probably reflecting a shift in feeding preferences due to different habitats (migration). The reason for this discrepancy between the bluefin tuna from the coastal areas of Japan and those from the Mediterranean Sea remains unclear. Details of yellowfin and albacore tuna migration around the coastal areas of Japan are unknown.

The level of Hg accumulation is generally positively correlated with trophic level as determined by $\delta^{15}N$ (*24*). In agreement, positive correlations were found between those in the bluefin and yellowfin tuna samples, while negative correlations were found in the albacore tuna samples and cetacean samples as reported previously (*7*). The reason for the discrepancy remains to be elucidated.

Acknowledgments

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Polychlorinated Biphenyl-Mediated Decrease in Serum Thyroxine Level in Rodents

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ABSTRACT:

Effects of Kanechlor-500 (KC500), a commercial polychlorinated biphenyl mixture, on the levels of serum thyroid hormones such as total thyroxine (T_4) and triiodothyronine were examined in male mice, hamsters, rats, and guinea pigs. Four days after a single intraperitoneal injection of KC500, significant decreases in the levels of the serum total T_4 and free T_4 occurred in all the animals examined, whereas a significant decrease in the level of serum triiodothyronine was observed only in guinea pigs among the animals examined. In addition, no significant change in the level of serum thyroid-stimulating hormone was observed in any of the rodents examined. A significant increase in the activity of hepatic T_4-UDP-glucuronosyltransferase after the KC500 administration occurred only in guinea pigs, whereas the increase in the amount of biliary [^{125}I]T_4 glucuronide after an intravenous injection of [^{125}I]T_4 to the KC500-pretreated animals occurred only in rats. On the other hand, in all the rodents examined, KC500-pretreatment promoted the clearance of [^{125}I]T_4 from the serum and led to a significant increase in the steady-state distribution volumes of [^{125}I]T_4. Likewise, its pretreatment raised the concentration ratio (K_p value) of the liver to serum and the liver distribution of [^{125}I]T_4 in all the rodents tested. The present findings indicate that for the first time the KC500-mediated decrease in the serum T_4 level in mice, hamsters, rats and guinea pigs occurs mainly through an increase in the accumulation level of T_4 in the liver.

There are known species differences among experimental animals in responses to polychlorinated biphenyl (PCB)-derived toxicities, including endocrine disruption, impairments of the reproductive and immune systems, and teratogenicity (Safe, 1994). The species differences might be attributed to the differences in the metabolic patterns of PCB congeners and/or the PCB-mediated induction of drug-metabolizing enzymes (Duignan et al., 1987, 1988).

In general, PCBs, including 3,3′,4,4′,5-pentachlorobiphenyl (CB126) and Aroclor 1254, have the ability to decrease serum thyroid hormone levels in rats and mice, and the decreases are thought to occur through the induction of thyroxine (T_4)- UDP-glucuronosyltransferases (UDPGTs) (Barter and Klaassen, 1994; Van Birgelen et al., 1995; Craft et al., 2002), especially UGT1A1 and UGT1A6 (Visser, 1996). However, we have previously found the following: the PCB-mediated reduction of the serum T_4 level in rats and mice is not necessarily correlated with an increase in hepatic T_4 glucuronidation activity (Kato et al., 2003); Kanechlor-500 (KC500) treatment results in a significant decrease in the level of serum total T_4 not only in Wistar rats but also in Gunn rats (UGT1A-deficient Wistar rats) (Kato et al., 2004, 2007); and the KC500-mediated decrease in rats occurs through an increase in the accumulation level of T_4 in the liver rather than an increase in hepatic T_4-UDPGT activity (Kato et al., 2007). However, to date, only limited data are available that would help to explain the mechanism of the PCB-mediated decrease in the level of serum thyroid hormone and its species difference.

In the present study, we examined the KC500-mediated biological alterations, such as decreases in the levels of serum thyroid hormones, induction of hepatic T_4-UDPGT, and increase in hepatic accumulation level of T_4, in mice, hamsters, rats, and guinea pigs. On the basis of the obtained results, a mechanism underlying the PCB-mediated decrease in serum T_4 level was further discussed.

Materials and Methods

Chemicals. Panacete 810 (medium-chain triglycerides) was purchased from Nippon Oils and Fats Co. Ltd. (Tokyo, Japan). The [^{125}I]T_4 [>95% radiochemical pure as determined by high-performance liquid chromatography (HPLC), specific activity: 150 μCi/μg T_4], radiolabeled at the 5′-position of the outer ring, was obtained from PerkinElmer Life and Analytical Sciences (Waltham, MA). The KC500 used in the present experiments contains

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ABBREVIATIONS: PCB, polychlorinated biphenyl; CB126, 3,3′,4,4′,5-pentachlorobiphenyl; T_4, thyroxine; UDPGT, UDP-glucuronosyltransferase; KC500, Kanechlor-500; HPLC, high-performance liquid chromatography; T_3, triiodothyronine; TSH, thyroid-stimulating hormone; TBG, thyroxine binding protein; TTR, transthyretin.

2,2′,5,5′-tetrachlorobiphenyl (5.6% of total PCBs), 2,2′,3,5′,6-pentachlorobiphenyl (6.5%), 2,2′,4,5,5′-pentachlorobiphenyl (10%), 2,3,3′,4′,6-pentachlorobiphenyl (7.4%), 2,3′,4,4′,5-pentachlorobiphenyl (7.7%), 2,2′,3,4,4′,5′-hexachlorobiphenyl (5.6%), and 2,2′,4,4′,5,5′-hexachlorobiphenyl (5.4%) as major PCB congeners (Haraguchi et al., 2005). All of the other chemicals used were obtained commercially at the highest grade of purity.

Animal Treatments. Male ddY mice (28–36 g), male Syrian hamsters (95–120 g), male Wistar rats (160–200 g), and male Hartley guinea pigs (400–540 g) were obtained from Japan SLC, Inc. (Shizuoka, Japan). They were housed three or four per cage with free access to commercial chow and tap water, maintained on a 12-h dark/light cycle (8:00 AM to 8:00 PM light) in an air-controlled room (temperature, 24.5 ± 1°C; humidity, 55 ± 5%), and handled with animal care under the guidelines of the University of Shizuoka (Shizuoka, Japan). Mice received a single intraperitoneal injection of KC500 at the desired doses (6.25, 12.5, 25, 37.5, 50, and 100 mg/kg), and the other animals received an intraperitoneal injection of KC500 (37.5 or 100 mg/kg) dissolved in Panacete 810 (5 ml/kg). Control animals were treated with vehicle alone (5 ml/kg).

In Vivo Study. All animals were killed by decapitation 4 days after the intraperitoneal administration of KC500. The liver was removed, and hepatic microsomes were prepared according to the method of Kato et al. (1995) and stored at −85°C until use. Blood was collected from each animal between 10:30 and 11:30 AM. After clotting at room temperature, serum was separated by centrifugation and stored at −50°C until use.

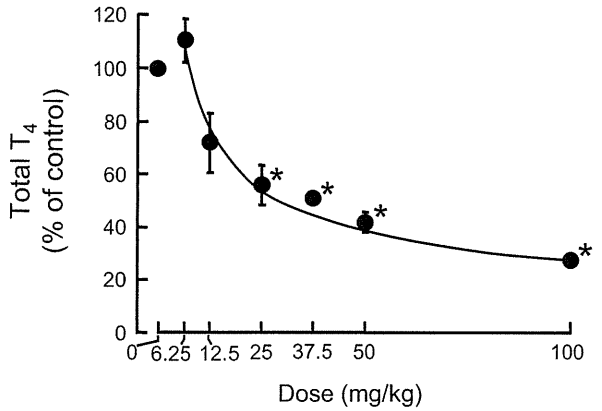

FIG. 1. Effect of graded doses of KC500 on the level of serum total T_4 in mice. Mice were killed 4 days after the intraperitoneal administration of KC500 at the various doses indicated, and the level of serum total T_4 was measured as described under *Materials and Methods*. Constitutive level: 3.24 ± 0.25 μg/dl (n = 5). Each point represents the mean ± S.E. (vertical bars) for four to six mice. *, $P < 0.05$, significantly different from the control.

Analysis of serum hormones. Levels of total T_4, free T_4, total triiodothyronine (T_3), and thyroid-stimulating hormone (TSH) were measured by radioimmunoassay using a Total T4 and Free T4 kit (Diagnostic Products Corporation, Los Angeles, CA), T-3 RIABEAD (Dainabot Co., Ltd., Tokyo, Japan), and the rat TSH [^{125}I] Biotrak assay system (GE Healthcare, Little Chalfont, Buckinghamshire, UK), respectively.

Hepatic microsomal T_4-UDPGT activity. The amount of hepatic microsomal protein was determined by the method of Lowry et al. (1951) with bovine serum albumin as a standard. The activity of microsomal UDPGT toward T_4 (T_4-UDPGT activity) was determined by the methods of Barter and Klaassen (1992).

Ex Vivo Study. Four days after intraperitoneal administration of KC500, the animals were anesthetized with saline solution (2 ml/kg) containing sodium pentobarbital (25 mg/ml) and potassium iodide (1 mg/ml). The femoral artery was cannulated (polyethylene tube SP8, SP10, and SP31; Natsume Inc., Tokyo, Japan) and primed with heparinized saline (33 units/ml). The bile duct was cannulated and then the animal's body was warmed to 37°C. Fifteen minutes later, the animals were given [^{125}I]T_4 (15 μCi /ml i.v.) dissolved in saline containing 10 mM NaOH and 1% normal animal serum. The doses of [^{125}I]T_4 were 0.1 ml for mice, 0.6 ml for hamsters, 1 ml for rats, and 2 ml for guinea pigs, respectively. The dose of [^{125}I]T_4 administered was calculated based on the dose used for rats by Vansell and Klaassen (2001).

Clearance of [^{125}I]T_4 from serum. Clearance of [^{125}I]T_4 from serum was measured according to the method of Oppenheimer et al. (1968). In brief, after the administration of [^{125}I]T_4, a portion (0.1–0.3 ml) of blood was sampled from the artery at the indicated times, and serum was prepared and stored at −50°C until use. Two aliquots (15 μl each) of each serum were used for determination of the level of [^{125}I]T_4 by a gamma counter (Cobra II Auto-Gamma 5002; PerkinElmer Life and Analytical Sciences).

Biliary excretion of [^{125}I]T_4. After the administration of [^{125}I]T_4, bile was collected in glass tubes on ice for 2 h at 30-min intervals. Bile volume was determined gravimetrically. For analysis of biliary total [^{125}I]T_4 level, two aliquots (10–30 μl each) were taken from each bile sample for determination of [^{125}I]T_4 level by a gamma counter (Cobra II Auto-Gamma 5002). The amount of biliary [^{125}I]T_4 glucuronide was determined with HPLC as described by Vansell and Klaassen (2001). In brief, a portion (10–20 μl) of bile was added to 2 volumes of methanol and kept at −20°C for 1 h to precipitate protein. After the mixture was centrifuged at 12,000g (4°C) for 10 min, the resultant supernatant was collected for HPLC analysis. The HPLC analysis was performed by using a ChromSpher C18 column (10 × 0.3 cm; Chrompack, Inc., Raritan, NJ) in combination with both a ChromSep reverse-phase guard column (10 × 2 mm; Chrompack, Inc.) and Adsorbosphere C18 reverse-phase guard column (7.5 × 4.6 mm; Alltech Associates, Deerfield, IL). Then, 0.02 M ammonium acetate, pH4.0, containing 16 to 45% of acetonitrile solution was used for elution of [^{125}I]T_4 glucuronide; 16% of acetonitrile was used as the initial solution for 6 min, and then the elution solution was changed by a linear increase to 27% over 12 min, held for 4 min, followed by a linear

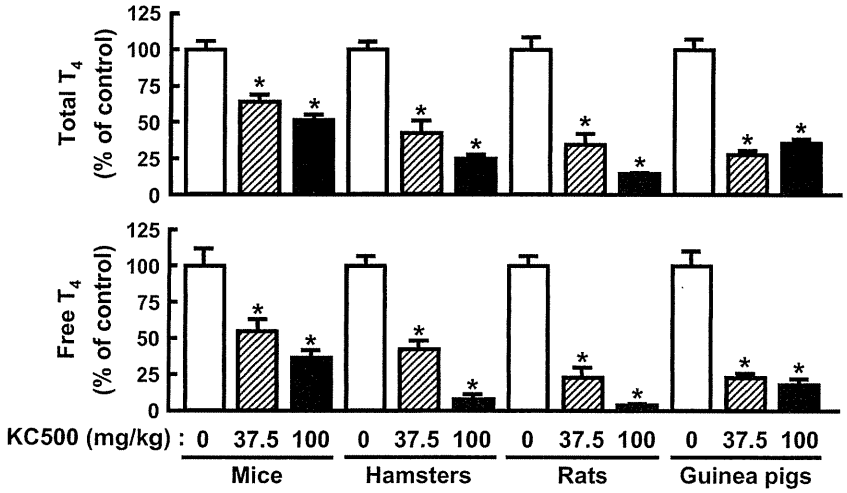

FIG. 2. Effects of KC500 on the levels of serum total T_4 and free T_4 in animals. Animals were killed 4 days after the intraperitoneal administration of KC500 (37 and 100 mg/kg), and levels of serum thyroid hormones were measured as described under *Materials and Methods*. Constitutive levels: total T_4, 2.98 ± 0.17 (mice, n = 8), 2.67 ± 0.17 (hamsters, n = 8), 3.73 ± 0.32 (rats, n = 6), and 2.05 ± 0.16 μg/dl (guinea pigs, n = 5); free T_4, 0.43 ± 0.05 (mice, n = 8), 1.08 ± 0.08 (hamsters, n = 8), 1.47 ± 0.11 (rats, n = 6), and 1.44 ± 0.16 ng/dl (guinea pigs, n = 5). Each column represents the mean ± S.E. (vertical bars) for four to eight animals. *, $P < 0.05$, significantly different from each control.

increase to 45% over 5 min, and held for 11 min. The levels of biliary [^{125}I]T_4 glucuronide were determined by a Radioisotope Detector 171 (Beckman Coulter, Fullerton, CA).

To further identify [^{125}I]T_4 glucuronides, the disappearance of a peak responsible for [^{125}I]T_4 glucuronides by treatment with β-glucuronidase was examined. A portion (100 μl) of bile was incubated for 4 h at 37°C with β-glucuronidase (250 units) in 100 mM phosphate buffer (100 μl, pH 6.8), and the reaction was stopped by addition of 50 μl of methanol and cooling on ice. After the reaction mixture was centrifuged at 12,000*g* (4°C) for 10 min, the resultant supernatant was collected and used for the HPLC analysis of [^{125}I]T_4 derivatives.

Analysis of [^{125}I]T_4 bound to serum proteins. The levels of serum [^{125}I]T_4-thyroxine-binding protein (TBG), [^{125}I]T_4-albumin, and [^{125}I]T_4-transthyretin (TTR) complexes were determined according to the method of Davis et al. (1970). In brief, serum was diluted in 100 mM phosphate buffer, pH 7.4, containing 1 mM EDTA, 1 mM dithiothreitol, and 30% glycerol, and the diluted serum was subjected to electrophoresis on 4 to 20% gradient native polyacrylamide gels (PAG Mid "Daiichi" 4/20; Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan). The electrophoresis was performed at 4°C for 11 h at 20 mA in 0.025 M Tris buffer, pH 8.4, containing 0.192 M glycine. The human albumin and TTR incubated with [^{125}I]T_4 were also applied on the gel as references. After the electrophoresis, the gel was dried and autoradiographed for 20 h at room temperature using Imaging Plate 2040 (Fuji Photo Film Co., Ltd., Tokyo, Japan). The levels of [^{125}I]T_4-TBG, [^{125}I]T_4-albumin, and [^{125}I]T_4-TTR in serum were determined by counting the corresponding gel fractions identified with the Bio Imaging Analyzer (BAS-2000II IP Reader; Fuji Photo Film Co., Ltd.).

Tissue distribution of [^{125}I]T_4. Tissue distribution of [^{125}I]T_4 was assessed according to the modified method of Oppenheimer et al. (1968). In brief, at 60 min after administration of [^{125}I]T_4 to KC500-pretreated animals, blood was sampled from the abdominal aorta. Then, the cerebrum, cerebellum, pituitary gland, thyroid gland, sublingual gland, submandibular gland, thymus, heart, lung, liver, kidney, adrenal gland, spleen, pancreas, testis, prostate gland, seminal vesicle, stomach, duodenum, jejunum, ileum, cecum, brown fat, skeletal muscle, bone marrow, skin, spinal cord, and fat were removed and weighed. Radioactivities in serum and the tissues were determined by a gamma counter (Cobra II Auto-Gamma 5002), and amounts of [^{125}I]T_4 in the tissues were calculated as ratios to the amount in serum.

Statistics. The data obtained were statistically analyzed according to the Student's *t* test or Dunnett's test after analysis of variance. In addition, clearance of [^{125}I]T_4 from the serum, amount of biliary [^{125}I]T_4 glucuronide, and the binding level of [^{125}I]T_4 to serum proteins were statistically analyzed according to the Newman-Keuls test after analysis of variance. The pharmacokinetic parameters of [^{125}I]T_4 were estimated with noncompartmental methods as described previously (Tabata et al., 1999).

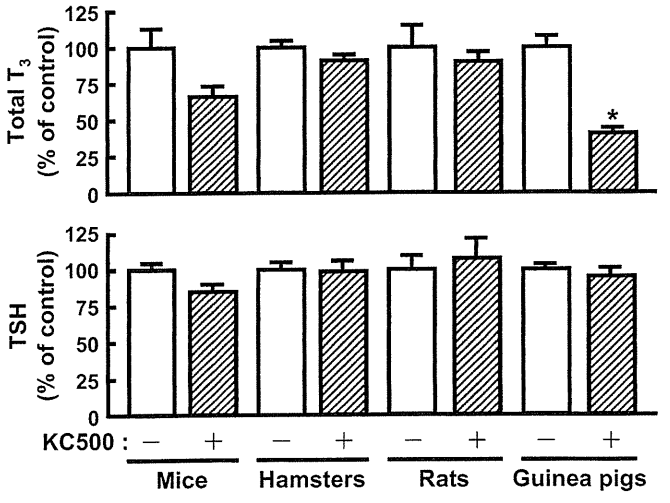

FIG. 3. Effects of KC500 on the levels of serum total T_3 and TSH in animals. Animals were killed 4 days after the intraperitoneal administration of KC500 (37 mg/kg), and levels of serum thyroid hormones were measured as described under *Materials and Methods*. Constitutive levels: total T_3, 0.38 ± 0.05 (mice, n = 6), 0.46 ± 0.02 (hamsters, n = 6), 0.59 ± 0.09 (rats, n = 6), and 0.25 ± 0.02 ng/ml (guinea pigs, n = 6); TSH, 3.50 ± 0.19 (mice, n = 6), 3.46 ± 0.22 (hamsters, n = 6), 5.45 ± 0.61 (rats, n = 6), and 2.40 ± 0.07 ng/ml (guinea pigs, n = 5). Each column represents the mean ± S.E. (vertical bars) for five to six animals. *, P < 0.05, significantly different from each control.

Results

Serum Hormone Levels. A dose effect of KC500 on the level of serum total T_4 was first examined in mice 4 days after the chemical treatment (Fig. 1). Serum total T_4 levels were significantly decreased by the treatment with KC500 at doses of over 25 mg/kg, and the decrease occurred in a dose-dependent fashion up to 100 mg/kg. The 50% effective dose of KC500 for decreasing the level of serum total T_4 was approximately 37.5 mg/kg. Therefore, 37.5 and/or 100 mg/kg were selected as doses of KC500 in the present experiments.

Treatments of mice, hamsters, rats, and guinea pigs with KC500 at a dose of 37.5 mg/kg decreased the total T_4 levels to 63, 43, 35, and 27%, respectively, of the corresponding controls (Fig. 2). Treatment at a dose of 100 mg/kg resulted in a more effective decrease in the

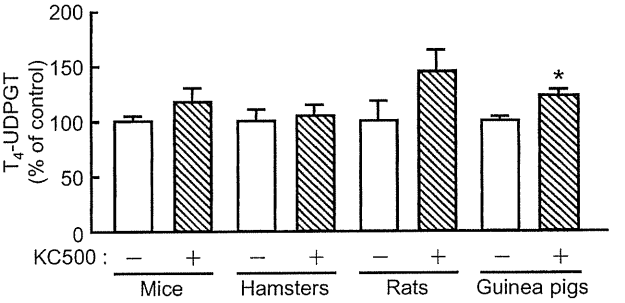

FIG. 4. Effect of KC500 on the activity of hepatic microsomal T_4-UDPGT in animals. Animals were killed 4 days after the intraperitoneal administration of KC500 (37 mg/kg), and hepatic microsomes from individual animals were used for the T_4-UDPGT enzyme assay, as described under *Materials and Methods*. Constitutive levels: T_4-UDPGT, 21.22 ± 1.02 (mice, n = 6), 21.83 ± 2.36 (hamsters, n = 6), 15.39 ± 2.96 (rats, n = 6), and 25.15 ± 1.04 pmol/mg of protein/min (guinea pigs, n = 6). Each column represents the mean ± S.E. (vertical bars) for five to six animals. *, P < 0.05, significantly different from each control.

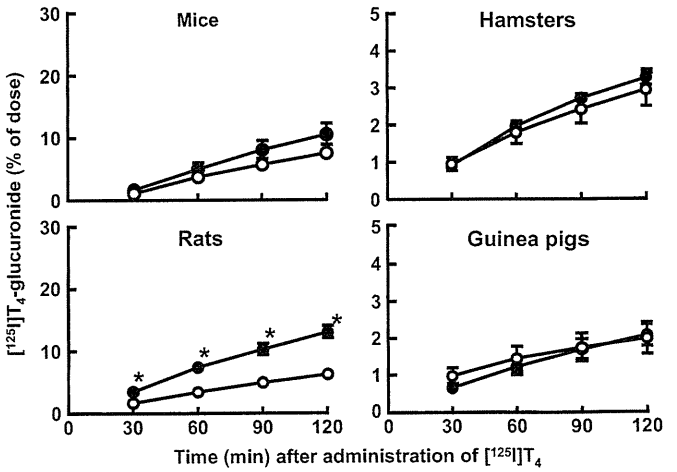

FIG. 5. Effect of KC500 on the amount of the biliary [^{125}I]T_4 glucuronide in animals. KC500 (37.5 mg/kg) was intraperitoneally given to animals, and 96 h after the KC500 treatment, a portion of [^{125}I]T_4 (15 μCi/ml i.v.) was administered to animals, as described under *Materials and Methods*. The level of [^{125}I]T_4 glucuronide excreted was measured in bile collected at 30-min intervals after the intravenous administration of [^{125}I]T_4. Each point represents the mean ± S.E. (vertical bars) for three to seven animals. *, P < 0.05, significantly different from each control. —○—, control; —●—, KC500.

rodents, with the exception of guinea pigs. Likewise, the serum free T_4 level was markedly decreased by KC500 (37.5 mg/kg) in all the rodents examined: 56% of control in mice, 43% of control in hamsters, 24% of control in rats, and 24% of control in guinea pigs. The decrease in each animal species was greater when the KC500 treatment was used at the higher dose (100 mg/kg).

A significant decrease in serum total T_3 level by the treatment with KC500 at a dose of 37.5 mg/kg occurred only in guinea pigs among the species of animals examined (Fig. 3). On the other hand, no significant change in the level of serum TSH by the KC500 treatment was observed in any animals examined (Fig. 3).

Hepatic T_4-UDPGT. The effects of KC500 on hepatic microsomal T_4-UDPGT activity were examined in mice, hamsters, rats, and guinea pigs. A significant increase in the activity of hepatic T_4-UDPGT by the treatment with KC500 at a dose of 37.5 mg/kg was observed in only guinea pigs among the rodents examined (Fig. 4).

Biliary Excretion of [^{125}I]T_4 Glucuronide. Effects of pretreatment of KC500 (37.5 mg/kg) on biliary excretion of T_4 glucuronide were examined in mice, hamsters, rats, and guinea pigs. After intravenous injection of [^{125}I]T_4 to the KC500-pretreated animals, biliary excretion levels of T_4 glucuronide were measured. A significant increase in the biliary excretion level was observed only in rats among the species of animals examined (Fig. 5).

Clearance of [^{125}I]T_4 from Serum. After an intravenous administration of [^{125}I]T_4 to the KC500 (37.5 or 100 mg/kg)-pretreated mice, hamsters, rats, and guinea pigs, serum concentrations of [^{125}I]T_4 in the animals were measured at the indicated times (Fig. 6). KC500 (100 mg/kg)-pretreatment clearly enhanced the clearance of [^{125}I]T_4 from the serum in all the animals tested. Within 5 min after the administration of [^{125}I]T_4, concentration of serum [^{125}I]T_4 in mice, hamsters, rats, and guinea pigs were approximately 69, 32, 28, and 54% of the corresponding control levels, respectively, and the decreases remained up to 120 min later. When the animals were pretreated with KC500 at a dose of 37.5 mg/kg, clear promotion of the clearance of [^{125}I]T_4 was observed in the animals with the exception of mice.

The serum pharmacokinetic parameters of the [^{125}I]T_4 estimated from these data (Fig. 6) were summarized in Table 1. The mean total body clearance of [^{125}I]T_4 and steady-state volumes of distribution in the KC500 (100 mg/kg)-pretreated mice, hamsters, rats, and guinea pigs increased, compared with the corresponding control animals, although these increases were observed in the KC500 (37.5 mg/kg)-pretreated rats and guinea pigs but not in the mice and hamsters. The steady-state volumes of distribution in the KC500 (100 mg/kg)-pretreated mice, hamsters, rats, and guinea pigs increased to 1.5, 4.0, 4.1, and 1.8 times over the corresponding control animals, respectively (Table 1).

Tissue Distribution of [^{125}I]T_4. Effects of KC500 (100 mg/kg)-pretreatment on the tissue-to-serum concentration ratio (K_p value) and the distribution level of [^{125}I]T_4 in tissues after the administration of [^{125}I]T_4 were examined in mice, hamsters, rats, and guinea pigs. In all animals examined, the thyroid gland, liver, kidney, stomach, duodenum, and jejunum had K_p values over 1 (Fig. 7), and the K_p value was the greatest in the thyroid gland and liver among the examined tissues, with the exception of kidney in guinea pigs. Pretreatment with KC500 resulted in significant increases in the K_p values of thyroid gland, liver, kidney, and duodenum in all the animals examined (Fig. 7).

In KC500-untreated (control) animals, the accumulation level of [^{125}I]T_4 in the liver was the highest among the tissues examined (Fig. 8). In all the animals examined, pretreatment with KC500 (100 mg/kg) resulted in significant increases in the accumulation level of [^{125}I]T_4 in the liver. More than 34, 55, 58, and 17% of the [^{125}I]T_4

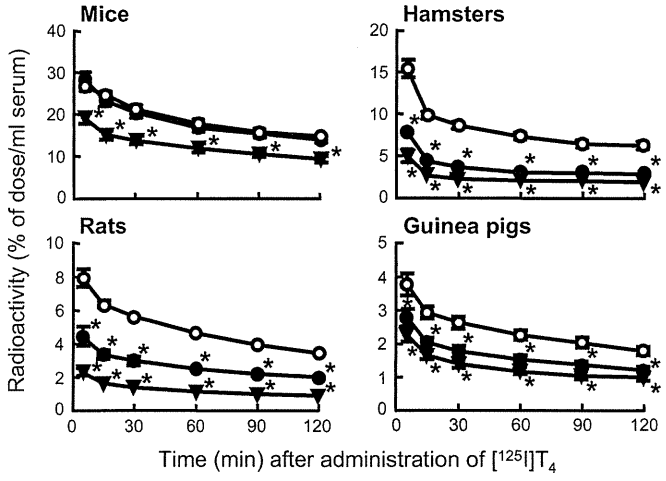

FIG. 6. Effects of KC500 on the clearance of [^{125}I]T_4 from serum in animals. KC500 (37.5 and 100 mg/kg) was intraperitoneally given to animals, and 96 h after the KC500 treatment, a portion of [^{125}I]T_4 (15 μCi/ml i.v.) was administered to the animals, as described under *Materials and Methods*. The amount of serum [^{125}I]T_4 was measured at the indicated times after the intravenous administration of [^{125}I]T_4. Each point represents the mean ± S.E. (vertical bars) for four to eight animals. *, $P < 0.05$, significantly different from each control. —○—, control; —●—, KC500 37.5 mg/kg; —▼—, KC500 100 mg/kg.

TABLE 1

Pharmacokinetic parameters for [^{125}I]T_4 after the administration of [^{125}I]T_4 to the KC500-pretreated animals

The pharmacokinetic parameters of [^{125}I]T_4 were calculated from the data in Fig. 6 with noncompartmental methods as described previously (Tabata et al., 1999). The values shown are expressed as the mean ± S.E. for four to 11 animals.

Animal	Pretreatment	Dose	Mean Total Body Clearance × 100	Distribution Volume
		mg/kg	*ml/min*	*ml*
Mice	Control		1.56 ± 0.15	4.46 ± 0.25
	KC500	37.5	1.65 ± 0.20	4.69 ± 0.31
	KC500	100	2.66 ± 0.22*	6.52 ± 0.51*
Hamsters	Control		3.63 ± 0.32	10.46 ± 0.53
	KC500	37.5	5.35 ± 0.93	25.76 ± 1.21*
	KC500	100	8.19 ± 1.24*	42.04 ± 4.50*
Rats	Control		7.83 ± 0.39	15.62 ± 0.68
	KC500	37.5	13.23 ± 1.46*	31.81 ± 3.31*
	KC500	100	30.75 ± 3.27*	64.06 ± 4.58*
Guinea pigs	Control		13.73 ± 0.91	35.70 ± 2.77
	KC500	37.5	20.65 ± 1.54*	54.70 ± 4.70*
	KC500	100	23.42 ± 2.17*	65.42 ± 4.98*

* $P < 0.05$, significantly different from each control.

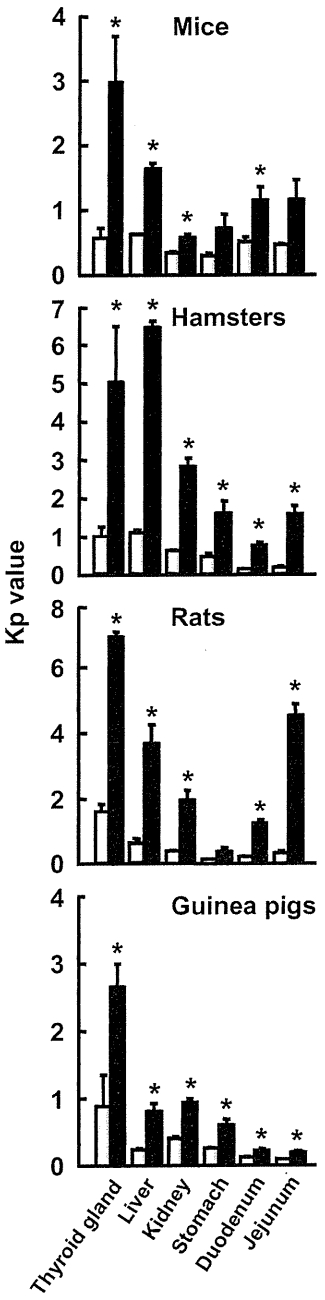

FIG. 7. Tissue-to-serum concentration ratio (K_p value) of [^{125}I]T_4 in various tissues after administration of [^{125}I]T_4 to KC500-pretreated animals. KC500 (100 mg/kg i.p.) was given to animals, and 96 h after the KC500 treatment, [^{125}I]T_4 was intravenously administered to the animals. At 60 min after the [^{125}I]T_4 administration, the radioactivity in each tissue was measured, as described under *Materials and Methods*. Each column represents the mean ± S.E. (vertical bars) for three to six animals. *, $P < 0.05$, significantly different from each control. □, control; ■, KC500.

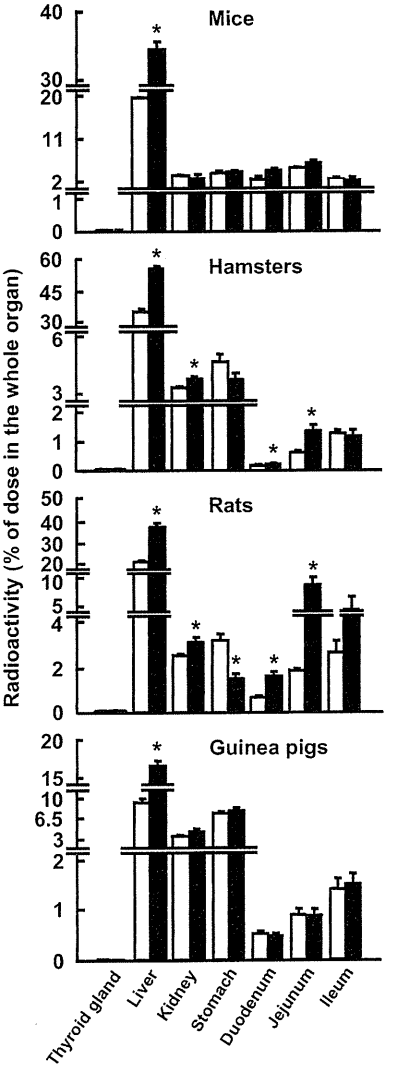

FIG. 8. Tissue distribution of [^{125}I]T_4 after administration of [^{125}I]T_4 to KC500-pretreated animals. Experimental protocols were the same as those described in the legend of Fig. 7. Each column represents the mean ± S.E. (vertical bars) for three to six animals. *, $P < 0.05$, significantly different from each control. □, control; ■, KC500.

dosed were accumulated in the liver in the KC500-pretreated mice, hamsters, rats, and guinea pigs, respectively (Fig. 8). In addition, the accumulation level per gram of liver was also increased in the KC500-pretreated mice, hamsters, rats, and guinea pigs, compared with the corresponding control animals (Table 2). Furthermore, KC500-pretreatment led to significant increases in the liver weight in mice, rats, and guinea pigs, but not in hamsters (Table 3).

Serum Proteins Bound to [^{125}I]T_4. The effects of pretreatment with KC500 (100 mg/kg) on the binding of [^{125}I]T_4 to serum proteins, such as TBG, albumin, and TTR, were examined in mice, hamsters, rats, and guinea pigs (Fig. 9). In KC500-pretreated hamsters, the level of serum [^{125}I]T_4-TTR complex slightly de-

TABLE 2

Accumulation of [^{125}I]T_4 in the KC500-pretreated mice, hamsters, rats, and guinea pigs livers

The radioactivity in the liver was measured at 60 min after the [^{125}I]T_4-administration, as described under *Materials and Methods*. Accumulation levels of the liver in the control mice, hamsters, rats, and guinea pigs were 292620 ± 8873 (n = 4), 577975 ± 51307 (n = 4), 568665 ± 16375 (n = 6), and 121496 ± 4234 (n = 4) cpm/g wet liver, respectively. The values shown are expressed as the mean ± S.E. for four to six animals.

Animal	[^{125}I]T_4	
	Control	KC500
	% of dose/g liver	
Mice	10.03 ± 0.13	15.34 ± 0.90*
Hamsters	6.93 ± 0.59	10.41 ± 1.03*
Rats	2.75 ± 0.12	3.95 ± 0.33*
Guinea pigs	0.45 ± 0.02	0.76 ± 0.05*

* $P < 0.05$, significantly different from each control.

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TABLE 3

Liver weights after the administration of KC500 to animals

Animals were killed 4 days after the intraperitoneal administration of KC500 (100 mg/kg), and the liver weight was measured. The values shown are expressed as the mean ± S.E. for four to six animals.

Animal	Relative Liver Weight	
	Control	KC500
	% of b.wt.	
Mice	5.08 ± 0.09	5.74 ± 0.21*
Hamsters	4.27 ± 0.30	4.64 ± 0.33
Rats	3.74 ± 0.08	4.65 ± 0.17*
Guinea pigs	3.81 ± 0.17	4.36 ± 0.11*

* $P < 0.05$, significantly different from each control.

creased, whereas the binding level of $[^{125}I]T_4$ to serum albumin slightly increased. In mice, no such effects of KC500-pretreatment were observed. In rats and guinea pigs, the KC500-pretreatment resulted in significant decreases in the level of $[^{125}I]T_4$-TTR complex, whereas it led to significant increases in the level of $[^{125}I]T_4$ bound to albumin. In addition, most of the $[^{125}I]T_4$ released from $[^{125}I]T_4$-TTR complex in KC500-pretreated animals, with the exception of guinea pigs, bound to serum albumin. In the KC500-pretreated guinea pigs, most of the released $[^{125}I]T_4$ was detected as free (serum protein unbound) $[^{125}I]T_4$.

Discussion

In the present study, we found that treatment with KC500 promoted accumulation of T_4 in several tissues, especially the liver, and resulted in a drastic decrease in the levels of serum total T_4 and free T_4 not only in rats but also in mice, hamsters, and guinea pigs. Incidentally, we have previously reported the KC500-induced decreases in the level of serum total T_4 in the Wistar and Gunn rats (Kato et al., 2004, 2007).

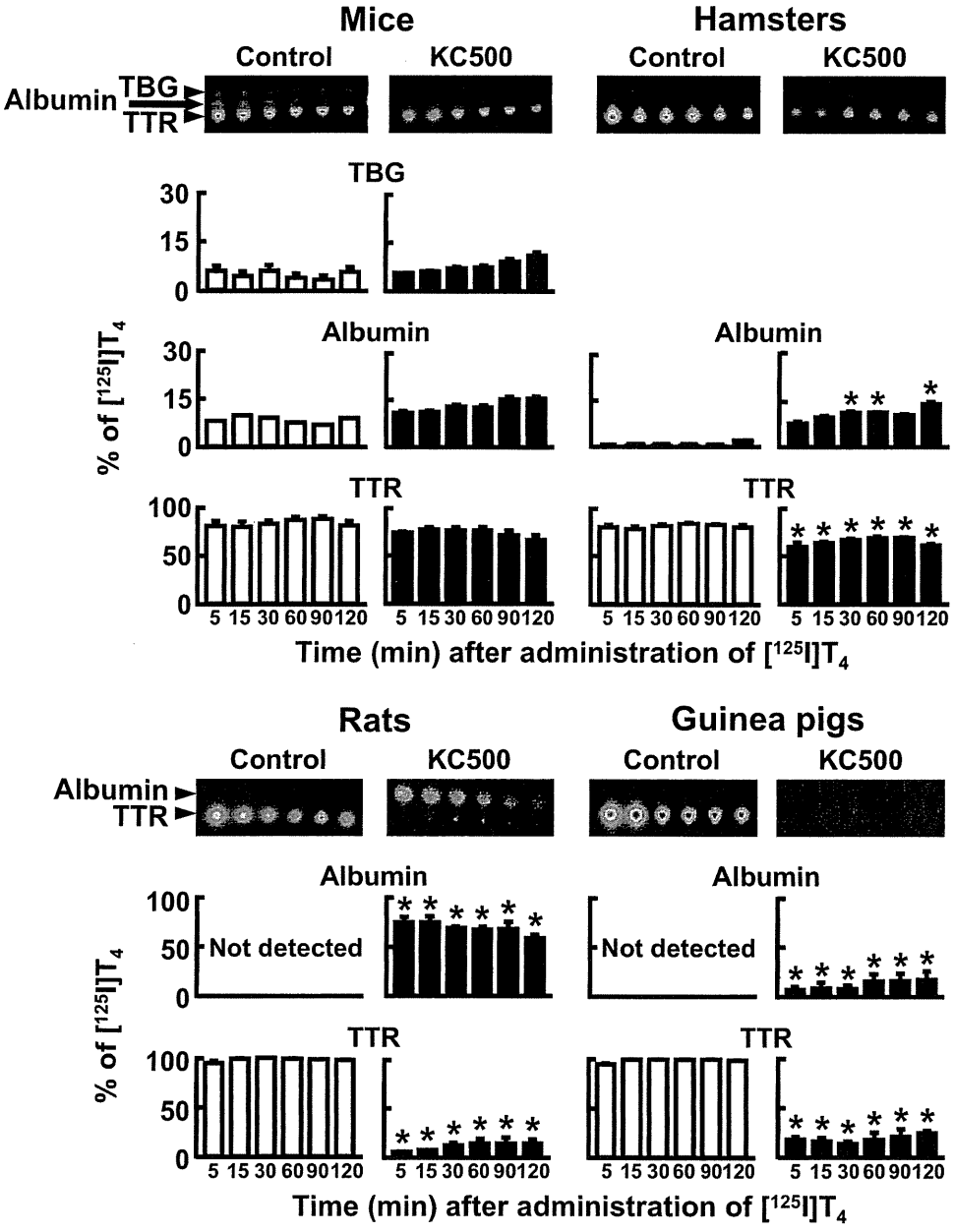

FIG. 9. Effect of KC500 on the binding of $[^{125}I]T_4$ to serum proteins in animals. KC500 (100 mg/kg i.p.) was given to animals, and 96 h after the KC500 treatment $[^{125}I]T_4$ was intravenously administered to the animals. The amounts of $[^{125}I]T_4$ bound to the serum proteins 60 min after $[^{125}I]T_4$ administration were assessed by the method described under *Materials and Methods*. Each column represents the mean ± S.E. (vertical bars) for three to four animals. *, $P < 0.05$, significantly different from each control.

A possible explanation for the PCB-induced decrease in serum thyroid hormones is the hepatic T_4-UDPGT-dependent mechanism, which is generally considered because T_4-UDPGT inducers, such as Aroclor 1254, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, and CB126, show strong activities for decreasing the levels of serum total thyroid hormones in rats (Barter and Klaassen, 1994; Van Birgelen et al., 1995; Schuur et al., 1997). However, between mice and rats treated with a T_4-UDPGT inducer, the differences in magnitude of the decreases in the level of serum total T_4 is not necessarily correlated with that of hepatic T_4-UDPGT activity (Craft et al., 2002; Hood et al., 2003; Kato et al., 2003). More recently, we have demonstrated that KC500 treatment resulted in significant decreases in the level of serum total T_4 not only in Wistar rats but also in Gunn rats (UGT1A-deficient Wistar rats) (Kato et al., 2004, 2007) and further indicated that the KC500-mediated decrease in rats occurred through an increase in the accumulation of T_4 in several tissues, especially the liver, rather than through an increase in hepatic T_4-UDPGT activity (Kato et al., 2007). In addition to the previous results, we showed that the activity of hepatic T_4-UDPGT was changed very little by KC500 treatment in mice, hamsters, and rats, although a KC500-mediated decrease in the serum total T_4 level was observed in all the species of animals examined. In addition, no significant changes in the excretion level of biliary T_4 glucuronide were observed in KC500-pretreated mice, hamsters, and guinea pigs. All the results obtained herein strongly suggest that the KC500-induced decrease in the serum T_4 level in mice, hamsters, rats, and guinea pigs primarily occurs in a T_4-UDPGT-independent manner.

KC500 treatment led to no significant change in the level of serum TSH in mice, hamsters, rats, and guinea pigs, although serum TSH is considered as one of the factors regulating the level of serum total T_4. These results are similar with those in previous reports on the effect of PCBs on the level of serum TSH in rats (Liu et al., 1995; Hood et al., 1999; Hallgren et al., 2001; Kato et al., 2004, 2007).

The factors regulating the level of serum total T_4, hepatic type-I iodothyronine deiodinase, and sulfotransferase are also known. However, hepatic type-I iodothyronine deiodinase activity was significantly decreased by KC500 in rats and hamsters, and furthermore, no significant change in the enzyme activity by treatment with KC500 occurred in either mice or guinea pigs (data not shown). No significant change in the activity of hepatic sulfotransferase was observed in the KC500-treated mice, hamsters, rats, and guinea pigs (data not shown). Therefore, a KC500-mediated decrease in the serum T_4 level seems to occur in the type-I iodothyronine deiodinase- and sulfotransferase-independent pathways.

Another possible mechanism for the PCB-induced decrease in the level of serum total T_4 is the TTR-associated pathway, which might be considered because PCB and its ring-hydroxylated metabolites act as T_4 antagonists to TTR (Lans et al., 1993; Brouwer et al., 1998; Meerts et al., 2002; Kato et al., 2004). Thus, competitive inhibition by PCB and/or its metabolites might decrease the level of serum total T_4 through an increase in the level of free T_4 and promotion of T_4 excretion. However, no such competitive inhibition by KC500 was observed in ddY mice, although the significant decrease in the level of [^{125}I]T_4 bound to serum TTR and the increase in the level of [^{125}I]T_4 bound to serum albumin occurred in KC500-pretreated hamsters, rats, and guinea pigs. In hamsters, rats, and guinea pigs, but not mice, KC500-mediated inhibition of the T_4-TTR formation might lead to changes in the tissue distribution of T_4. Therefore, to clarify this point, we administered [^{125}I]T_4 to KC500-pretreated mice, hamsters, rats, and guinea pigs and measured the levels of [^{125}I]T_4 in their tissues. Marked increases in the mean total body clearance of [^{125}I]T_4 and in the steady-state distribution volume of [^{125}I]T_4 were observed in the KC500 (100 mg/kg)-pretreated mice, hamsters, rats, and guinea pigs. Similar increases were demonstrated to occur in KC500 (intraperitoneal injection at a dose of 10 mg/kg once daily for 10 days)-pretreated rats (Kato et al., 2007). The tissue-to-serum concentration ratio (K_p value) was greater in the tissues, especially thyroid gland, liver, kidney, stomach, and small intestine, of KC500-pretreated animals than in those of the corresponding control animals. In addition, more than 34, 55, 58, and 17% of the [^{125}I]T_4 dosed were accumulated in the liver of the KC500-pretreated mice, hamsters, rats, and guinea pigs, respectively.

In conclusion, we demonstrate for the first time that a KC500-mediated decrease in serum T_4 occurs not only in rats (Kato et al., 2004, 2007) but also in mice, hamsters, and guinea pigs. We also hypothesize that the PCB-induced decrease occurs through an increase in accumulation (transportation from serum to liver) of T_4 in the liver rather than through induction of hepatic T_4-UDPGT. Furthermore, we suggest that the increased accumulation in the liver is attributed, at least in part, to the PCB- and its metabolite(s)-mediated inhibition of formation of the serum T_4-TTR complex.

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A Possible Mechanism for the Decrease in Serum Thyroxine Level by a 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin-Like Polychlorinated Biphenyl Congener, 3,3′,4,4′,5-Pentachlorobiphenyl in Mice

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ABSTRACT:

Serum total thyroxine (T_4) and free T_4 levels were markedly decreased 7 days after treatment with 3,3′,4,4′,5-pentachlorobiphenyl (CB126) (2.5 mg/kg i.p.) in 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-sensitive C57BL/6 mice but not in TCDD-resistant DBA/2 mice. At the same time, the level and activity of hepatic T_4-UDP-glucuronosyltransferase (T_4-UGT) were significantly increased in C57BL/6 mice but not in DBA/2 mice. Furthermore, the amounts of biliary [^{125}I]T_4 and [^{125}I]T_4 glucuronide after injection of [^{125}I]T_4 were increased by CB126 pretreatment in C57BL/6 mice but not in DBA/2 mice. Clearance of [^{125}I]T_4 from serum was also promoted by CB126 pretreatment in C57BL/6 mice but not in DBA/2 mice. On the other hand, no significant changes in the steady-state volumes of distribution of [^{125}I]T_4 and in the concentration ratio (K_p value) of the liver to serum by CB126 pretreatment were observed in either strain of mice. Because liver weight was increased by CB126 pretreatment in C57BL/6 mice but not in DBA/2 mice, hepatic total [^{125}I]T_4 was increased only in C57BL/6 mice. The present findings indicate that CB126-mediated decrease in serum T_4 occurs through the increase in hepatic T_4-UGT and the enhanced accumulation of hepatic T_4 along with development of liver hypertrophy.

Most polychlorinated biphenyl (PCB) congeners, such as 2,3′,4,4′,5-pentachlorobiphenyl, 3,3′,4,4′,5-pentachlorobiphenyl (CB126), and 2,2′,4,4′,5,5′- and 2,3,3′,4,4′,5-hexachlorobiphenyls, are known to decrease the levels of serum thyroid hormone and to increase the activities of hepatic drug-metabolizing enzymes in rats and mice (Ness et al., 1993; Van Birgelen et al., 1995; Desaulniers et al., 1999; Craft et al., 2002). As a possible mechanism for the PCB-mediated decrease in serum thyroid hormone, the induction of hepatic UDP-glucuronosyltransferases (UGTs), especially UGT1As, responsible for thyroid hormone metabolism and the competition of thyroxine (T_4) and the PCB for binding to transthyretin (TTR) have been proposed (Brouwer et al., 1998; Craft et al., 2002). In addition, hydroxylated PCBs show a high binding affinity for serum TTR (Lans et al., 1993; Brouwer et al., 1998; Ucán-Marín et al., 2009). The decrease in serum T_4 by Aroclor 1254, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), and TCDD-like PCBs, CB126, in rats is reported to occur mainly through the induction of the UGT (T_4-UGT) responsible for T_4 glucuronidation through an aryl hydrocarbon receptor-mediated mechanism (Barter and Klaassen, 1994; Van Birgelen et al., 1995; Schuur et al., 1997). However, we have demonstrated that a single and consecutive treatments with Kanechlor-500, a commercial PCB mixture, resulted in a significant decrease in serum total T_4 not only in Wistar but also in Gunn rats (UGT1A-deficient Wistar rats) (Kato et al., 2004, 2007), and further indicated that the Kanechlor-500-mediated decrease occurs through increased accumulation of T_4 in several tissues, especially the liver, rather than an increase in hepatic T_4-UGT activity (Kato et al., 2007).

In the present study, therefore, to determine the mechanism for the decrease in serum thyroid hormone by a TCDD-like PCB, we selected CB126 (Fig. 1), because CB126 is a toxic coplanar PCB congener with a toxic equivalency factor of 0.1 (Safe, 1994) that generally exists with mixtures of multiple PCB congeners in the environment and also because it shows antiestrogenic effects in a human breast cancer cell line (Krishnan and Safe, 1993; Gierthy et al., 1997). We also examined a relationship between the decrease in serum total T_4 and the increase in the hepatic T_4-UGT. It has been reported that TCDD-induced glucuronidation is observed in rats but occurs only marginally in mice (Craft et al., 2002). In the present work, therefore, we examined the differences in the CB126-induced alteration of levels of thyroid hormones between TCDD-sensitive C57BL/6 mice and TCDD-insensitive DBA/2 mice. The results strongly suggest that the

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ABBREVIATIONS: PCB, polychlorinated biphenyl; CB126, 3,3′,4,4′,5-pentachlorobiphenyl; UGT, UDP-glucuronosyltransferase; T_4, thyroxine; TTR, transthyretin; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TSH, thyroid-stimulating hormone; TBG, thyroxine-binding globulin.

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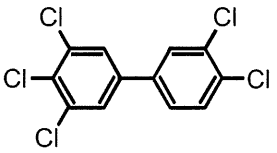

3,3',4,4',5-pentachlorobiphenyl
(CB126)

FIG. 1. Chemical structure of 3,3',4,4',5-pentachlorobiphenyl.

CB126-mediated decrease in serum total T_4 in mice occurs through the increase in hepatic T_4-UGT and the enhanced accumulation of T_4 in the liver.

Materials and Methods

Chemicals. Panacete 810 (medium-chain triglycerides) was purchased from Nippon Oils and Fats Co. Ltd. (Tokyo, Japan). The [^{125}I]T_4, radiolabeled at the 5'-position of the outer ring, was obtained from PerkinElmer Life and Analytical Sciences (Waltham, MA). CB126 were purchased from Cambridge Isotope Laboratories, Inc. (Cambridge, MA). All of the other chemicals used were obtained commercially at appropriate grades of purity.

Animal Treatments. Male C57BL/6 mice (18–31 g) and the DBA/2 mice (18–28 g) were obtained from Japan SLC., Inc. (Shizuoka, Japan). Male C57BL/6 and DBA/2 mice were housed three or four per cage with free access to commercial chow and tap water, maintained on a 12-h dark/light cycle (8:00 AM to 8:00 PM light) in an air-controlled room (temperature, 24.5 ± 1°C, humidity, 55 ± 5%), and handled under the animal care guidelines of the University of Shizuoka (Shizuoka, Japan). Mice received an intraperitoneal injection of CB126 (2.5 mg/kg) dissolved in Panacete 810 (5 ml/kg). Control animals were treated with vehicle alone (5 ml/kg).

In Vivo Study. Mice were killed by decapitation 7 days after the administration of CB126. The thyroid gland and liver were removed and weighed. Hepatic microsomes were prepared according to the method of Kato et al. (1995) and stored at −85°C until use. Blood was collected from each animal between 10:30 and 11:30 AM. After clotting at room temperature, serum was separated by centrifugation and stored at −50°C until use.

Analysis of serum hormones. Levels of total T_4, free T_4, and thyroid-stimulating hormone (TSH) were measured by radioimmunoassay using Total T4 and Free T4 kits (Diagnostic Products Corporation, Los Angeles, CA), and the recombinant TSH [^{125}I] Biotrak assay system (GE Healthcare UK, Ltd., Little Chalfont, Buckinghamshine, UK), respectively.

Hepatic microsomal enzyme assays. The amount of hepatic microsomal protein was determined by the method of Lowry et al. (1951) with bovine serum albumin as a standard. Microsomal *O*-dealkylase activities of 7-benzyloxy-, 7-ethoxy-, and 7-pentoxyresorufins were determined by the method of Burke et al. (1985). The activity of microsomal UGT toward T_4 (T_4-UGT activity) was determined by the method of Barter and Klaassen (1992).

Western blot analysis. The polyclonal antipeptide antibodies against the common region of rat UGT1A isoforms and specific antibodies against rat UGT1A1 and UGT2B1, which were established by Ikushiro et al. (1995, 1997), were used. Western blot analyses for microsomal UGT isoforms were performed by the method of Luquita et al. (2001). The bands of mouse Ugt1a1 and Ugt2b1, which correspond to rat UGT1A1 and UGT2B1, respectively, were detected using chemical luminescence (ECL detection kit, GE Healthcare UK, Ltd.), and the level of each protein was determined densitometrically with an LAS-1000 system (Fuji Photo Film. Co., Ltd., Tokyo, Japan).

Ex Vivo Study. At 7 days after treatment with CB126, the mice were anesthetized with saline solution (2 ml/kg) containing sodium pentobarbital (25 mg/ml) and potassium iodide (1 mg/ml). The femoral artery was cannulated (polyethylene tube SP8; Natsume Inc., Tokyo, Japan) and primed with heparinized saline (33 units/ml), and then the animal's body was warmed to 37°C. Fifteen minutes later, the mice were given 0.1 ml of [^{125}I]T_4 (15 μCi/ml) dissolved in saline containing 10 mM NaOH and 1% normal mouse serum intravenously. In addition, because bile was collected within 2.25 h after pentobarbital administration, little pentobarbital-mediated induction of the enzymes responsible for T_4 metabolism was expected.

Clearance of [^{125}I]T_4 from serum. Clearance of [^{125}I]T_4 from serum was measured according to the method of Oppenheimer et al. (1968). In brief, after the administration of [^{125}I]T_4, a portion (0.08 ml) of blood was sampled from the artery at the indicated times, and serum was prepared and stored at −50°C until use. An aliquot (15 μl) of serum was used for measurement of the [^{125}I]T_4 level by a gamma counter (Cobra II Auto-Gamma 5002; PerkinElmer Life and Analytical Sciences), and the assay was performed in duplicate.

Biliary excretion of total [^{125}I]T_4 and [^{125}I]T_4 glucuronide. After the administration of [^{125}I]T_4, bile was collected on ice for 2 h at 30-min intervals. Bile volume was determined gravimetrically. The amounts of total [^{125}I]T_4 and [^{125}I]T_4 glucuronide in bile were determined by the method of Vansell and Klaassen (2001). In brief, an aliquot (10 μl) of each bile sample was used for determining the total [^{125}I]T_4 level by a gamma counter (Cobra II Auto-Gamma 5002), and the assay was performed in duplicate. To measure the amount of [^{125}I]T_4 glucuronide in bile, a portion (10 μl) of each bile sample was added to 2 volumes of methanol and stored at −20°C for 1 h to precipitate protein. After the mixture was centrifuged at 12,000*g* (4°C) for 10 min, the resultant supernatant was collected for high-performance liquid chromatography analysis. This analysis was performed using a ChromSpher C18 column (10 × 0.3 cm) (Chrompack, Inc., Raritan, NJ) in combination with both a ChromSep reverse-phase guard column (10 × 2 mm) (Chrompack, Inc.) and an Adsorbosphere C18 reverse-phase guard column (7.5 × 4.6 mm) (Alltech Associates, Deerfield, IL). A solution of 0.02 mM ammonium acetate (pH 4.0) containing 16 to 45% acetonitrile was used to elute [^{125}I]T_4 glucuronide; 16% acetonitrile was used as a initial solution for 6 min, and then the elution solution was changed by a linear increase to 27% over 12 min, held for 4 min, followed by a linear increase to 45% over 5 min and held for 11 min. The level of biliary [^{125}I]T_4 glucuronide was determined by Radioisotope Detector 171 (Beckman Coulter, Fullerton, CA).

Analysis of [^{125}I]T_4 bound to serum proteins. The levels of serum [^{125}I]T_4-thyroxine-binding globulin (TBG), [^{125}I]T_4-albumin, and [^{125}I]T_4-TTR complexes were determined according to the method of Davis et al. (1970). In brief, serum was diluted in 100 mM phosphate buffer (pH 7.4) containing 1 mM EDTA, 1 mM dithiothreitol, and 30% glycerol, and the diluted serum was subjected to electrophoresis on 4 to 20% gradient native polyacrylamide gels (PAG Mid "Daiichi" 4/20; Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan). The electrophoresis was performed at 4°C for 11 h at 20 mA in the 0.025 M Tris buffer (pH 8.4) containing 0.192 M glycine. The human albumin and TTR, which were incubated with [^{125}I]T_4, were also applied on the gel as templates. After the electrophoresis, a gel was dried and radioautographed for 20 h at room temperature using Imaging Plate 2040 (Fuji Photo Film Co., Ltd.). The levels of [^{125}I]T_4-TBG, [^{125}I]T_4-albumin, and [^{125}I]T_4-TTR in serum were determined by counting the corresponding gel fractions identified from a bioimaging analyzer (BAS-2000II IP Reader; Fuji Photo Film Co., Ltd.).

Tissue distribution of [^{125}I]T_4. Tissue distribution of [^{125}I]T_4 was performed according to the modified method of Oppenheimer et al. (1968). In brief, at 5 min after administration of [^{125}I]T_4 to CB126-pretreated mice, blood was sampled from the abdominal aorta. Then, cerebrum, cerebellum, pituitary gland, thyroid gland, sublingual gland, submandibular gland, thymus, heart, lung, liver, kidney, adrenal gland, spleen, pancreas, testis, prostate gland, seminal vesicle, stomach, duodenum, jejunum, ileum, caecum, brown fat, skeletal muscle, bone marrow, skin, spinal cord, and fat were removed and weighed. Radioactivities in serum and the tissues were determined by a gamma counter (Cobra II Auto-Gamma 5002), and amounts of [^{125}I]T_4 in the tissues were shown as ratios to the amount in serum.

Statistics. The data obtained were analyzed statistically according to Student's *t* test or Dunnett's test after analysis of variance. In addition, clearance of [^{125}I]T_4 from serum, the amount of biliary [^{125}I]T_4 glucuronide, and the level of [^{125}I]T_4 bound to serum proteins were statistically analyzed according to the Newman-Keuls test after analysis of variance. The pharmacokinetic parameters of [^{125}I]T_4 were estimated with noncompartmental methods as described previously (Tabata et al., 1999).

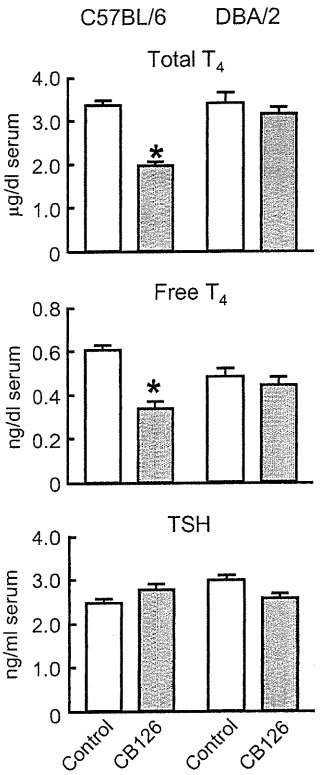

FIG. 2. Effects of CB126 on the levels of serum total T_4, free T_4, and TSH. Animals were killed 7 days after the administration of CB126 (2.5 mg/kg), and levels of serum thyroid hormones were measured, as described under *Materials and Methods*. Each column represents the mean ± S.E. (vertical bars) for four to seven animals. *, $P < 0.05$, significantly different from each control.

Results

Serum Hormone Levels. We performed preliminary experiments on the dose response (CB126: 0.25, 0.5, 1.0, 2.5, and 5.0 mg/kg) and time course (72, 120, and 168 h). On the basis of the results, we determined the suitable dose and time (CB126: 2.5 mg/kg, 7 days after the dosing). The effects of CB126 on the levels of serum thyroid hormones were examined in C57BL/6 and DBA/2 mice (Fig. 2). Serum total T_4 and free T_4 levels 7 days after the treatment with CB126 were markedly decreased in C57BL/6 mice but not in DBA/2 mice. On the other hand, no significant increase in the level of serum TSH by CB126 pretreatment was observed in either strain of mice.

Hepatic Drug-Metabolizing Enzymes. Effects of CB126 on hepatic microsomal activities of ethoxyresorufin *O*-dealkylase (Cyp1a1/2), benzyloxyresorufin *O*-dealkylase (Cyp2b1/2 and Cyp3a1/2), and pentoxyresorufin *O*-dealkylase (Cyp2b1/2) were examined in C57BL/6 and DBA/2 mice. Treatment of C57BL/6 mice with CB126 resulted in remarkable increases in hepatic microsomal enzyme activities: 73-fold for ethoxyresorufin *O*-dealkylase activity, 7-fold for pentoxyresorufin *O*-dealkylase activity, and 3-fold for benzyloxyresorufin *O*-dealkylase activity. On the other hand, no such CB126-mediated increase was observed in DBA/2 mice (Table 1).

Hepatic T_4-UGT. T_4 glucuronidation is reported to be primarily mediated by hepatic T_4-UGT, including UGT1A1 and UGT1A6, in the rat liver (Visser, 1996). Therefore, we examined the effects of CB126 on hepatic microsomal T_4-UGT activity in C57BL/6 and DBA/2 mice. The activity of hepatic T_4-UGT was significantly increased by CB126 in C57BL/6 mice but not in DBA/2 mice (Fig. 3).

The amounts of the proteins responsible for T_4-UGTs, including total Ugt1a, Ugt1a1, and Ugt2b1, in mice were determined by Western blot analysis. The amounts of the proteins responsible for the Ugt1a enzymes in the liver were significantly increased by CB126 in C57BL/6 mice but not in DBA/2 mice. The level of hepatic Ugt2b1 was decreased in C57BL/6 mice, whereas the level was not significantly changed in DBA/2 mice by CB126 treatment (Fig. 4).

TABLE 1

Effects of CB126 on the activity of hepatic microsomal alkoxyresorufin O-dealkylases in C57BL/6 and DBA/2 mice

Animals were killed 7 days after the administration of CB126 (2.5 mg/kg). Data represent the mean ± S.E. for four to five mice.

Resolfin *O*-Dealkylase	C57BL/6		DBA/2	
	Control	CB126	Control	CB126
	nmol resolfin formed/mg/protein/min			
7-Ethoxy-	0.20 ± 0.02	14.29 ± 0.56*	0.18 ± 0.01	0.21 ± 0.01*
7-Benzyloxy-	0.12 ± 0.02	0.38 ± 0.01*	0.07 ± 0.01	0.10 ± 0.01
7-Pentoxy-	0.02 ± 0.002	0.15 ± 0.01*	0.02 ± 0.001	0.03 ± 0.003*

* $P < 0.05$, significantly different from each control.

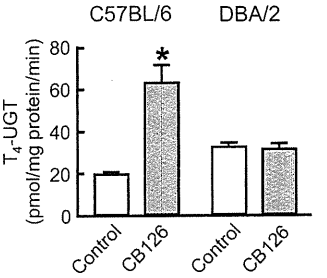

FIG. 3. Effect of CB126 on the activity of hepatic microsomal T_4-UGT. Hepatic microsomes from individual animals were used for the T_4-UGT enzyme assay, as described under *Materials and Methods*. Each column represents the mean ± S.E. (vertical bars) for four to seven animals. *, $P < 0.05$, significantly different from each control.

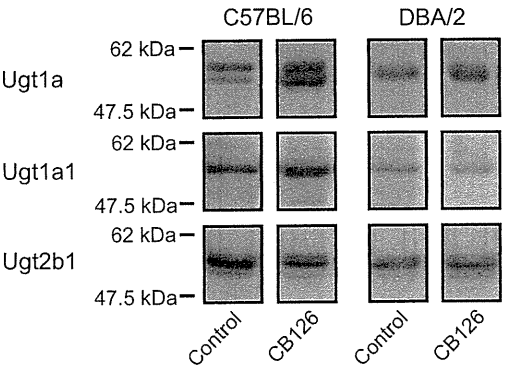

FIG. 4. Representative Western blot patterns for hepatic microsomal Ugt isoforms in CB126-treated mice. Hepatic microsomes from individual animals were used for Western blot analysis, as described under *Materials and Methods*.

Biliary Excretion of [^{125}I]T_4 and [^{125}I]T_4 Glucuronide. We examined effects of CB126 on the levels of biliary [^{125}I]T_4 and [^{125}I]T_4 glucuronide in C57BL/6 and DBA/2 mice. The amounts of biliary [^{125}I]T_4 and [^{125}I]T_4 glucuronide after intravenous injection of [^{125}I]T_4 were increased by CB126 pretreatment in C57BL/6 mice but not in DBA/2 mice (Fig. 5).

Serum Proteins Bound to [^{125}I]T_4. The effects of CB126 on the binding of [^{125}I]T_4 to serum proteins, such as TTR, albumin, and TBG, were examined in C57BL/6 and DBA/2 mice (Figs. 6 and 7). In CB126-pretreated C57BL/6 mice, the level of serum [^{125}I]T_4-TTR complex increased slightly, whereas the binding levels of [^{125}I]T_4 to serum albumin and TBG decreased slightly. In DBA/2 mice, no such effects of CB126 pretreatment were observed.

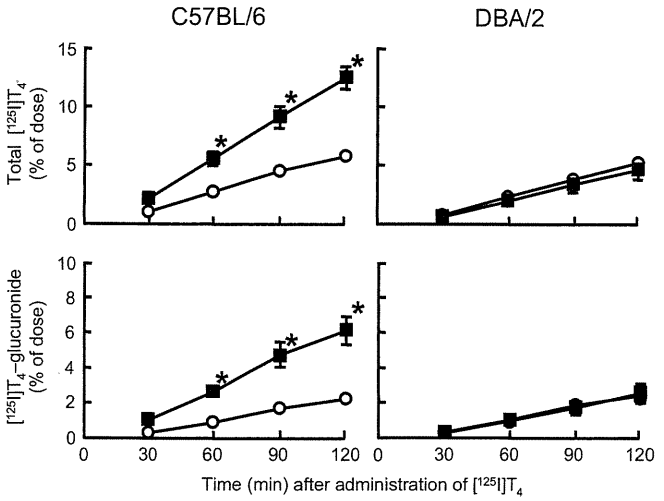

FIG. 5. Effects of CB126 on amounts of biliary total $[^{125}I]T_4$ and $[^{125}I]T_4$ glucuronide. The levels of total $[^{125}I]T_4$ and $[^{125}I]T_4$ glucuronide excreted were measured in the bile collected at 30-min intervals after the intravenous administration of $[^{125}I]T_4$. Each point represents the mean ± S.E. (vertical bars) for five to eight mice. *, $P < 0.05$, significantly different from each control. ○, control; ■, CB126.

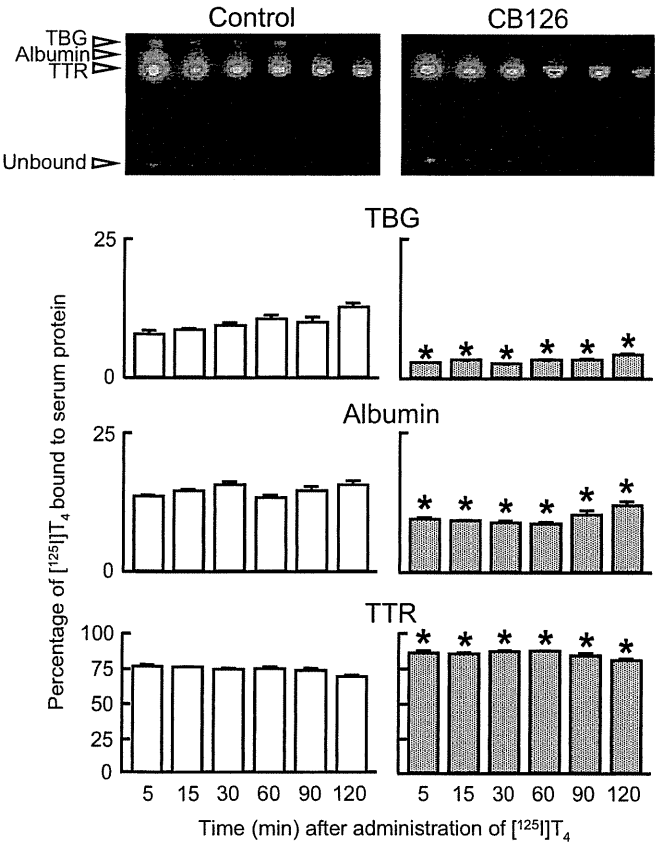

FIG. 6. Effects of CB126 on the binding of $[^{125}I]T_4$ to serum proteins in C57BL/6 mice. The amounts of $[^{125}I]T_4$ bound to the serum proteins 5 min after $[^{125}I]T_4$ administration were assessed by the method described under *Materials and Methods*. Each column represents the mean ± S.E. (vertical bars) for five to six animals. *, $P < 0.05$, significantly different from each control.

Clearance of $[^{125}I]T_4$ from Serum. After intravenous administration of $[^{125}I]T_4$ to the CB126-pretreated C57BL/6 and DBA/2 mice, concentrations of $[^{125}I]T_4$ in the serum were measured at the indicated times (Fig. 8). CB126 pretreatment resulted in a clear enhancement of

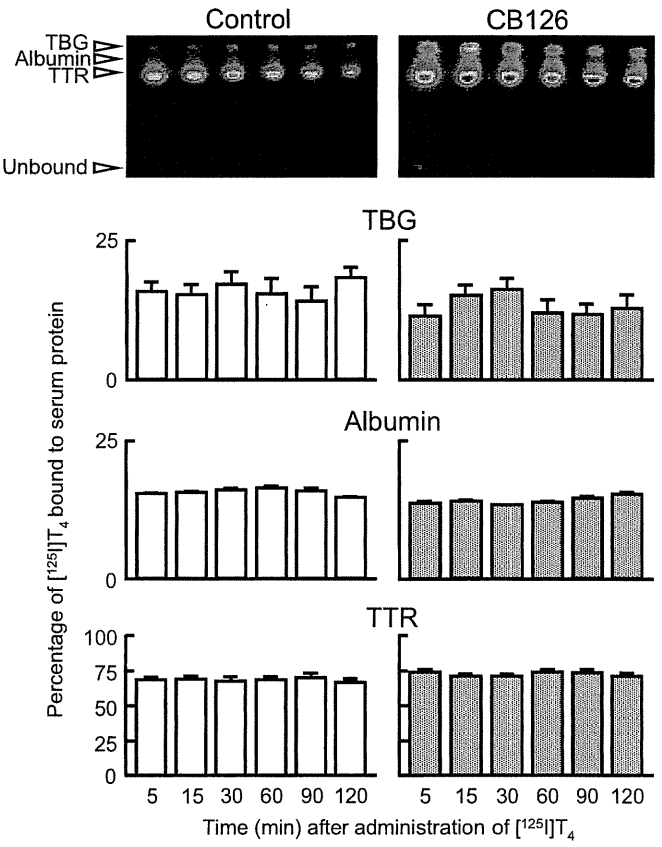

FIG. 7. Effects of CB126 on the binding of $[^{125}I]T_4$ to serum proteins in DBA/2 mice. Experimental protocols were the same as those described in the legend to Fig. 6. Each column represents the mean ± S.E. (vertical bars) for four to five animals.

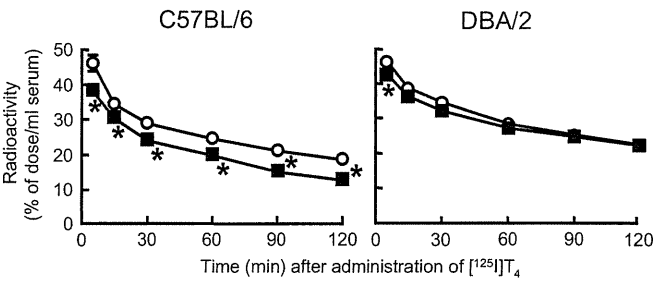

FIG. 8. Effects of CB126 on the clearance of $[^{125}I]T_4$ from serum. The amount of serum $[^{125}I]T_4$ was measured at the indicated times after the intravenous administration of $[^{125}I]T_4$. Each point represents the mean ± S.E. (vertical bars) for five to eight mice. *, $P < 0.05$, significantly different from each control. ○, control; ■, CB126.

the clearance of $[^{125}I]T_4$ from the serum in C57BL/6 mice but not in DBA/2 mice. The serum $[^{125}I]T_4$ level was decreased by approximately 35% of control level within 5 min, and the decrease remained up to 120 min later. The serum pharmacokinetic parameters of the $[^{125}I]T_4$ estimated from these data (Fig. 8) are summarized in Table 2. The mean total body clearance (Cl_{tb}) of $[^{125}I]T_4$ in the CB126-pretreated C57BL/6 mice increased 1.7-fold, compared with that in the control mice. On the other hand, no significant change in the steady-state volumes of distribution of $[^{125}I]T_4$ by CB126 pretreatment was observed in either strain of mice (Table 2).

Tissue Distribution of $[^{125}I]T_4$. Effects of CB126 pretreatment on the tissue/serum concentration ratio (K_p value) and tissue distribution level of $[^{125}I]T_4$ were examined in C57BL/6 and DBA/2 mice. The K_p

TABLE 2

Pharmacokinetic parameters for [^{125}I]T_4 after the administration of [^{125}I]T_4 to the CB126-pretreated mice

The data shown were calculated from the data in Fig. 8. The values shown are expressed as the mean ± S.E. for five to eight mice.

	C57BL/6		DBA/2	
	Control	CB126	Control	CB126
Mean total body clearance (ml/min)	0.015 ± 0.001	0.025 ± 0.002*	0.012 ± 0.0003	0.012 ± 0.001
Distribution volume (ml)	2.90 ± 0.17	3.06 ± 0.12	2.50 ± 0.08	2.72 ± 0.06

* $P < 0.05$, significantly different from each control.

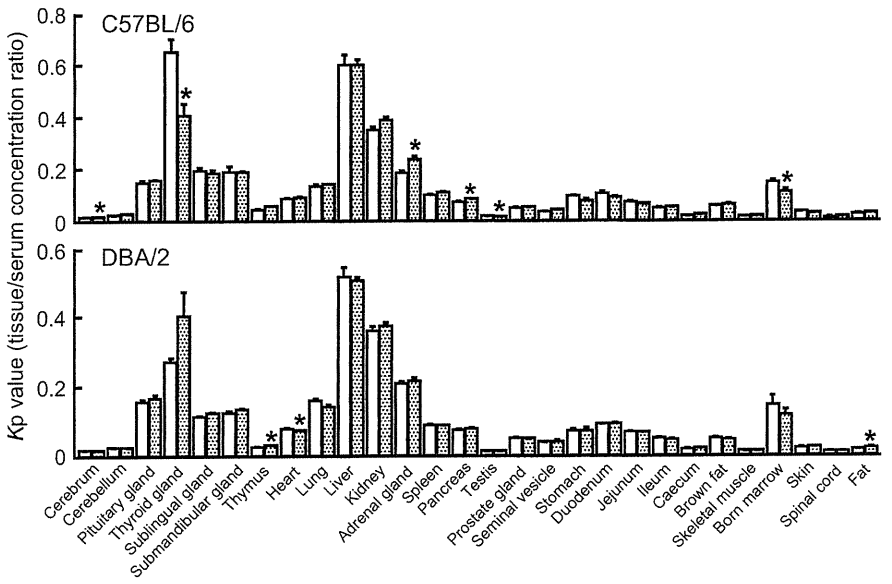

FIG. 9. Tissue/serum concentration ratio (K_p value) of [^{125}I]T_4 in various tissues after administration of [^{125}I]T_4 to CB126-pretreated mice. CB126 (2.5 mg/kg) was given to mice, and 168 h after the CB126 treatment, [^{125}I]T_4 was further administered to the mice. At 5 min after the [^{125}I]T_4 administration, the radioactivity in each tissue was measured, as described under *Materials and Methods*. Each column represents the mean ± S.E. (vertical bars) for five to seven animals. *, $P < 0.05$, significantly different from each control. □, control; ▨, CB126.

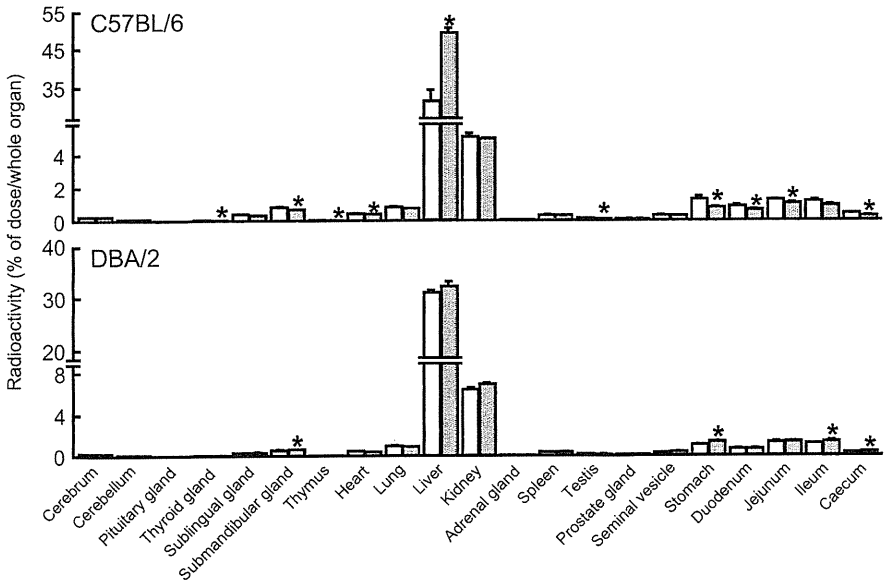

FIG. 10. Tissue distribution of [^{125}I]T_4 after the administration of [^{125}I]T_4 to CB126-pretreated mice. Experimental protocols were the same as those described in the legend to Fig. 9. Each column represents the mean ± S.E. (vertical bars) for five to seven animals. *, $P < 0.05$, significantly different from each control. □, control; ▨, CB126.

values in the thyroid gland, liver, and kidney were the greatest in either strain of control mice (Fig. 9). The K_p value in the thyroid gland was reduced by CB126 pretreatment in C57BL/6 mice but not in DBA/2 mice, whereas no such significant changes in the K_p values of the liver and kidney were observed in either strain of mice (Fig. 9).

In the control C57BL/6 and DBA/2 mice, accumulation of [^{125}I]T_4 was highest in the liver, among the tissues examined (Fig. 10). In C57BL/6 mice, pretreatment with CB126 resulted in an increase in hepatic total [^{125}I]T_4, and the accumulated level in the liver was to more than 49% of the [^{125}I]T_4 dosed (Fig. 10), whereas no significant change in liver accumulation (per gram of liver) of [^{125}I]T_4 by CB126 pretreatment was observed (Table 3). In DBA/2 mice, no significant change in accumulation of [^{125}I]T_4 in the liver by CB126 occurred. In addition, treatment of C57BL/6 mice with CB126 resulted in signif-

TABLE 3

Accumulation of [^{125}I]T_4 in the CB126-pretreated mouse livers

Radioactivity in the liver was measured at 5 min after [^{125}I]T_4 administration. Data represent the mean ± S.E. for five to seven mice.

	C57BL/6		DBA/2	
	Control	CB126	Control	CB126
[^{125}I]T_4 (%dose/g liver)	27.13 ± 1.06	24.16 ± 0.67	25.25 ± 1.02	23.59 ± 0.62

icant increases in weights of liver and thyroid gland and in significant decreases in weights of thymus and cecum. In DBA/2 mice, the CB126 treatment resulted in slight increases in weights of liver, kidney, seminal vesicle, stomach, jejunum, ileum, and cecum and in a significant decrease in weight of thymus (Table 4).

Discussion

In the present study, treatment with CB126 (a single intraperitoneal administration at a dose of 2.5 mg/kg) resulted in a significant decrease in serum total T_4 and free T_4 in C57BL/6 mice, but not in DBA/2 mice. The strain difference in the CB126-mediated decrease in the serum T_4 level was closely correlated with those in the increases in the activities of hepatic drug-metabolizing enzymes, including T_4-UGT and in accumulation of T_4 in the liver.

As a possible explanation for the TCDD-like PCB-induced decrease in serum thyroid hormones, a hepatic T_4-UGT-dependent mechanism was considered, because T_4-UGT inducers, such as TCDD and CB126, have strong activities for decreasing serum total thyroid hormones in rats (Van Birgelen et al., 1995; Schuur et al., 1997). Furthermore, we confirmed that the activity of hepatic T_4-UGT (Ugt1a and Ugt1a1) was significantly increased by CB126 treatment in C57BL/6 mice but not in DBA/2 mice and further found that the amounts of biliary [^{125}I]T_4 and [^{125}I]T_4 glucuronide after intravenous injection of [^{125}I]T_4 were increased by CB126 pretreatment in C57BL/6 mice but not in DBA/2 mice, suggesting that induction of hepatic T_4 removal is more likely at the level of basolateral transport of T_4. The previous reports and the present findings strongly suggest that induction of hepatic T_4-UGT is one of factors that mediate the decrease in serum T_4 level by CB126. In addition, our present result of a CB126-mediated decrease in the level of Ugt2b1 in C57BL/6 mice was consistent with previous results (Buckley and Klaassen, 2009).

However, among the rats and mice treated with a TCDD-like PCB, the difference in magnitude of the decrease in serum total T_4 dose not correlate with that of hepatic T_4-UGT activity (Craft et al., 2002; Hood et al., 2003). More recently, we have demonstrated a hepatic T_4-UGT-independent pathway for the PCB-mediated decrease in serum total thyroid hormones using Wistar and Gunn (T_4-UGT-deficient) rats and further indicated that an increase in the accumulation of T_4 in livers of PCB-treated rats is partially responsible for the decrease in serum total thyroid hormones including T_4 (Kato et al., 2007). In addition, serum TSH and hepatic type I iodothyronine deiodinase, which are important the factors regulating serum thyroid hormones, are not induced by PCBs in rats (Liu et al., 1995; Hood et al., 1999; Hallgren et al., 2001; Kato et al., 2004). Likewise, CB126 treatment did not increase serum TSH in either C57BL/6 and DBA/2 mice. Furthermore, enhanced accumulation of T_4 in the tissues, especially liver, was observed in CB126-pretreated C57BL/6 mice but not in CB126-pretreated DBA/2 mice, strongly suggesting that enhancement of the overall amount of T_4 in liver is one of the factors that mediate the PCB-induced decrease in serum total thyroid hormones in mice.

As a possible mechanism for CB126-mediated enhancement of T_4 accumulation in liver, a TTR-associated pathway might be considered, because the PCB and its hydroxylated metabolites act as competitors for T_4 for binding to TTR and because a decrease in T_4-TTR complex formation results in an increase in serum free T_4 and then in uptake of T_4 into the liver (Lans et al., 1993; Brouwer et al., 1998; Meerts et al., 2002; Kato et al., 2004). However, the present study concerning the fate of serum T_4 using [^{125}I]T_4 indicates that only a slight increase in the serum [^{125}I]T_4-TTR complex and only a slight

TABLE 4

Changes in body and tissue weights after the administration of CB126 to C57BL/6 and DBA/2 mice

Animals were killed 7 days after the administration of CB126 (2.5 mg/kg). Data represent the mean ± S.E. for five to seven mice.

	C57BL/6		DBA/2	
Body and Tissues	Control	CB126	Control	CB126
		g		
Body	25.9 ± 0.21	25.4 ± 0.20	25.2 ± 0.30	25.8 ± 0.53
Cerebrum	0.322 ± 0.004	0.311 ± 0.004	0.275 ± 0.005	0.268 ± 0.002
Cerebellum	0.094 ± 0.008	0.107 ± 0.0003	0.114 ± 0.002	0.118 ± 0.004
Pituitary gland	0.0011 ± 0.0001	0.0013 ± 0.0001	0.0013 ± 0.0002	0.0014 ± 0.0001
Thyroid gland	0.0019 ± 0.0001	0.0023 ± 0.0001*	0.0022 ± 0.0001	0.0023 ± 0.0002
Sublingual gland	0.048 ± 0.003	0.047 ± 0.002	0.054 ± 0.002	0.058 ± 0.003
Submandibular gland	0.092 ± 0.006	0.091 ± 0.003	0.094 ± 0.002	0.098 ± 0.002
Thymus	0.020 ± 0.002	0.008 ± 0.0007*	0.033 ± 0.001	0.029 ± 0.001*
Heart	0.112 ± 0.003	0.107 ± 0.002	0.117 ± 0.002	0.119 ± 0.003
Lung	0.129 ± 0.003	0.132 ± 0.002	0.121 ± 0.0030	0.123 ± 0.0019
Liver	1.149 ± 0.073	2.029 ± 0.030*	1.234 ± 0.034	1.366 ± 0.041*
Kidney	0.319 ± 0.008	0.330 ± 0.004	0.361 ± 0.011	0.407 ± 0.014*
Adrenal gland	0.0028 ± 0.0002	0.0027 ± 0.0003	0.0030 ± 0.0001	0.0030 ± 0.0002
Spleen	0.071 ± 0.005	0.063 ± 0.002	0.082 ± 0.001	0.090 ± 0.006
Testis	0.194 ± 0.004	0.189 ± 0.003	0.224 ± 0.007	0.233 ± 0.005
Prostate gland	0.040 ± 0.002	0.044 ± 0.003	0.046 ± 0.003	0.057 ± 0.004
Seminal vesicle	0.165 ± 0.010	0.171 ± 0.013	0.160 ± 0.010	0.194 ± 0.004*
Stomach	0.257 ± 0.026	0.262 ± 0.020	0.299 ± 0.023	0.438 ± 0.039*
Duodenum	0.186 ± 0.006	0.180 ± 0.007	0.147 ± 0.001	0.154 ± 0.009
Jejunum	0.390 ± 0.006	0.399 ± 0.023	0.401 ± 0.018	0.453 ± 0.009*
Ileum	0.511 ± 0.047	0.457 ± 0.024	0.488 ± 0.019	0.641 ± 0.050*
Cecum	0.477 ± 0.042	0.294 ± 0.009*	0.290 ± 0.004	0.390 ± 0.027*

* $P < 0.05$, significantly different from each control.

decrease in the binding level of [^{125}I]T_4 to serum albumin and TBG in CB126-pretreated C57BL/6 mice. In addition, although some hydroxylated metabolites of PCBs are known to show a higher capacity for binding to TTR (Lans et al., 1993; Brouwer et al., 1998; Ucán-Marín et al., 2009), the hydroxylated metabolites of CB126 were minimally detected in the serum and liver in CB126-pretreated C57BL/6 mice (data not shown), as described previously by Koga et al. (1990). The previous reports and the present findings indicate that a TTR-associated pathway is not considered a primarily mechanism for the CB126-mediated decrease in serum total T_4. However, it was found that CB126-induced liver hypertrophy occurred in C57BL/6 mice but not in DBA/2 mice, and only a small change was seen in the concentration of T_4 (per gram of tissue) in livers of both C57BL/6 and DBA/2 mice. Therefore, the CB126-induced increase in accumulation of hepatic T_4 in C57BL/6 mice is thought to occur mainly through development of liver hypertrophy.

However, the increases in tissue weights were not necessarily correlated with those in accumulation of [^{125}I]T_4 in the tissues of the CB126-pretreated mice. For example, no CB126-induced accumulation of [^{125}I]T_4 was observed in the thyroid gland in C57BL/6 mice. Although an exact mechanism for a liver-selective accumulation of [^{125}I]T_4 by CB126 remains unclear, the liver-selective apparatus for T_4 transportation might exist. In addition, CB126-induced development of liver hypertrophy was herein found to occur in C57BL/6 mice but not in DBA/2 mice, confirming that the development of liver hypertrophy occurs in an aryl hydrocarbon receptor-dependent pathway (Yoshizawa et al., 2007). Although it has been reported that CB126-induced development of liver hypertrophy occurs in the rat liver (Yoshimura et al., 1979), there has been no report, with the exception of this article, concerning the CB126-induced change in the level of T_4 in the liver. Therefore, it is unclear whether CB126-enhanced accumulation of [^{125}I]T_4 in the liver occurs specifically in mice. Furthermore, in DBA/2 mice, CB126 pretreatment resulted in increases in K_p values of [^{125}I]T_4 in the thymus and fat, whereas it did decrease the value in the heart. The mechanism for the tissue difference in the CB126-mediated changes in the K_p value remains unclear.

In conclusion, we demonstrate that the CB126-mediated decrease in serum T_4 in mice occurs not only through increases in hepatic drug-metabolizing enzymes, especially T_4-UGT, but also through development of liver hypertrophy.

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1．公害防止のための法律

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- 規制対象と規制物質を知る．
- 規制の方法とねらいを知る．
- 近年の改正点とポイント
- コンプライアンスの確保が必要となっている．

Key Words　環境基準，排出基準，汚染者負担原則，無過失責任主義

□ 公害防止のための法律の概要

日本における環境保全，公害防止のための法律は環境基本法（平成5年法律第91号）に基づく．かつては公害対策基本法で公害対策を規定し，自然環境保全法で自然環境対策を規定していた．これは公害対策の視点のみでは環境保全問題の複雑化に対応できないことから環境基本法の制定につながった．しかしながら公害関係法令は基本的に大きな違いはない．

公害対策基本法の制定は昭和42年になされ，大気汚染，水質汚濁，騒音，振動，地盤沈下および悪臭の6つを公害として掲げた．環境基準の設定，公害防止計画の策定が盛り込まれた．さらに公害関係法令は第64回国会（公害国会，昭和45年）において，公害問題対策を徹底するために改正，整備された．公害対策基本法の改正を含む公害関係14法案が提出され，そのすべてが可決成立した．当初，公害対策基本法の第1条では「生活環境の保全については，経済の健全な発展との調和が図られるようにするものとする」（経済発展との調和条項）とされていたが削除され，公害防止のために環境保全を経済活動より優先する姿勢を明確にしたものである．また公害の範囲に土壌汚染が加わり，典型七公害となった．これは環境基本法第2条に引き継がれており，大気汚染防止法，水質汚濁防止法，土壌汚染対策法，騒音規制法，振動規制法，悪臭防止法，工業用水法などにより規制が実施されている．

本稿では特に重要な大気汚染防止法，水質汚濁防止法，土壌汚染対策法についてポイントを解説する．

□ 大気汚染防止法

大気汚染防止法において，国内全域を対象として一律の基準が定めており，さらに公害問題の地域性を考慮して，地方公共団体が条例によってより厳しい排出基準を定めることができる．また環境基準が健康保護，生活環境保全のうえで，排出規制を通して維持されることが望ましい施策目標の基準として定められている．固定発生源から排出，飛散する大気汚染物質について，物質ごと，施設の種類ごとに排出基準が定められており，事業者は発生施設の設置にあたって事前の届け出を行う必要がある．事業者は実際の排出濃度，総量の測定を実施し，必要な場合には報告，立ち入り検査を受け入れなければならない．

規制対象はばい煙，揮発性有機化合物，粉じん，有害大気汚染物質である．ばい煙は，ボイラー，廃棄物焼却炉等における燃料や鉱石等の燃焼に伴い発生する硫黄酸化物（SOx），ばいじん（スス），また精錬や燃焼施設より発生する有害物質（カドミウム，塩素，フッ素，鉛，窒素酸化物（NOx））を含む．このうちSOx，NOxは総量規制も設定される．緊急時の措置として，光化学オキシダントによる大気汚染が深刻な状態になったときは，都道府県知事は，一般にその事態を周知

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させるとともに，排出者に対して，排出量の削減を要請することとなっている．京都府の例では，住民や小中高等学校，幼稚園等関係者に伝達を図り，大規模排出者に排出量の削減を要請し，光化学大気汚染が原因とみられる被害を保健所，市町において把握する対策が策定されている[1]．平成21年の光化学大気汚染によると思われる被害の届け出は，全国12県で合計910人であった．揮発性有機化合物（VOC）はトルエン，キシレン，酢酸エチルなどの大気中に排出され，または飛散したときに気体である有機化合物を指し，2004年から規制されている．SOx，NOxによる光化学オキシダント，浮遊粒子状物質の発生にVOCが寄与するため，対策がなされている．特定施設と大規模施設は使用施設の設置にあたり届け出，また排出物質の測定義務が求められる．一方で自主的取組みを促進させるため（ベストミックス），事業者がVOCの排出抑制設備を取得した場合に税制優遇措置，特別融資を受けられる制度が平成17年から設けられている．粉じんは人の健康に被害を生じるおそれのある物質を特定粉じん，それ以外の粉じんを一般粉じんとして定められている．粉じん発生施設の設置もやはり届け出が必要である．特定粉じんは石綿が指定されているのみである．事業場の敷地境界基準として10本/リットル，建築物解体時の除去，囲い込み，封じ込め作業に関する基準があり，測定義務と遵守が求められている．有害大気汚染物質は，低濃度であっても長期的な摂取により健康影響が生ずるおそれのある物質のことを指す．確固たる証拠がまだないことから，基本的に自主的な排出抑制の取組みを求めており，排出状況の把握，国による科学的知見の充実させることになっている．そのうちベンゼン，トリクロロエチレン，テトラクロロエチレンは指定物質と指定されており，健康影響の未然防止のためクリーニング施設などに排出抑制基準が定められている．

大気汚染防止法では違反に対して，改善命令を経ず罰する直罰規定の導入されており，また無過失であっても健康被害が生じた場合における事業者の損害賠償責任を定め（無過失責任），被害者の保護を図ることとされている．しかし，このような規制による公害防止の取組みに反して，環境管理データの改ざんや虚偽報告が近年生じている．平成18年5月22日に神戸製鋼所は加古川製鉄所と神戸製鉄所で大気汚染防止法で定めるSOx，NOxの排出基準超過に加えて，記録保管用データの改ざんを行っていたことを公表した[2]．このことから大気汚染防止法及び水質汚濁防止法の一部を改正する法律（平成22年法律第31号）が制定された．これによりばい煙の測定結果の改ざん，破棄等に対する罰則の創設，また改善命令等の要件の見直しがなされ，改善命令等の発動要件のうち「その継続的な排出により人の健康又は生活環境に係る被害を生ずると認めるとき」が削除され，「排出基準等に適合しないばい煙を継続して排出するおそれがあると認めるとき」は，施設の構造の改善命令を出すことができるとされた．

□ 水質汚濁防止法

水質汚濁防止法において特定事業場に対して排水規制を課している．平成20年度において排水規制の対象となる特定事業場の数は全体で約27万7,000であり，もっとも多い業種は旅館業で約25%を占めており，次いで畜産農業，自動式車両洗浄施設であった．一律排水基準では健康項目で重金属，有機塩素溶剤などの有害物質を，生活環境項目で生物化学的酸素要求量（BOD），ノルマルヘキサン抽出物質含有量（脂質），大腸菌群数が定められている．また同様の項目が維持すべき環境基準として定められている．平成20年度公共用水域水質測定結果では，全国の類型指定水域の3,331水域（河川2,560，湖沼181，海域590）BODの環境基準の達成状況は，河川で92.3%，湖沼で53.0%，海域で76.4%となっており，湖沼では水質改善が進んでいない．下水道への放流について下水道法において定められており，水質基準項目はほぼ同じであるが，基準値が異なる．このほか要監視項目が定められており，「人の健康の保護に関連する物質ではあるが，公共用水域等における検出状況等からみて，直ちに環境基準とはせず，引き続き知見の集積に努めるべき物質」として，クロロホルム，フェニトロチオン，ニッケルなど25物質が定められている．また要

調査項目は，個別物質ごとの水環境リスクは比較的大きくない，または不明であるが，環境中での検出状況や複合影響等の観点からみて，水環境リスクに関する知見の集積が必要な物質として，アクリルアミド，アルキルフェノール，エンドスルファンなど工業原料，農薬など300物質が選定されており，情報収集，調査が実施されている．

規制事業者の排水基準の順守義務を負い，排水測定，記録の保管義務がある．また都道府県は排水監視義務があり，適宜立ち入り調査を実施し，必要に応じて改善命令，排水停止命令を出す．一律基準のほか，地方自治体は条例により，対象となっていない事業所，項目を追加する「横出し規制」を認められており，基準を強化する「上乗せ規制」も可能である．大気汚染防止法と同様に違反に対する直罰規定，無過失責任主義が導入されている．一方で，平成17年2月，3月にJFEスチール東日本製鉄所と昭和電工千葉事業所が排水データを改ざんしていたことが発覚した．このことから大気汚染防止法とともに改正が行われて，データ改ざんへの罰則の追加，改善命令の用件の見直しが行われた．

□ 土壌汚染対策法

土壌汚染対策法において土壌汚染の状況を把握するため，汚染の可能性のある土地について，一定の契機をとらえて調査を行い，汚染が合った場合に健康被害の防止のため，汚染の除去等の措置命令を行うとしている．

調査対象は，使用が廃止された有害物質使用特定施設の敷地，土壌汚染により人の健康被害が生ずるおそれがあると都道府県知事が認める土地であり，土地の所有者等は，環境大臣が指定する指定調査機関に調査させて，結果を報告しなければならない．土壌における環境基準設定物質は水質汚濁防止法における健康保護に関する環境基準の対象物質にほぼ等しく，重金属や塩素系溶剤などである．汚染の除去等の措置命令として，都道府県知事は，所有者等に対し，汚染の除去等の措置を命令することができ，汚染原因者が明らかな場合は，汚染原因者に措置を命令することができる．汚染対策には立ち入り制限，覆土，舗装，汚染土壌の封じ込め，浄化，掘削除去などがあるが，もっぱら掘削除去が行われ，多大な費用を要する．汚染の除去等の措置に要した費用の請求は，汚染原因者に費用を請求する．土壌汚染指定区域内の土地を宅地造成，土地の掘削，土壌の採取などの形質変更する場合は，都道府県知事に届け出し，施行方法が基準に適合しないと認めるときは，計画の変更を命令する．

土壌汚染対策法の一部を改正する法律（平成21年法律第23号）が公布され，大きな改正となった．近年，事業体の自主調査によっても土壌汚染が判明する場合も多く，この場合に土地所有者等が都道府県知事に区域の指定を申請することになった．また3,000 m^2以上の土地の形質変更を行おうとする場合に，都道府県知事が土壌汚染のおそれがあると認めた範囲について，土壌調査義務が設けられた．改善命令については，土壌汚染の摂取経路があり，健康被害が生ずるおそれがあるため，汚染の除去等の措置が必要な区域を「要措置区域」とし，除去等の命令がなされる．一方で土壌汚染の摂取経路がなく，健康被害が生ずるおそれがないため，汚染の除去等の措置が不要な区域を「形質変更時要届出区域」として区別するようになった．ヒトへの曝露経路を十分に遮断できれば，必ずしも土壌の入れ替えまで行うことはないという考え方を明確にしている．また汚染土壌の適正処理の確保のため，汚染土壌を措置実施区域外へ搬出する場合に，都道府県知事への事前届け出，基準遵守，汚染土壌処理業許可事業者への委託を義務付けられた．

環境汚染が生じた場合，その現状復帰は多大な費用を要する．かつては廃棄物，下水道のように国ひいては国民が負担してきた（共同負担原則）．しかし経済協力開発機構は昭和47年「環境政策の国際経済的側面に関する指導原則」を採択し，「汚染者負担原則」を提起した．日本においては昭和48年に公害健康被害補償法（昭和45年法律第133号）が制定された．近年では島根県馬潟団地周辺水路の底質ダイオキシン類汚染について，産業廃棄物処理業者や工場が浄化費用を負担して浄化が行われている．このような負担が多大であることが，事業体にとって，汚染を防止するよう

にインセンティブを与えることが期待されている．

□ 今後の課題

以上のような対策が現在行われているが，基準の達成にはまだ遠い．近年では発展の著しい中国からの越境汚染も考慮する必要もあり，日中韓三ヵ国環境大臣の合意に基づく研究協力も始まっている．土壌汚染については未然防止の観点から，有害物質の使用施設の廃止時点の調査のみではなく，調査機会を増やすことが重要となっていくであろう．

文　献

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