (45) [M^+-Cl_2]．

(2) 代謝物の合成

CB 105 代謝物の合成も Cadogan の方法[3)]で行った．すなわち，3,4-dichloroaniline と 2,3,6-あるいは 2,3,4-trichloroanisole を，または 2,3,4-trichloroaniline と 2,6-あるいは 2,3-dichloroanisole を加えて，110 °C，24 時間反応させた．反応後，CB 105 と同様にアルミナカラムおよび HPLC で精製した．

4-CH_3O (MeO) -2,3,5,3',4'-PenCB：^{1}H-NMR (500 MHz，chloroform-*d*) δ 7.52 (1 H，d，J=8.1 Hz，5'-H)，7.47 (1 H，d，J=2.2 Hz，2'-H)，7.28 (1 H，s，6-H)，7.22 (1 H，dd，J=8.3 Hz，2.0 Hz，6'-H)，3.95(3 H，s，4-MeO)；MS (EI) m/z (relative intensity) 354 (100) [M^+]，356 (160) [M^++2]，358 (105) [M^++

4], 360 (38) [M^++6], 339 (62) [M^+−CH_3], 311 (29) [M^+− $COCH_3$], 241 (25).

5-MeO-CB 105: ^{1}H-NMR (500 MHz, chloroform-*d*) δ 7.530 (1 H, d, J=8.33 Hz, 5'-H), 7.488(1 H, d, J=2.02 Hz, 2'-H), 7.251(1 H, dd, J=8.33 Hz, 2.02 Hz, 6'-H), 6.774 (1 H, s, 6-H); MS (EI) m/z (relative intensity) 354 (100) [M^+], 356 (162) [M^++2], 358 (83) [M^++4], 360 (30) [M^++6], 311 (32) [M^+−$COCH_3$].

6-MeO-CB 105: ^{1}H-NMR(500 MHz, chloroform-*d*) δ 7.622 (1 H, d, J=8.07 Hz, 5'-H), 7.423 (1 H, d, J=2.02 Hz, 2'-H), 7.159 (1 H, dd, J=8.07 Hz, 2.02 Hz, 6'-H), 7.101 (1 H, s, 5-H); MS (EI) m/z (relative intensity) 354 (100) [M^+], 356 (148) [M^++2], 358 (105) [M^++4], 360 (37) [M^++6], 304 (87) [M^+−CH_3Cl], 284 (24) [M^+−Cl_2], 241 (31).

4'-MeO-2,3,4,3',5'-PenCB: ^{1}H-NMR (500 MHz, chloroform-*d*) δ 7.445 (1 H, d, J=8.33 Hz, 5-H), 7.325 (2 H, s, 2'-H and 6'-H), 7.147(1 H, d, J=8.33 Hz, 6-H), 3.967(3 H, s, CH_3); MS (EI) m/z (relative intensity) 354 (100) [M^+], 356 (162) [M^++2], 358 (105) [M^++4], 360 (40) [M^++6], 339 (65) [M^+−CH_3], 311 (30) [M^+−$COCH_3$], 241 (50).

5'-MeO-CB 105: ^{1}H-NMR(500 MHz, chloroform-*d*) δ 7.454 (1 H, d, J=8.32 Hz, 5-H), 7.175 (1 H, d, J=8.32 Hz, 6-H), 7.086 (1 H, d, J=2.02 Hz, 2'-H), 6.845 (1 H, d, J=2.02 Hz, 6'-H); MS (EI) m/z (relative intensity) 354(100) [M^+], 356(173) [M^++2], 358(100) [M^++4], 360 (42) [M^++6], 311 (29) [M^+−$COCH_3$], 241 (30).

2. 薬物投与

Golden syrian 系雄性ハムスター (体重約 80 g) および Wistar 系雄性ラット (体重約 170 g) に, P 450 誘導剤として PB(80 mg/kg/day), あるいは MC (20 mg/kg/day) を 3 日間腹腔内に投与した. PB は生理食塩水に, MC はコーン油に溶解した. PB および MC の最終投与日の翌日に, CB 105 を 3 mg/body で腹腔内に投与した.

3. 代謝物の分離

CB 105 投与後 5 日間の糞を採取し, 60 °C で 24 時間乾燥し, コーヒーミルで粉砕後, ソックスレー抽出器を用いて, クロロホルムで 14 時間抽出を行った.

一方, 採血は, CB 105 投与後 5 日目にハムスターあるいはラットの頸動脈より行った. 血液を直接, セパクリーン-A (血清分離剤入スピッツ: 栄研機材) に入れて, 血清を分離し, これにつき代謝物の分析を行った. すなわち, 血清 0.5 ml に 0.5 M 硫酸溶液 0.25 ml を添加した後, クロロホルム-メタノール (2 : 1) 1 ml 及び n-ヘキサン 3 ml による抽出を 2 回行い, これらをジアゾメタンでメチル化後, GC/ECD および GC/MS に供した.

4. 代謝物の分析

代謝物の分析には ECD 付 HP 5890 Series II ガスクロマトグラフ (Hewlett-Packard 製) を用いた. GC/ECD の条件は次の通りである. 3 種類のカラム, すなわち, ① DB-1 fused silica capillary column (15 m×0.25 mm i.d., 0.25 μm 膜厚, J&W Scientific 製), ② DB-1 fused silica capillary column (30 m×0.25 mm i.d., 0.25 μm 膜厚, J&W Scientific 製) および ③ MSP 50 fused silica capillary column (50 m×0.25 mm i.d., 0.1 μm 膜厚, 東京化成製) を使用した. オーブン温度は 200〜240 °C で, さらに注入口温度は 250 °C, 検出器温度は 250°C に固定し, キャリアーガスとして N_2 (1 ml/min) を用いた.

5. その他の分析機器

(1) GC/MS

マススペクトルの測定は質量分析計付 HP 5980 ガスクロマトグラフ (Hewlett-Packard 製) を用いて, EI モードで行った. GC/MS は次の 2 通りの条件を用いて行った. ① カラム, DB-1 fused silica capillary column (15 m × 0.25 mm i.d., 0.25 μm 膜厚, J&W Scientific 製); オーブン温度, 190 °C; 注入口温度, 250 °C; 検出器温度, 280 °C; キャリアーガス, He (1 ml/min). ② カラム, MPS 50 fused silica capillary column (50 m x 0.25 mm id, 0.1 μm 膜厚, 東京化成製)); オーブン温度, 100 °C→(20 °C/min)

→ 200 °C→ (5 °C/min) → 280 °C；キャリアーガス，He gas.

(2) ^{1}H-NMR

スペクトルの測定は日本電子製の JEOL GSX-500 (500 MHz) を用いて行った．試料は，重クロロホルムに溶解し，テトラメチルシランを内部標準物質として用いた．

実 験 結 果

1．ラット糞中および血中代謝物の検出

まず，CB 105 投与ラットにおける 5 日間の糞中代謝物を調べた．糞のクロロホルム抽出物をメチル化後，GC/ECD (15 m DB-1 カラム) に供した結果，未処理群において，未変化体 (7.79 min) 以外に，代謝物と思われる 3 本のピークがそれぞれ保持時間 11.82 min，15.85 min および 16.27 min に検出された (Fig. 1 a)．これらを仮に M-1，M-2 および M-3 とする．また，PB 及び MC 前処理群のいずれにおいても未処理群と全く同様に 3 種類のピークが検出された．一方，CB 105 投与後 5 日目の血中代謝物を同じ条件で分析したところ，いずれの群においても未変化体以外に，M-1 と全く同じ保持時間 11.82 min に代謝物と思われる 1 本のピークが検出された．これを以下 M-4 とした (Fig. 1 b)．

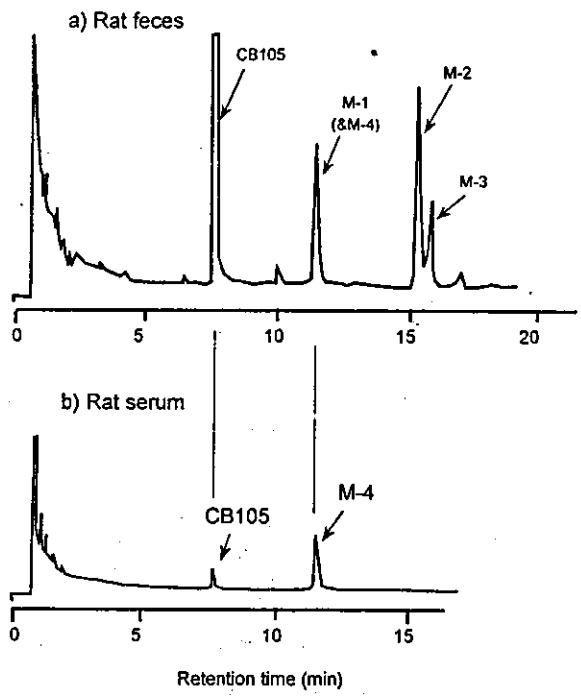

Fig. 1　Gas chromatograms of the methylated derivatives of CB105 metabolites detected in the feces (a) and serum (b) of untreated rats.

2．ハムスター糞中および血中代謝物の検出

次に，未処理，PB および MC 前処理ハムスターにつき，CB 105 投与後 5 日間の糞中代謝物および 5 日目の血中代謝物を調べた．Fig. 2 に未処理ハムスターから得られた代謝物 (メチル化体) のガスクロマトグラムを示す．Fig. 2 a に示すように，糞中代謝物として M-2 および M-3 はラットと同様に検出されたが，M-1 はほとんど検出されなかった．一方，血中代謝物の場合，ラットと同様に，未変化体とともに M-4 が検出された (Fig. 2 b)．なお，PB および MC 前処理ハムスターの場合にも，未処理ハムスターと同じ代謝パターンを示した (データ未掲載)．

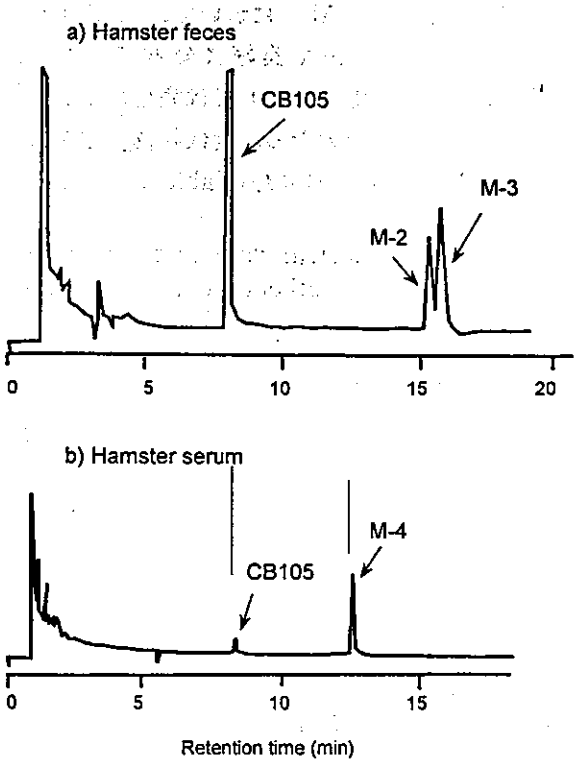

Fig. 2　Gas chromatograms of the methylated derivatives of CB105 metabolites detected in the feces (a) and serum (b) of untreated hamsters.

3．代謝物の化学構造

GC/MS の結果，M-1～M-4 のメチル誘導体はいずれも分子量 354 を有していたことから，OH 体であることが明らかになった．このうち糞中代

謝物 M-1 および血中代謝物 M-4 は保持時間がいずれも 11.82 min と全く同じであることから，本当に同一のものであるかを検討した．長さ 15 m の DB-1 カラムを用いた GC/MS の結果から，M-1 および M-4 のマススペクトルはいずれも破片イオン [M^+-15] を示したことから，4 または 4'位の塩素が 5 または 5'位に NIH 転位をしたと思われる 4-OH 体あるいは 4'-OH 体のメチル誘導体であると推定された (Table 1)．そこで，合成標品の 4-MeO-2,3,3',4',5-PenCB と 4'-MeO-2,3,3',4,5'-PenCB を同様の条件で測定したところ，DB-1 (15 m) カラムにおける保持時間およびマススペクトルが全く同じで区別できなかった．次に，長さ 50 m の MPS 50 カラムに換えて両化合物の分離を試みた．その結果，Fig. 3 に示すように，4-MeO 体と 4'-MeO 体をそれぞれ保持時間 29.88 min と 30.12 min に分離することができた．そこで，血中代謝物 M-4 のメチル化体につき，同様の条件で測定したところ，保持時間 29.88 min であった．これらの結果から，M-4 および M-1 はそれぞれ 4-OH-2,3,3',4',5-PenCB および 4'-OH-2,3,3',4,5'-PenCB であることが示唆された．

一方，M-2 および M-3 については，DB-1 カラムでは長さ 15～30 m で良好に分離できたが，MPS 50 カラム (長さ 50 m) では完全に重なった (データ未掲載)．そのため，両代謝物の分析には，以下 DB-1 カラムを用いた．Table 1 に示したように，マススペクトルをみると M-2 および M-3 のいずれにおいても特徴的なイオン [M^+-43] が観察されたことから，5-OH 体あるいは 5'-OH 体のメチル誘導体と推定された．そこで，合成標品とマススペクトルおよび GC における保持時間を比較したところ，M-2 および M-3 はそれぞれ5'-OH-CB 105 及び 5-OH-CB 105 であることが明らかとなった．

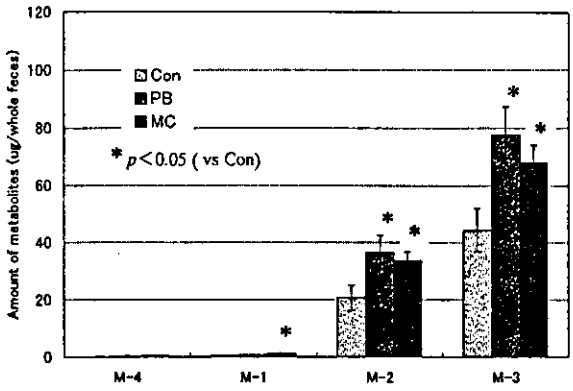

Fig. 4 Effect of P450 inducer on the amount of CB105 metabolites in hamster feces

3．糞中代謝物に及ぼす P 450 誘導剤の影響

Fig. 4 に，ハムスター糞中代謝物の定量結果 (μg/全糞) を示した．M-1 と M-4 の定量には MPS 50 カラム (長さ 50 m) を，また M-2 および M-3 の定量には DB-1 カラム (長さ 30 m) を使用した．まず，未処理ハムスター糞中代謝物を調べたところ，それらの生成比は，M-4：M-1：M-2：

Table 1 Mass spectral data and retention times of the methylated CB105 metabolites and the synthetic authentic compounds in GC/MS

Compound	Molecular weight	Mass spectral data					Retention time (min)
		[M^+]	[M^+-15]	[M^+-43]	[M^+-50]	[M^+-70]	
CB105(feces)	324	100	-	-	-	45	6.41
M-1 (feces)	354	100	62	41	-	-	10.17
M-2 (feces)	354	100	-	44	-	-	14.00
M-3 (feces)	354	100	-	26	-	-	14.45
M-4 (serum)	354	100	55	31	-	-	10.17
2'-CH_3O-CB105	354	100	-	-	144	31	8.10
4-CH_3O-2,3,3',4',5-PenCB	354	100	62	29	-	-	10.17
4'-CH_3O-2,3,3',4,5'-PenCB	354	100	65	30	-	-	10.17
6-CH_3O-CB105	354	100	-	-	87	24	10.94
5'-CH_3O-CB105	354	100	-	26	-	-	14.01
5-CH_3O-CB105	354	100	-	32	-	-	14.46

GC/MS： column (DB-1, 15m)； oven temp.(190°C).

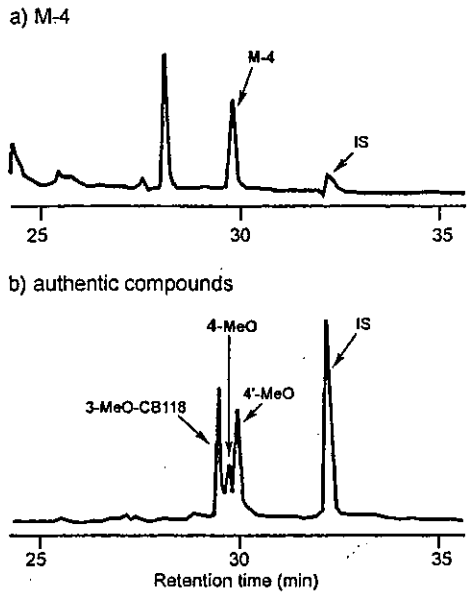

Fig. 3 Gas chromatograms of the methylated M-4 detected in the serum (a) and of three authentic compounds (b). 3'-MeO-2,2',3,4,4',5-hexachlorobiphenyl was used as an internal standard (IS).

M-3 = 0.2：1：39：84 であり，M-4 はほとんど検出されなかった．また，主代謝物は M-2 と M-3 であったが，M-3 の方が M-2 の約 2 倍と多かった．さらに，P 450 誘導剤前処理ハムスターでは，4 種類の代謝物はいずれも PB 前処理で未処理の 1.7 倍前後に，また MC 前処理では 1.5 倍前後というように，ほぼ同じ割合で有意に増加していた．なお，M-4 および M-1 に関しては，PB よりは MC 前処理でより多く増加した．

次に，ラット糞中代謝物の定量結果 (μg/全糞) を Fig. 5 に示した．未処理ラットの糞中代謝物の生成比は M-4：M-1：M-2：M-3 = 0.7：1：19：6.7 であり，M-2 が最も多く生成されていた．また，P 450 誘導剤前処理ラットでは，4 種類の代謝物はいずれもほぼ同じ割合で生成量が増加しており，PB 前処理で未処理の 2～3 倍，また MC 前処理では約 3 倍であった．ただし，M-4 の生成は MC 前処理で約 7 倍に顕著に増加していた．

4．血中代謝物に及ぼす P 450 誘導剤の影響

CB 105 投与後 5 日目の血中代謝物を定量し，ラットとハムスターで比較した (Fig. 6)．その結果，ラット血中の M-4 濃度は未処理で 0.28 μg/ml serum であったが，PB 前処理により全く影響を受けなかった．また，MC 前処理でも未処理の約 1.4 倍に過ぎなかった．一方，ハムスター血中の M-4 濃度は未処理で 0.39 μg/ml serum とラットの 1.4 倍と高かったが，さらに PB 前処理により未処理の 1.8 倍に，MC 前処理で 2.6 倍に高くなっていた．

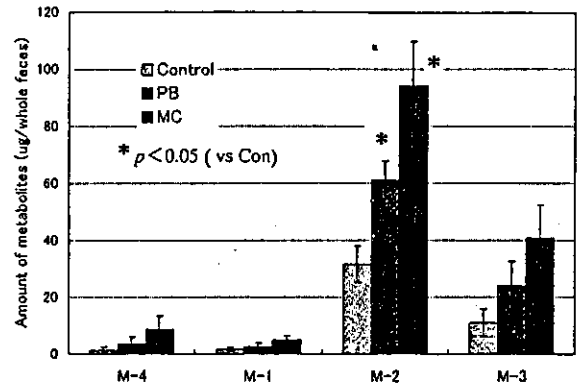

Fig. 5 Effect of P450 inducer on the amount of CB105 metabolites in rat feces

考　察

CB 105 投与ハムスターの糞中および血中代謝物を調べ，代謝パターンをラットと比較した．その結果，両動物においても，糞中から 4 種類の一水酸化体 (M-1～M-4) と血中から M-4 のみが検出された．これらの構造は Klasson-Wehler ら[8] および原口ら[5] の報告と一致して，M-1, M-2, M-3 および M-4 はそれぞれ，4'-OH-2,3,3',4,5'-PenCB，5'-OH-CB 105，5-OH-CB 105 および 4-OH-2,3,3',4',5-PenCB であった．以上の結果から，本 PCB の代謝経路として，Fig. 7 に示すよう

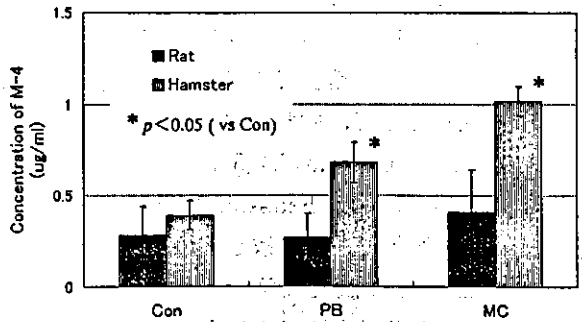

Fig. 6 Effect of P450 inducer on the concentration of M-4 in the serum of rats and hamsters.

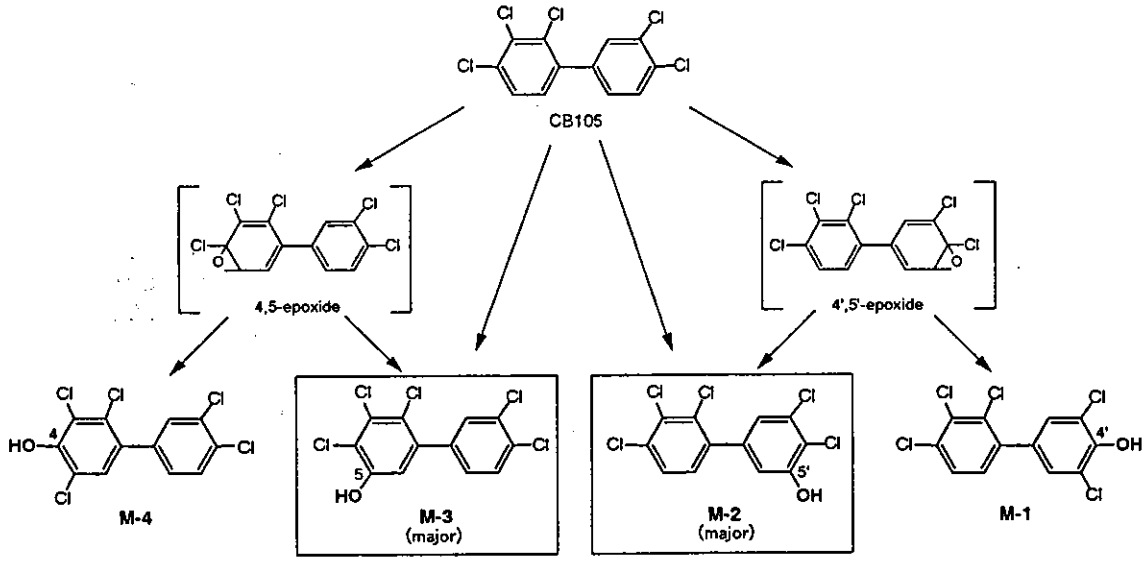

Fig. 7 Postulated metabolic pathways of CB105 in rats and hamsters

に，三塩素置換ベンゼン環の4あるいは5位への酸化および二塩素置換ベンゼン環の4'あるいは5'位への酸化が明らかとなった．主な糞中代謝物は両動物で，M-2 (5'-OH 体) および M-3 (5-OH 体) であった．M-2 と M-3 の生成量をみると，両動物で大きな種差が観察された．すなわち，ラットでは M-2 の方が M-3 の約3倍であったのに対し，ハムスターでは逆に M-3 の方が M-2 の約2倍であった．一方，M-1 および M-4 は，ハムスターでは糞中から痕跡程度しか検出されなかった．また，PB および MC 前処理により数倍に増加はしたものの，M-2 および M-3 の生成量に比較すると数10分の1であった．以上の結果からラットとは逆に，ハムスターでは3',4'-二塩素置換ベンゼン環よりは，2,3,4-三塩素置換ベンゼン環の方が酸化されやすいことが示唆された．また，M-1 および M-4 の生成はアレンオキシド中間体経由した後，NIH 転位したことを示している[10)16)21)]が，今回の結果から両動物において中間体経由よりは直接水酸化反応の方が優位であることが示唆された．

血中代謝物として，両動物において，未変化体以外に M-4 (4-OH-2,3,3',4',5-PenCB) のみが検出された．この事実も，Klasson-Wehler ら[8)]および原口ら[5)]の報告とよく一致していた．ただし，ハムスター血中 M-4 濃度はラットの数倍と高かった．また，この値は PB および MC 前処理でそれぞれ約2倍および約3倍に増加した．Brouwer ら[1)2)4)]は，4-OH-3,5-二塩素置換の PCB，例えば，4-OH-3,3',4',5-TCB 等は血中の甲状腺ホルモン輸送タンパクである transthyretin と高い親和性を有することから，血中によく保持されることを報告している．今回の結果は，生成された M-4 がラットに比べ，より選択的に血中に移行することを意味しており，その原因としてハムスター transthyretin の M-4 に対する親和性がラットより高いのかもしれない．実際，Klasson-Wehler ら[8)]は，M-4 濃度をマウスとミンクで比較し，マウスがミンクの4～5倍高いことを報告している．また，なぜ，M-1 (4'-OH-3',5'-二塩素置換の PCB) よりも，M-4 (4-OH-2,3,5-三塩素置換の PCB) の方が選択的に血中に局在しているかという点は現在のところ，不明である．

原口ら[5)]は，CB 105 投与ラットの血液，肝，肺，腎および脂肪組織中の代謝物を調べたところ，いずれの組織でも M-4 のみを検出し，その濃度は血液＞肝＝肺＞腎＞脂肪組織の順であることを報告した．本研究において，ハムスターでは血液と糞しか調べていないが，ハムスターにおいても，ラットと同様に，主代謝物の M-2 と M-3 は糞中へ，M-4 は血液をはじめとした各組織へと分布することが示唆された．

CB 105 代謝に関与する P 450 分子種についての情報を得るために，PB あるいは MC 前処理して代謝パターンの変動を観察した．一般に，MC 型誘導能を有する coplanar PCB は MC 誘導性 P 450 によって，PB 型誘導能を有する mono-あ

るいはdi-ortho-PCBはPB誘導性P450によって代謝される[17]. CB 105は混合型誘導能を有していることから，両方のP450によって代謝されると予想されたが，今回予想と一致してハムスターの全代謝物はPBおよびMC前処理のいずれでも1.5～1.7倍に増加した．これまでの精製P450を用いたPCB代謝研究[11)-13)]から，ハムスター肝ではPB誘導性のHPB-1 (CYP 2 B)およびMC誘導性のCYP1A2とCYP2A8が関与することが明らかになっているが，CB 105の場合もこれらのP450が有力であろう．一方，ラットにおいても同様に，全代謝物の生成がPBおよびMC前処理で2～3倍に促進された．この結果から，CB 105の場合にも，これまでに二塩素化ビフェニル[7]やTCB[6)12)15)]で明らかにされているPB誘導性のCYP2B1とCYP2B2およびMC誘導性のCYP1A1が関与することが示唆された．なお，M-4およびM-1の生成はMC前処理で強く促進されたことから，ハムスターではCYP1A2とCYP2A8の関与が，またラットではCYP1A1の関与が強く示唆された．

総　括

1．未処理，PBおよびMC前処理のハムスターおよびラットにCB 105 (3 mg/body)を腹腔内に投与し，糞中および血中代謝物の検出・定量を行った．

2．ハムスターおよびラット糞中代謝物として，4種類すなわち，M-1, M-2, M-3およびM-4が検出された．これらは合成標品との比較により，それぞれ4'-OH-2,3,3',4,5'-PenCB, 5'-OH-CB 105, 5-OH-CB 105および4-OH-2,3,3',4',5-PenCBであることが明らかとなった．両動物ともに，主代謝物はM-2とM-3であったが，ハムスターではM-3が，ラットではM-2が多く生成された．また，ラットに比べハムスター糞中ではM-1およびM-4が著しく少なかった．

3．CB 105代謝に及ぼすP450誘導剤の影響を調べたところ，ハムスターの糞中代謝物はPBおよびMC前処理により，いずれも1.5～1.7倍に増加した．ラットでは同様に2～3倍に増加した．

4．ハムスターおよびラット血中代謝物として，未変化体とともにM-4が検出された．次にP450誘導剤の影響を調べたところ，血中M-4濃度はラットであまり変動しなかったのに対し，ハムスターではPB前処理で未処理の1.8倍に，またMC前処理で2.6倍に増加した．

以上の結果から，ハムスターではラットとは逆に，3',4'-二塩素置換ベンゼン環よりは，2,3,4-三塩素置換ベンゼン環の方が酸化されやすいこと，また，生成されたM-4はラットに比べ，より選択的に血中に分布することが示唆された．

謝　辞

本研究は厚生科学研究費補助金（生活安全総合研究）および科学研究費補助金（基盤研究 (C) (2), No. 14572119, No. 15510058）に一部負うものである．ここに記して謝意を表します．

文　献

1) Brouwer A, Klasson-Wehler E, Bokdam M, Morse DC and Traag WA: Competitive inhibition of thyroxin binding to transthyretin by monohydroxy metabolites of 3,4,3',4'-tetrachlorobiphenyl. Chemosphere 20: 1257-1262, 1990.

2) Brouwer A and van den Berg KJ: Binding of a metabolite of 3,4,3',4'-tetrachlorobiphenyl to transthyretin reduces serum vitamin A by inhibiting the formation of the protein complex carrying both retinol and thyroxin. Toxicol Appl Pharmacol 85: 301-312, 1986.

3) Cadogan JIG: A convenient new method of aromatic arylation. J. Chem. Soc.: 4257-4258, 1962.

4) Darnerud PO, Morse D, Klasson-Wehler E and Brouwer A: Binding of a 3,3',4,4'- tetrachlorobiphenyl (CB-77) metabolite to fetal transthyretin and effects on fetal thyroid hormone levels in mice. Toxicology 106: 105-114, 1996.

5) Haraguchi K, Kato Y, Kimura R and Masuda Y: Hydroxylation and methylthiolation of mono-ortho-substituted polychlorinated biphenyls in rats: identification of metabolites with tissue affinity. Chem Res Toxicol 11: 1508-1515, 1998.

6) Ishida C, Koga N, Hanioka H, Saeki KE and Yoshimura H: Metabolism in vitro of 3,4,3', 4'- and 2,5,2',5'-tetrachlorobiphenyls by rat liver microsomes and highly purified cyto-

chrome P-450. J. Pharmacobio-Dyn. 14: 276-284, 1991.
7) Kaminsky LS, Kennedy MW, Adams SM and Guengerich FP: Metabolism of dichlorobiphenyls by highly purified isozymes of rat liver cytochrome P-450. Biochemistry 20: 7379-7384, 1981.
8) Klasson-Wehler E, Lindberg L, Jonsson CJ and Bergman A: Tissue retention and metabolism of 2,3,4,3',4'-pentachlorobiphenyl in mink and mouse. Chemosphere 27: 2397-2412, 1993.
9) Kociba RJ and Cabey O: Comparative toxicology and biologic activity of chlorinated dibenzo-p-dioxins and furans relative to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Chemosphere 14: 649-660, 1985.
10) Koga N, Beppu M and Yoshimura H: Metabolism in vivo of 3,4,5,3',4'-pentachlorobiphenyl and toxicological assessment of the metabolite in rats. J. Pharmacobio-Dyn. 13: 497-506, 1990.
11) Koga N, Kikuichi N, Kanamaru T, Ariyoshi N, Oguri K and Yoshimura H: Hamster liver cytochrome P450 (CYP2A8) as a 4-hydroxylase for 2,5,2',5'-tetrachlorobiphenyl. Biochem. Biophys. Res. Commun. 225: 685-688, 1996.
12) Koga N, Kikuichi N, Kanamaru T, Kuroki H, Matsusue K, Ishida C, Ariyoshi N, Oguri K and Yoshimura H: Metabolism of 2,3',4',5-tetrachlorobiphenyl by cytochrome P450 from rats, guinea pigs and hamsters. Chemosphere 37: 1985-1904, 1998.
13) Koga N, Kikuichi-Nishimura N, Hara T, Harada N, Ishii Y, Yamada H, Oguri K and Yoshimura H: Purification and characterization of a newly identified isoform of cytochrome P450 responsible for 3-hydroxylation of 2,5,2',5'-tetrachlorobiphenyl in hamster liver. Arch. Biochem. Biophys. 312: 464-470, 1995.
14) Koga N, Kikuichi-Nishimura N and Yoshimura H: Effect of cytochrome P450 inducers on liver microsomal metabolism of tetrachlorobiphenyls in rats, guinea pigs and hamsters. Biol. Pharm. Bull. 18: 705-710, 1995.
15) Koga N, Nishimura N, Kuroki H, Masuda Y and Yoshimura H: Metabolism of 3,5,3',5'-tetrachlorobiphenyl by rat liver microsomes and purified P4501A1. Xenobiotica 24: 775-783, 1994.
16) Koga N, Shin'yama A, Ishida C, Hanioka N and Yoshimura H: A new metabolite of 2,4,3',4'-tetrachlorobiphenyl in rat feces. Chem. Pharm. Bull. 40: 3338-3339, 1992.
17) 古賀信幸，吉村英敏：PCB と関連化学物質の代謝並びに代謝物の毒性．小栗一太，赤峰昭文，古江増隆編：油症研究 −30 年の歩み−，pp 93-110，九州大学出版会，2000.
18) Kuroki H and Masuda Y: Structures and concentrations of the main components of polychlorinated biphenyls retained in patients with Yusho. Chemosphere 6: 469-474, 1997.
19) Parkinson A, Safe SH, Robertson LW, Thomas PE, Ryan DE, Reik LM and Levin W: Immunochemical quantitation of cytochrome P-450 isozymes and epoxide hydrolase in liver microsomes from polychlorinated or polybrominated biphenyl-treated rats. - A study of structure-activity relationships. J. Biol. Chem. 258: 5967-5976, 1983.
20) Yamamoto H, Yoshimura H, Fujita M and Yamamoto T: Metabolic and toxicologic evaluation of 2,3,4,3',4'-pentachlorobiphenyl in rats and mice. Chem. Pharm. Bull. 24: 2168-2174, 1976.
21) Yoshimura H, Yonemoto Y, Yamada H, Koga N, Oguri K and Saeki S: Metabolism in vivo of 3,4,3',4'-tetrachlorobiphenyl and toxicological assessment of the metabolites in rats. Xenobiotica 17: 897-910, 1987.
22) Yoshimura H, Yoshihara S, Ozawa N and Miki M: Possible correlation between induction modes of hepatic enzymes by PCBs and their toxicity in rats. Ann. N. Y. Acad. Sci. 320: 179-192, 1979.

(受付 2003-3-31)

Pure Appl. Chem., Vol. 75, Nos. 11–12, pp. 2555–2561, 2003.

Workshop 6.2

Hormonally active agents and plausible relationships to adverse effects on human health*

Tohru Inoue‡

Center for Biological Safety and Research, National Institute of Health Sciences, 1-18-1 Kamiyohga, Setagaya-ku, Tokyo 158-8501, Japan

Abstract: A hormonally active compound was first identified in the book *Silent Spring* by Rachel Carson in 1962, implicating the effect of pesticides such as DDT and the derivatives. Nearly four decades later, the book *Our Stolen Future* by Theo Colborn et al., and other pertinent publications have revisited and broadened the issue regarding a variety of possible chemicals and the area exposed. Translation and publication became available in Japan within the last four years. Since then, Japan joined the member countries involved in the global issue of endocrine disruptors, the "environmental hormone".

Although a significant number of chemicals possessing a hormone-like action have been recognized for many years, and the action of their biological plausibility related to the receptor-mediated effects strongly suggests possible human effects comparable to hormonal changes in wildlife, little is known about evidences or adversities in experimental animals and humans. The most essential key to resolving these dilemmas may be to understand the mechanism of actions (i.e., a possible low-dose issue). In other words, the mechanism at the low-dose effect may be resolved simultaneously by the mechanism of three major questions linked to the low-dose issue; namely, threshold, possible oscillation, and additive and/or synergistic action.

INTRODUCTION

The objective of this paper is to summarize all currently available information on hormonally active agents and plausible relationships to adverse effects on human health from the standpoints of the mechanisms of action of these chemicals.

It is not uncommon to come across agrochemicals and industrial chemicals that have hormone-mimic effects. These chemicals, the so-called "environmental hormones", often accumulate at detectable levels in the environment, and it has been feared that they may have adverse effects not only on wildlife but also on human beings. Following reports of feminization and decreased colony size of wild creatures, and reports suggesting a possible association of these chemicals with abnormalities of reproductive organs and oncogenesis in humans, attention has focused on the possibility that these occurrences may be associated with exposure to endocrine-disrupting chemicals (EDCs). In this connection, we would like to draw the attention of the reader to a Japanese translation of the book *Our Stolen Future*, written by Theo Colborn et al.

*Report from a SCOPE/IUPAC project: Implication of Endocrine Active Substances for Human and Wildlife (J. Miyamoto and J. Burger, editors). Other reports are published in this issue, *Pure Appl. Chem.* 75, 1617–2615 (2003).

‡Tel.: +81-3-3700-1564; Fax: +81-3-3700-1622; E-mail: tohru@nihs.go.jp

This paper will review the subjects related to EDCs, the courses of arguments regarding the possible hazards of these chemicals, and current medical subjects pertaining to them.

CHEMICALS WITH HORMONE-MIMIC ACTIONS

Substances with hormone-mimic effects can be divided into four groups:

- hormones found in vivo;
- medicines with hormone-mimic actions manufactured for use in hormonal therapy, etc.;
- plant hormones known to exert phytoestrogen-like actions; and
- chemicals found in environments that can interact with hormone receptors.

In addition, substances that do not interact with hormone receptors but exert effects on gonads by their modifying effects on steroid metabolism may be deemed as hormone-mimics in the broader sense of the term. In this paper, however, emphasis shall be placed on the hormone-mimic actions mediated by receptors that play essential roles in the mechanism of actions of hormone-mimics.

CHARACTERISTICS OF THE RECEPTOR-MEDIATED ACTIONS OF HORMONE-MIMICS

The receptor-mediated actions of hormone-mimics are fundamentally characterized by the similarity in structures of the receptors involved, crossing the barrier of animal species. These characteristics allow us to speculate the possibility that the actions of these chemicals exerted in nature may also occur in humans.

Second, since similarities in the structure of various sex steroids and hormones are also known, it is possible that each individual hormone-mimic exerts diverse effects by acting on male hormone receptors, female hormone receptors, and nuclear receptors (including many orphan receptors), etc.

Third, many of these chemicals are excreted from the living body in the form of conjugated inactive substances instead of as degraded metabolites. They may also be eliminated in the unchanged form. Therefore, if feces and urine containing these substances are eliminated into river water, it is plausible to imagine that even inactivated hormones can sometimes become active and exert hormone-mimic actions in the environment. This is one of the characteristics unique to this class of chemicals.

Receptor-mediated responses involve many unresolved questions. Various undefined elements may be involved, including the relationship between receptor binding and signals, the relationship between receptor-ligand binding (ligand: substances that can bind to receptors) and the dissociation of ligands from receptors, signal cross-talks, involvement of unknown nuclear receptors, etc.

The actions of these chemicals add to the effects of intrinsic hormones. For this reason, these chemicals may exert their actions in a way different from that known for other chemicals that do not have structural or functional counterparts in vivo. For example, stimulation of hormone receptors by these extrinsic chemicals may modify homeostasis in vivo, leading to down-modulation of the physiological stimulation of these receptors by the intrinsic ligands. Therefore, the influence of the continued effects of environmental hormones needs special study.

PITFALL IN THE EFFECTS OF HORMONE-MIMICS

We must distinguish the interactions of endocrine hormone-mimics with hormone receptors from the hazards caused to endocrine tissue. Bearing this in mind, let us now summarize the problems related to the effects of hormone-mimics.

Antagonistic effects maintaining homeostasis

The endocrine system is regulated by homeostatic mechanisms. It is not uncommon for the effects of small amounts of hormone-mimics to interfere slightly with these mechanisms, often with no adverse influence; this is well known. However, this is not always the case. There seems to be a group of genes that act antagonistically to each other in the maintenance of homeostasis.

With the uterotrophic assay, which is used to check for estrogenic activity, the ovary is removed in advance and the blood level of the intrinsic female hormone is reduced to the minimum. Under the thus-created extremely shrinking state of the uterus, the test substance (a chemical or hormone) is administered to evaluate for its effects on the inflation of the uterus. This test (checking for growth of the uterus in ovariectomized animals) is designed to evaluate the hormone activity and effects of hormone-mimics under conditions of blockade of homeostasis.

This test method itself is valid. However, there is no sufficient rational evidence that indicates that the responses observed under such indirect control conditions of the living body can serve as an indicator of the health hazards of hormone-mimics. Although the ovo-testes seen in lower vertebrates may be used if the effects observed were to be valid as such an indicator, there is no consensus on what is valid as an indicator of the health hazards of EDCs when mammals are used as experimental animals.

Down-regulation of the expression of receptors

It is known that the expression of gene-encoding receptors is down-regulated by continuous stimuli, leading to reduced receptor activity. This can lead to a paradoxical outcome wherein the effects observed in the presence of low levels of a substance are not seen at high levels of the same substance. If this phenomenon occurred in individual organisms, the dose–response relationship will be nonlinear.

This means that extrapolation of results obtained at high levels of the chemicals, to conditions where low levels of the same substance are present, would be difficult. It is needed to test the validity of this hypothesis; analysis of the mechanisms underlying this phenomenon if the hypothesis were indeed valid, are thus important. Studies to resolve these questions are now under way.

Data gap concerning the effects of female hormones

In mature women, there are high levels of physiological hormones in vivo, and these are subject to cyclic control. It has been proposed that girls with inadequate physical growth begin menstruation at lower ages and undergo sexual maturation earlier than usual, and that hormone-mimics in these subjects can precipitate breast cancer.

The weak links in this hypothesis have been pointed out, and it has been shown experimentally that estrogen by itself may be teratogenic, although this tendency has been shown to be weak. It is known that organisms are programmed such that excessive exposure to estrogens during the intrauterine period or other developmental stages is avoided.

There are many open questions as to the mechanism by which mature females remain physiologically stable, even when exposed daily to high levels of estrogen (400 pM/l). Some additional dramatic effects may be needed to disturb this homeostatic physiology.

Multigeneration tests and effects on fetuses

It has been shown that exposure to hormones or hormone-mimics during intrauterine or early neonatal periods can lead to irreversible changes in the pattern of development. This susceptibility period is short, extending from the 13th gestational day to about one week after birth. These effects are the so-called "intrauterine window effects."

In animal studies involving observation of experimental animals for two or more generations, no effects of EDCs have been demonstrated. The question therefore arises as to why window effects are observed during the short period mentioned above. It is unknown whether or not these effects really do occur, and if they do, how they are produced.

Delayed growth of the thalamic nucleus specific to males (called sexual dimorphic nucleus) is seen in male rats treated with female hormones. We may say that under conditions of homeostasis of the physiological hormones in mature individuals, exposure to dose levels that usually cause only reversible changes can lead to irreversible changes, if the exposure occurs during genesis, morphogenesis, or functional development. However, there are no ample data endorsing this view in humans.

Considering the biological plausibility inferred from the experimental data accumulated to date*, we may say that there are no sufficient data that clearly rule out this view. Close attention has therefore been paid to these effects in children.

New theories of methodology, focusing on effects in fetuses and children, are now being developed, primarily in the United States, or the World Health Organization, within the framework of children's program, etc.

HEALTH HAZARDS AT LOW LEVELS OF EXPOSURE

Chemicals used for agriculture or industrial purposes are marketed, in general, only after their effects on living beings have been investigated. We may therefore understand that they are used on the premise that the possibility of these chemicals exerting hazardous effects on health at relatively high-dose levels has been almost ruled out. Nevertheless, problems with EDCs have begun to be highlighted. These problems may not be confined to those related to the accumulation of these substances through food chains in the ecosystem, but also to the additional possibility that these chemicals may exert effects at low-dose levels even if they have been declared safe at high-dose levels. The latter possibility may apply, however, only to some cases and not to others.

We may say that a major issue pertaining to EDCs that must be resolved urgently is whether or not they pose health hazards at low-dose levels. This issue can be summarized into the following three questions:

- presence/absence of threshold level;
- presence/absence of synergistic or additive effects; and
- possibility of extrapolation of high-dose effects to low-dose levels (i.e., presence/absence of a linear dose–response relationship).

No clear-cut answers have as yet emerged to these questions. Considering the above-mentioned characteristics of the effects of hormones, it is plausible to imagine how difficult it may be to resolve these questions.

To determine if these chemicals exerted hazardous effects on health at low-dose levels, the following basic questions may need to be considered; their biological plausibility is hardly denied.

- Regarding the presence or absence of threshold levels, it seems likely that many chemicals suspected of being EDCs can easily permeate across the cell membrane, which is composed of phospholipids. Therefore, assuming that one receptor molecule reacts with one chemical molecule, the lower limit of the dose level exerting the chemical's effects would be extremely low.

  Of course, since the probability of the binding of a ligand to the receptor will be low if the dose level is low, we cannot say that there is no threshold level for the effects seen in the low-

*Biological plausibility: Likelihood of a phenomenon as judged by considering the difference or similarity of elements of reactions in individual organisms, on the basis of the results of a series of related biological experiments. (Cf. probability)

dose-level range. In fact, for bisphenol A (which has been attracting close attention because of its hazardous effects on health at low-dose levels), the presence/absence of a threshold level has not yet been reported. It seems rational, therefore, to assume that these health hazards occur in a very low-dose-level range.

- If we consider not only the affinity of each substance for the receptor, but also the nonlinearity of responses (e.g., waveform responses as a result of reduced receptor expression following an increase in dose level), it is possible to assume that there are U-shaped or reverse U-shaped reactions, or oscillational dose–response curves. Interim data endorsing such a view are being accumulated.
- Regarding the possibility of synergistic or additive effects, the observation of additive effects among different nuclear receptors has been reported. Data yielded by analysis of interactions between receptor signals also suggest such a possibility. In fact, the dose–response curves for some composite materials were reported to be additive, but not synergistic.

Thus, the questions on health hazards at low-dose levels have several aspects:

- type of receptor-mediated actions of the hormone mimics;
- diverse reactive characteristics on the part of the receptors;
- diverse modification during expression of intracellular signals; and
- factors involved in irreversible changes related to morphogenesis and functional development.

Resolution of all these aspects of the question will lead to clarification of the mechanism of actions of the substances from each of the aforementioned standpoints. While these questions are among the hottest research themes at present, they are certainly unlikely to be resolved easily.

At a workshop held in North Carolina, USA, in October 2000, health hazards of chemicals at low-dose levels were discussed. Investigators for and against the possibility of these substances posing health hazards at low-dose levels gave detailed accounts of their studies, and no definitive conclusions could be reached, as the arguments of both sides appeared to be tenable.

This means that reports affirming the plausibility of these substances posing health hazards at low-dose levels in animal experiments cannot be immediately rejected. The workshop concluded by pointing out the necessity of paying attention to the possible hazards on fetuses and neonates.

HEALTH HAZARDS OF HORMONE-MIMICS TO HUMANS

The possibility of health hazards of hormone-mimics to human beings have not been supported by adequate epidemiological data, and the number of cases for which the data clearly endorse such effects is quite small. The U.S. National Research Council (NRC) emphasizes the necessity of conducting further epidemiological studies on this topic (NRC, 1999).

In conclusion, this paper summarizes the current knowledge concerning the health hazards of hormone-mimics to humans. Reports dealing with the effects of these substances on humans are confined to those pertaining to the effects of dioxins and polychlorinated biphenyls (PCBs); the validity and usefulness of these results have not yet been established.

The following information is based on case studies conducted to date.

Health hazards of dioxins

Regarding health hazards of dioxins, two-year dosing studies revealed weight loss and liver damage, and three-generation reproductive studies in rats disclosed intrauterine death and a decrease in litter size. Onset of endometriosis in rhesus monkeys has also been reported.

A causal relationship of EDCs to the following episodes in humans has been suggested: biased male-to-female ratio in children born in the dioxin-exposed Seveso area of Italy, and increased inci-