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**Polychlorinated Biphenyl-Mediated Decrease in Serum Thyroxine Level in Rodents**

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Abbreviations: PCB, polychlorinated biphenyl; KC500, Kanechlor-500; CB126, 3,3',4,4',5-pentachlorobiphenyl; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; UDPGT, UDP-glucuronosyltransferase; T<sub>4</sub>, thyroxine; T<sub>3</sub>, triiodothyronine; TSH, thyroid-stimulating hormone; HPLC, high-performance liquid chromatography; TBG, thyroxine binding protein; TTR, transthyretin.

## 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, while 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, while 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 for the first time indicate that 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.

## Introduction

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 (Duigman et al., 1987, 1988).

In general, PCBs, including 3,3',4,4',5-pentachlorobiphenyl (CB126) and Aroclor 1254, have abilities to decrease serum thyroid hormone levels in rats and mice, and the decreases are thought to occur through the induction of thyroxine (T<sub>4</sub>)-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 that the PCB-mediated reduction of the serum T<sub>4</sub> level in rats and mice is not necessarily correlated with an increase in hepatic T<sub>4</sub> glucuronidation activity (Kato et al., 2003), that Kanechlor-500 (KC500)-treatment results in a significant decrease in the level of serum total T<sub>4</sub> not only in Wistar rats but also in Gunn rats (UGT1A-deficient Wistar rats) (Kato et al., 2004, 2007), and that the KC500-mediated decrease in rats occurs through an increase in the accumulation level of T<sub>4</sub> in the liver rather than an increase in hepatic T<sub>4</sub>-UDPGT activity (Kato et al., 2007). To date, however, only limited data are available which 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,

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such as decreases in the levels of serum thyroid hormones, induction of hepatic T<sub>4</sub>-UDPGT, and increase in hepatic accumulation level of T<sub>4</sub>, in mice, hamsters, rats, and guinea pigs. On the basis of the obtained results, a mechanism underlying the PCB-mediated decrease in serum T<sub>4</sub> 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<sub>4</sub> (greater than 95% radiochemical pure as determined by HPLC, specific activity: 150  $\mu$ Ci / $\mu$ g T<sub>4</sub>), radiolabeled at the 5'-position of the outer ring, was obtained from Perkin Elmer Life and Analytical Sciences (Waltham, MA). The KC500 used in the present experiments contains 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 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  $\pm$  1°C; humidity, 55  $\pm$  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).

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**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  $-85^{\circ}\text{C}$  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  $-50^{\circ}\text{C}$  until use.

*Analysis of serum hormones.* Levels of total  $\text{T}_4$ , free  $\text{T}_4$ , total triiodothyronine ( $\text{T}_3$ ), and thyroid-stimulating hormone (TSH) were measured by radioimmunoassay using a Total  $\text{T}_4$  and Free  $\text{T}_4$  kit (Diagnostic Products Corporation; Los Angeles, CA), T-3 RIABEAD (DAINABOT Co., Ltd, Tokyo, Japan), and the rTSH [ $^{125}\text{I}$ ] Biotrak assay system (GE Healthcare, Little Chalfont, Buckinghamshire, UK), respectively.

*Hepatic microsomal  $\text{T}_4$ -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  $\text{T}_4$  ( $\text{T}_4$ -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

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body was warmed to 37°C. Fifteen minutes later, the animals were given intravenously [<sup>125</sup>I]T<sub>4</sub> (15 μCi /ml) dissolved in saline containing 10 mM NaOH and 1 % normal animal serum. The doses of [<sup>125</sup>I]T<sub>4</sub> were 0.1 ml for mice, 0.6 ml for hamsters, 1 ml for rats and 2 ml for guinea pigs, respectively. These dose of [<sup>125</sup>I]T<sub>4</sub> administered was calculated on the base of the dose used for rats by Vansell and Klaassen (2001).

*Clearance of [<sup>125</sup>I]T<sub>4</sub> from serum.* Clearance of [<sup>125</sup>I]T<sub>4</sub> from serum was measured according to the method of Oppenheimer et al. (1968). In brief, after the administration of [<sup>125</sup>I]T<sub>4</sub>, 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 [<sup>125</sup>I]T<sub>4</sub> by a gamma counter (COBRA II AUTO-GAMMA 5002; Perkin Elmer Life and Analytical Sciences).

*Biliary excretion of [<sup>125</sup>I]T<sub>4</sub>.* After the administration of [<sup>125</sup>I]T<sub>4</sub>, bile was collected in glass tube on ice for 2 h at 30-min intervals. Bile volume was determined gravimetrically. For analysis of biliary total [<sup>125</sup>I]T<sub>4</sub> level, two aliquots (10-30 μl each) were taken from each bile sample for determination of [<sup>125</sup>I]T<sub>4</sub> level by a gamma counter (COBRA II AUTO-GAMMA 5002; PerkinElmer Life and Analytical Sciences). The amount of biliary [<sup>125</sup>I]T<sub>4</sub> glucuronide was determined with high-performance liquid chromatography (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 12,000 g (4°C) for 10 min, the resultant supernatant was collected for HPLC analysis. The HPLC analysis was performed using a ChromSpher C18 column (10×0.3 cm) (Chrompack, Inc., Raritan,



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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 [<sup>125</sup>I]T<sub>4</sub> 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 increase to 45% over 5 min and held for 11 min. The levels of biliary [<sup>125</sup>I]T<sub>4</sub> glucuronide were determined by a Radioisotope Detector 171 (Beckman Coulter, Fullerton, CA).

To further identify [<sup>125</sup>I]T<sub>4</sub> glucuronides, the disappearance of a peak responsible for [<sup>125</sup>I]T<sub>4</sub> 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 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 [<sup>125</sup>I]T<sub>4</sub> derivatives.

*Analysis of [<sup>125</sup>I]T<sub>4</sub> bound to serum proteins.* The levels of serum [<sup>125</sup>I]T<sub>4</sub>-thyroxine binding protein (TBG), [<sup>125</sup>I]T<sub>4</sub>-albumin, and [<sup>125</sup>I]T<sub>4</sub>-transthyretin ([<sup>125</sup>I]T<sub>4</sub>-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

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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<sub>4</sub> 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.). The levels of [ $^{125}$ I]T<sub>4</sub>-TBG, [ $^{125}$ I]T<sub>4</sub>-albumin, and [ $^{125}$ I]T<sub>4</sub>-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<sub>4</sub>.* Tissue distribution of [ $^{125}$ I]T<sub>4</sub> was assessed according to the modified method of Oppenheimer et al. (1968). In brief, at 60 min after administration of [ $^{125}$ I]T<sub>4</sub> 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; Perkin Elmer Life and Analytical Sciences), and amounts of [ $^{125}$ I]T<sub>4</sub> 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<sub>4</sub> from the serum, amount of biliary [ $^{125}$ I]T<sub>4</sub> glucuronide, and the binding level of [ $^{125}$ I]T<sub>4</sub> to serum proteins were statistically analyzed according to the Newman-Keuls test after

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analysis of variance. The pharmacokinetic parameters of [ $^{125}\text{I}$ ]T<sub>4</sub> were estimated with noncompartmental methods as described previously (Tabata et al., 1999).

## 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 (Figure 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 ( $ED_{50}$ ) of KC500 for decreasing the level of serum total  $T_4$  was about 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 (Figure 2). Treatment at a dose of 100 mg/kg resulted in a more effective decrease in the 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 (Figure 3). On the other hand, no significant change in the level of serum TSH by the KC500 treatment was observed in any animals examined (Figure 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<sub>4</sub>-UDPGT by the treatment with KC500 at a dose of 37.5 mg/kg was observed in only guinea pigs among the rodents examined (Figure 4).

**Biliary Excretion of [<sup>125</sup>I]T<sub>4</sub> Glucuronide.** Effects of pretreatment of KC500 (37.5 mg/kg) on biliary excretion of T<sub>4</sub>-glucuronide were examined in mice, hamsters, rats and guinea pigs. After intravenous injection of [<sup>125</sup>I]T<sub>4</sub> to the KC500 -pretreated animals, biliary excretion levels of T<sub>4</sub>-glucuronide were measured. A significant increase in the biliary excretion level was observed only in rats among the species of animals examined (Figure 5).

**Clearance of [<sup>125</sup>I]T<sub>4</sub> from Serum.** After an intravenous administration of [<sup>125</sup>I]T<sub>4</sub> to the KC500 (37.5 or 100 mg/kg)-pretreated mice, hamsters, rats and guinea pigs, serum concentrations of [<sup>125</sup>I]T<sub>4</sub> in the animals were measured at the indicated times (Figure 6). KC500 (100 mg/kg)-pretreatment clearly enhanced the clearance of [<sup>125</sup>I]T<sub>4</sub> from the serum in all the animals tested. Within 5 min after the administration of [<sup>125</sup>I]T<sub>4</sub>, concentration of serum [<sup>125</sup>I]T<sub>4</sub> in mice, hamsters, rats and guinea pigs were about 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 [<sup>125</sup>I]T<sub>4</sub> was observed in the animals with the exception of mice.

The serum pharmacokinetic parameters of the [<sup>125</sup>I]T<sub>4</sub> estimated from these data (Figure 6) were summarized in Table 1. The mean total body clearance of [<sup>125</sup>I]T<sub>4</sub> and steady-state volumes of distribution in the KC500 (100 mg/kg)-pretreated mice, hamsters, rats and guinea pigs increased, as compared with the corresponding control

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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-times, 4.0-times, 4.1-times and 1.8-times over the corresponding control animals, respectively (Table 1).

**Tissue Distribution of [<sup>125</sup>I]T<sub>4</sub>.** Effects of KC500 (100 mg/kg)-pretreatment on the tissue-to-serum concentration ratio (K<sub>p</sub> value) and the distribution level of [<sup>125</sup>I]T<sub>4</sub> in tissues after the administration of [<sup>125</sup>I]T<sub>4</sub> were examined in mice, hamsters, rats and guinea pigs. In all animals examined, the thyroid gland, liver, kidney, stomach, duodenum and jejunum had K<sub>p</sub> values over 1 (Figure 7), and the K<sub>p</sub> 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<sub>p</sub> values of thyroid gland, liver, kidney and duodenum in all the animals examined (Figure 7).

In KC500-untreated (control) each species of animals, the accumulation level of [<sup>125</sup>I]T<sub>4</sub> in the liver was the highest among the tissues examined (Figure 8). In all the animals examined, pretreatment with KC500 (100 mg/kg) resulted in significant increases in the accumulation level of [<sup>125</sup>I]T<sub>4</sub> in the liver. More than 34%, 55%, 58% and 17% of the [<sup>125</sup>I]T<sub>4</sub> dosed were accumulated in the liver in the KC500-pretreated mice, hamsters, rats and guinea pigs, respectively (Figure 8). In addition, the accumulation level per g of liver was also increased in the KC500-pretreated mice, hamsters, rats and guinea pigs, as compared with the corresponding control animals (Table 2). Furthermore, KC500-pretreatment led to significant increases in the liver

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weight in mice, rats, and guinea pigs, but not in hamsters (Table 3).

**Serum Proteins Bound to [<sup>125</sup>I]T<sub>4</sub>.** The effects of pretreatment with KC500 (100 mg/kg) on the binding of [<sup>125</sup>I]T<sub>4</sub> to serum proteins, such as TBG, albumin, and TTR, were examined in mice, hamsters, rats and guinea pigs (Figure 9). In KC500-pretreated hamsters, the level of serum [<sup>125</sup>I]T<sub>4</sub>-TTR complex slightly decreased, while the binding level of [<sup>125</sup>I]T<sub>4</sub> 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 [<sup>125</sup>I]T<sub>4</sub>-TTR complex, while it led to significant increases in the level of [<sup>125</sup>I]T<sub>4</sub> bound to albumin. In addition, most of [<sup>125</sup>I]T<sub>4</sub> released from [<sup>125</sup>I]T<sub>4</sub>-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 [<sup>125</sup>I]T<sub>4</sub> was detected as free (serum protein-unbound) [<sup>125</sup>I]T<sub>4</sub>.

## 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).

As a possible explanation for the PCB-induced decrease in serum thyroid hormones, a hepatic  $T_4$ -UDPGT-dependent mechanism is generally considered because  $T_4$ -UDPGT inducers, such as Aroclor 1254, TCDD, and CB126, show strong activities for decreasing the levels of serum total thyroid hormones in rats (Barter and Klaassen, 1994; Schuur et al., 1997; Van Birgelen et al., 1995). 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 herein 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



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all the species of animals examined. In addition, no significant changes in the excretion level of biliary T<sub>4</sub>-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<sub>4</sub> level in mice, hamsters, rats, and guinea pigs primarily occurs in a T<sub>4</sub>-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<sub>4</sub>. These results are similar with those in previous reports on the effect of PCBs on the level of serum TSH in rats (Hallgren et al., 2001; Hood et al., 1999; Liu et al., 1995, Kato et al., 2004, 2007).

The factors regulating the level of serum total T<sub>4</sub>, 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 also observed in the KC500-treated mice, hamsters, rats, and guinea pigs (data not shown). Therefore, a KC500-mediated decrease in the serum T<sub>4</sub> level seems to occur in the type-I iodothyronine deiodinase- and sulfotransferase-independent pathways.

As another possible mechanism for the PCB-induced decrease in the level of serum total T<sub>4</sub>, a TTR-associated pathway might also be considered because PCB and its ring-hydroxylated metabolites act as T<sub>4</sub> 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<sub>4</sub> through an

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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, 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 and further propose a hypothesis 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

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serum T<sub>4</sub>-TTR complex.

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### **Footnotes**

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### Legends for figures

Fig. 1. Effect of graded doses of KC500 on the level of serum total T<sub>4</sub> 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<sub>4</sub> was measured as described in *Materials and Methods*. Constitutive level:  $3.24 \pm 0.25$  µg/dl ( $n=5$ ). Each point represents the mean  $\pm$  S.E. (vertical bars) for four to six mice. \* $P<0.05$ , significantly different from the control.

Fig. 2. Effects of KC500 on the levels of serum total T<sub>4</sub> and free T<sub>4</sub> 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 in *Materials and Methods*. Constitutive levels: total T<sub>4</sub>,  $2.98 \pm 0.17$  (mice,  $n=8$ ),  $2.67 \pm 0.17$  (hamsters,  $n=8$ ),  $3.73 \pm 0.32$  (rats,  $n=6$ ) and  $2.05 \pm 0.16$  µg/dl (guinea pigs,  $n=5$ ); free T<sub>4</sub>,  $0.43 \pm 0.05$  (mice,  $n=8$ ),  $1.08 \pm 0.08$  (hamsters,  $n=8$ ),  $1.47 \pm 0.11$  (rats,  $n=6$ ) and  $1.44 \pm 0.16$  ng/dl (guinea pigs,  $n=5$ ). Each column represents the mean  $\pm$  S.E. (vertical bars) for four to eight animals. \* $P<0.05$ , significantly different from each control.

Fig. 3. Effects of KC500 on the levels of serum total T<sub>3</sub> 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 in *Materials and Methods*. Constitutive levels: total T<sub>3</sub>,  $0.38 \pm 0.05$  (mice,  $n=6$ ),  $0.46 \pm 0.02$  (hamsters,  $n=6$ ),  $0.59 \pm 0.09$  (rats,  $n=6$ ) and  $0.25 \pm 0.02$  ng/ml (guinea pigs,  $n=6$ ); TSH,  $3.50 \pm$

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0.19 (mice,  $n=6$ ),  $3.46 \pm 0.22$  (hamsters,  $n=6$ ),  $5.45 \pm 0.61$  (rats,  $n=6$ ) and  $2.40 \pm 0.07$  ng/ml (guinea pigs,  $n=5$ ). Each column represents the mean  $\pm$  S.E. (vertical bars) for five to six animals.  $*P<0.05$ , significantly different from each control.

Fig. 4. Effect of KC500 on the activity of hepatic microsomal T<sub>4</sub>-UDP-GT 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<sub>4</sub>-UDP-GT enzyme assay, as described in *Materials and Methods*. Constitutive levels: T<sub>4</sub>-UDP-GT,  $21.22 \pm 1.02$  (mice,  $n=6$ ),  $21.83 \pm 2.36$  (hamsters,  $n=6$ ),  $15.39 \pm 2.96$  (rats,  $n=6$ ) and  $25.15 \pm 1.04$  pmol/mg protein/min (guinea pigs,  $n=6$ ). Each column represents the mean  $\pm$  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 [<sup>125</sup>I]T<sub>4</sub>-glucuronide in animals. KC500 (37.5 mg/kg) was intraperitoneally given to animals, and 96 h after the KC500-treatment, a portion of [<sup>125</sup>I]T<sub>4</sub> (15  $\mu$ Ci/ml) was further intravenously administered to animals, as described in *Materials and Methods*. The level of [<sup>125</sup>I]T<sub>4</sub>-glucuronide excreted was measured in bile collected at 30-min intervals after the intravenous administration of [<sup>125</sup>I]T<sub>4</sub>. Each point represents the mean  $\pm$  S.E. (vertical bars) for three to seven animals.  $*P<0.05$ , significantly different from each control. —○—, control; —●—, KC500.

Fig. 6. Effects of KC500 on the clearance of [<sup>125</sup>I]T<sub>4</sub> from serum in animals. KC500 (37.5 and 100 mg/kg) was intraperitoneally given to animals, and 96 h after the

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KC500-treatment, a portion of [ $^{125}\text{I}$ ]T<sub>4</sub> (15  $\mu\text{Ci/ml}$ ) was further intravenously administered to the animals, as described in *Materials and Methods*. The amount of serum [ $^{125}\text{I}$ ]T<sub>4</sub> was measured at the indicated times after the intravenous administration of [ $^{125}\text{I}$ ]T<sub>4</sub>. Each point represents the mean  $\pm$  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.

Fig. 7. Tissue-to-serum concentration ratio (K<sub>p</sub> value) of [ $^{125}\text{I}$ ]T<sub>4</sub> in various tissues after administration of [ $^{125}\text{I}$ ]T<sub>4</sub> to KC500-pretreated animals. KC500 (100 mg/kg) was intraperitoneally given to animals, and 96 h after the KC500-treatment, [ $^{125}\text{I}$ ]T<sub>4</sub> was further intravenously administered to the animals. At 60 min after the [ $^{125}\text{I}$ ]T<sub>4</sub> administration, the radioactivity in each tissue was measured, as described in *Materials and Methods*. Each column represents the mean  $\pm$  S.E. (vertical bars) for three to six animals. \* $P < 0.05$ , significantly different from each control. □, control; ■, KC500.

Fig. 8. Tissue distribution of [ $^{125}\text{I}$ ]T<sub>4</sub> after administration of [ $^{125}\text{I}$ ]T<sub>4</sub> to KC500-pretreated animals. Experimental protocols were the same as those described in the legend of Fig. 7. Each column represents the mean  $\pm$  S.E. (vertical bars) for three to six animals. \* $P < 0.05$ , significantly different from each control. □, control; ■, KC500.

Fig. 9. Effect of KC500 on the binding of [ $^{125}\text{I}$ ]T<sub>4</sub> to serum proteins in animals. KC500 (100 mg/kg) was intraperitoneally given to animals, and 96 h after the KC500-treatment, [ $^{125}\text{I}$ ]T<sub>4</sub> was further intravenously administered to the animals. The amounts of [ $^{125}\text{I}$ ]T<sub>4</sub> bound to the serum proteins 60 min after [ $^{125}\text{I}$ ]T<sub>4</sub> administration were assessed by the

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method as described in *Materials and Methods*. Each column represents the mean  $\pm$  S.E. (vertical bars) for three to four animals. \* $P < 0.05$ , significantly different from each control.

Table 1. Pharmacokinetic parameters for [<sup>125</sup>I]T<sub>4</sub> after the administration of [<sup>125</sup>I]T<sub>4</sub> to the KC500-pretreated animals

| Animal      | Pretreatment | Dose<br>(mg/kg) | Mean total body<br>clearance × 100<br>(ml/min) | Distribution<br>volume<br>(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*                  |

The pharmacokinetic parameters of [<sup>125</sup>I]T<sub>4</sub> were calculated from the data in Figure 6 with noncompartmental methods as described previously (Tabata et al., 1999). The values shown expressed as the mean ± S.E. for four to eleven animals. \*P<0.05 significantly different from each control.

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Table 2. Accumulation of [<sup>125</sup>I]T<sub>4</sub> in the KC500-pretreated mice, hamsters, rats and guinea pigs livers

| Animal      | [ <sup>125</sup> I]T <sub>4</sub> (% of dose/g liver) |               |
|-------------|---|---------------|
|             | Control   | KC500         |
| 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*  |

The radioactivity in the liver was measured at 60 min after the [<sup>125</sup>I]T<sub>4</sub>-administration, as described in *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. \**P*<0.05, significantly different from each control.

Table 3. Liver weights after the administration of KC500 to animals

| Animal      | Relative liver weight (% of body weight) |              |
|-------------|--|--------------|
|             | Control                                  | KC500        |
| 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* |

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

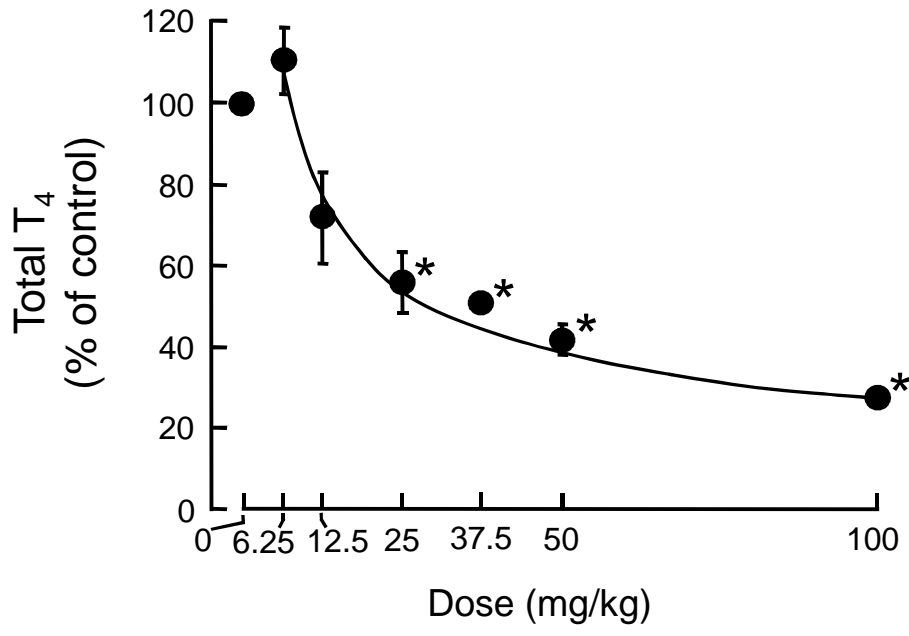


Fig. 1



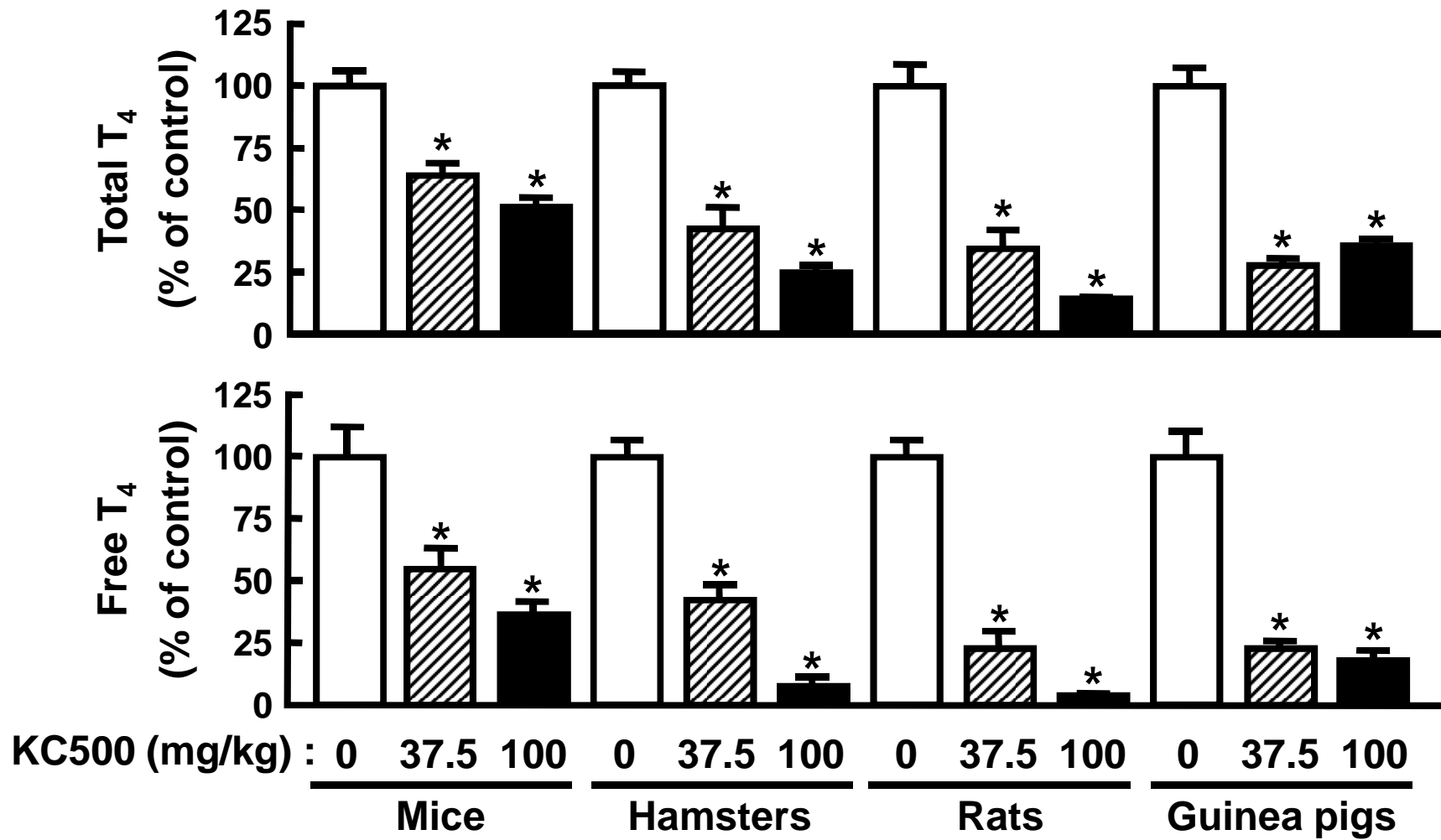


Fig. 2

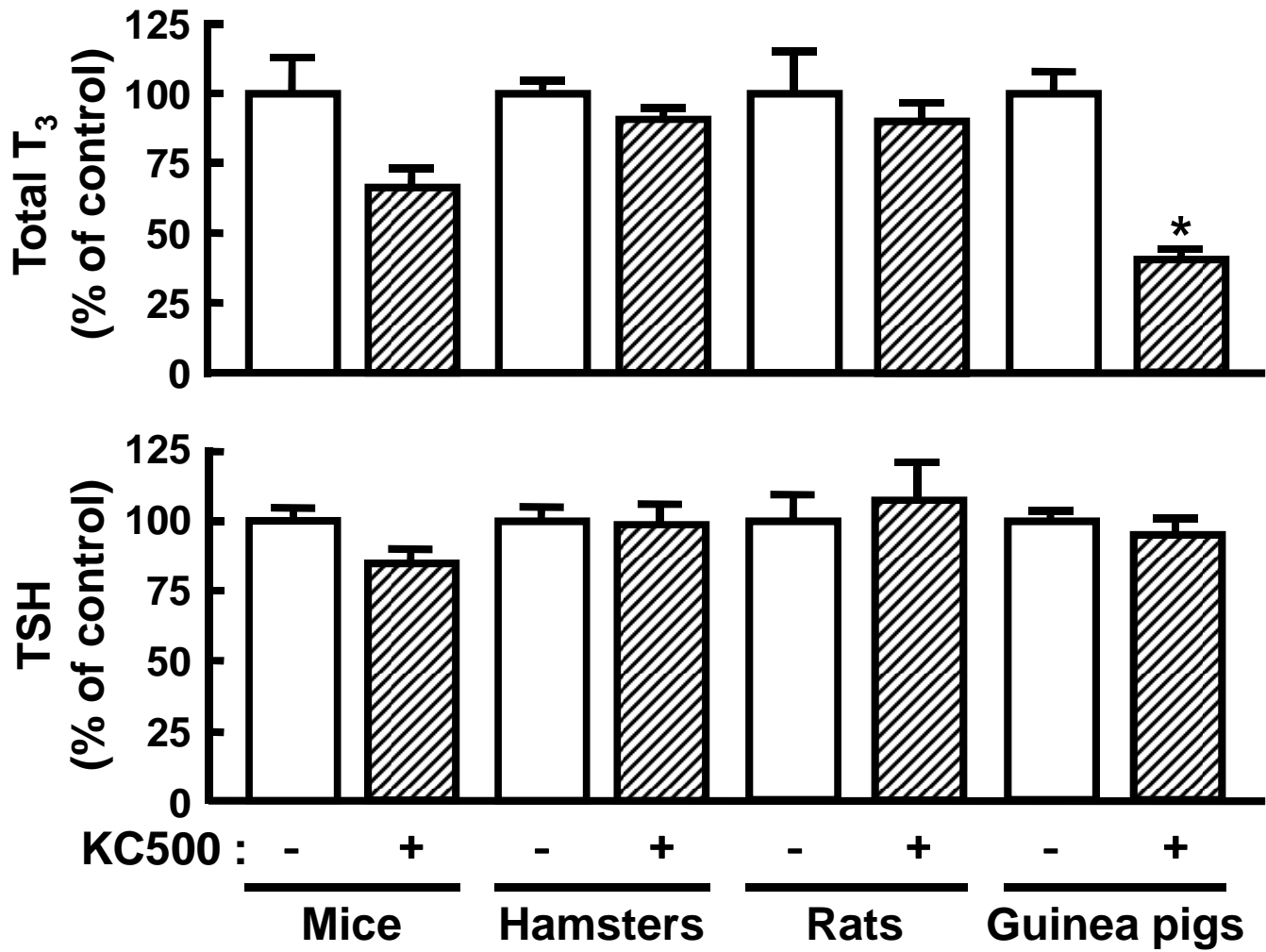


Fig. 3

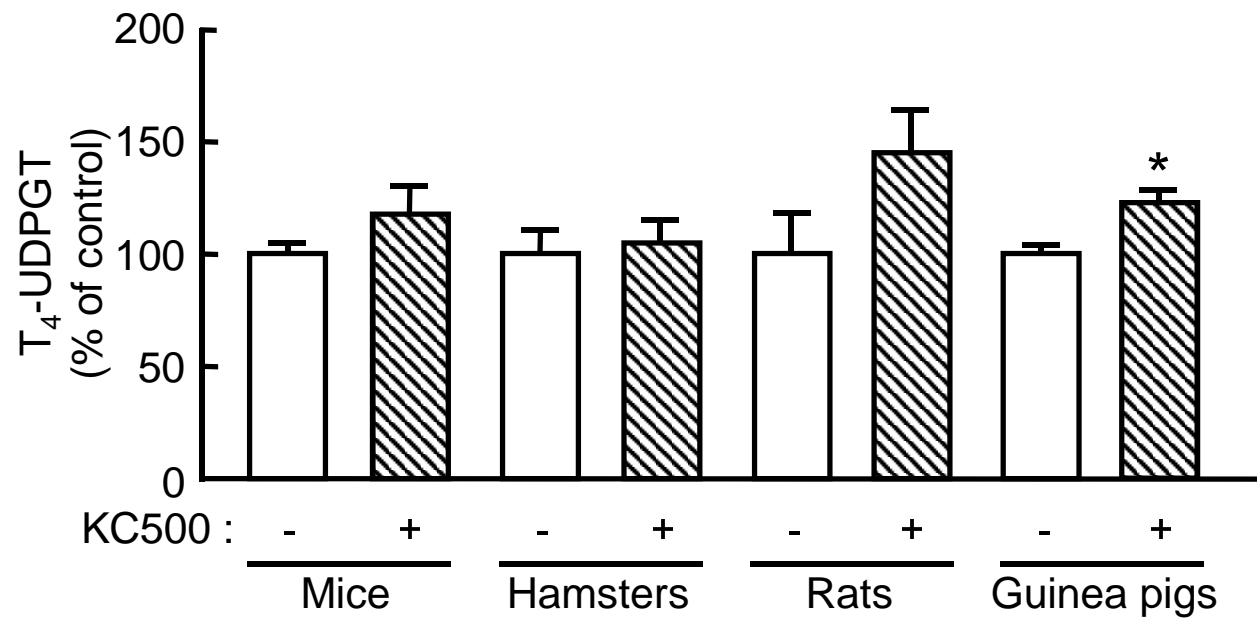


Fig. 4

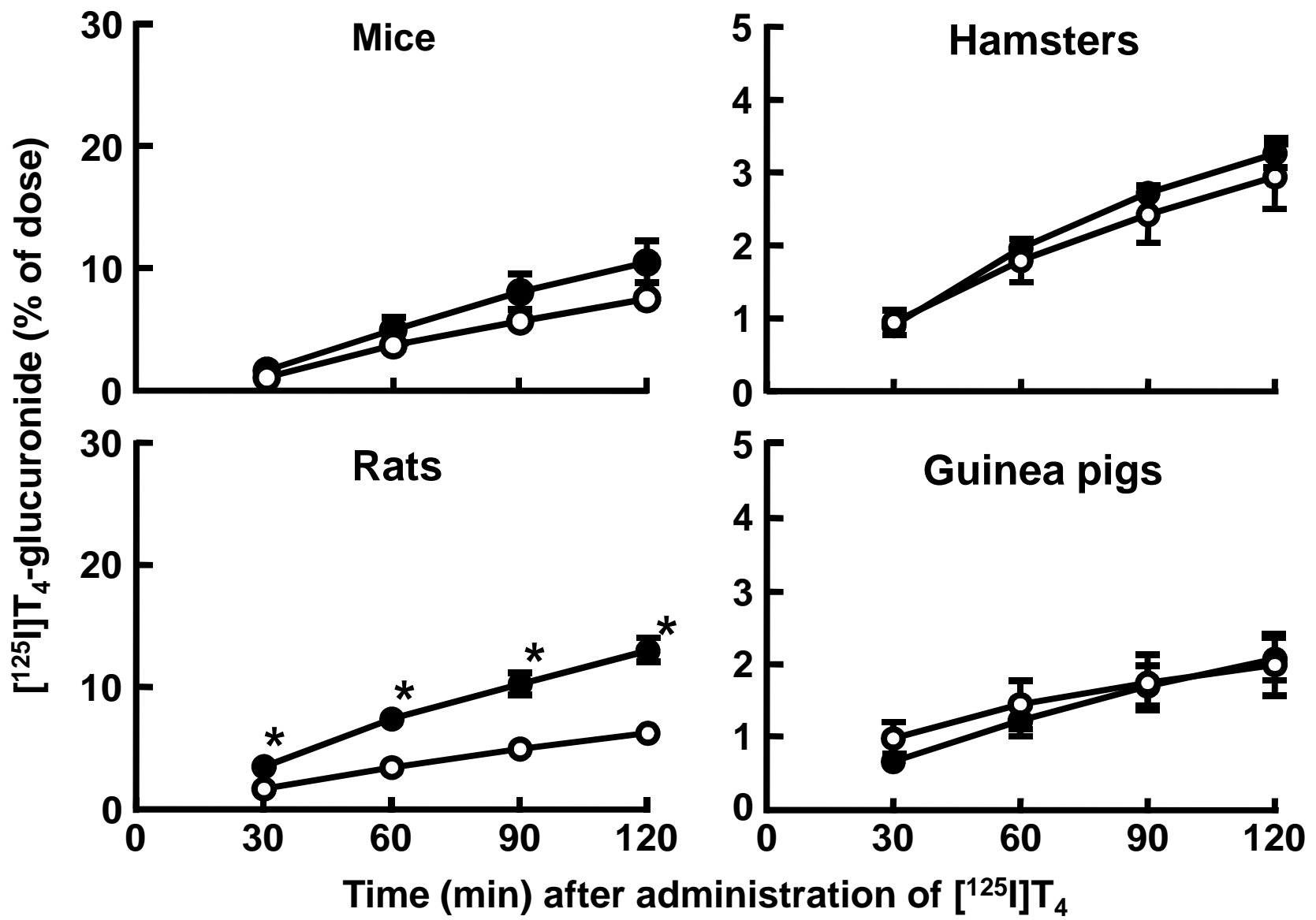


Fig. 5

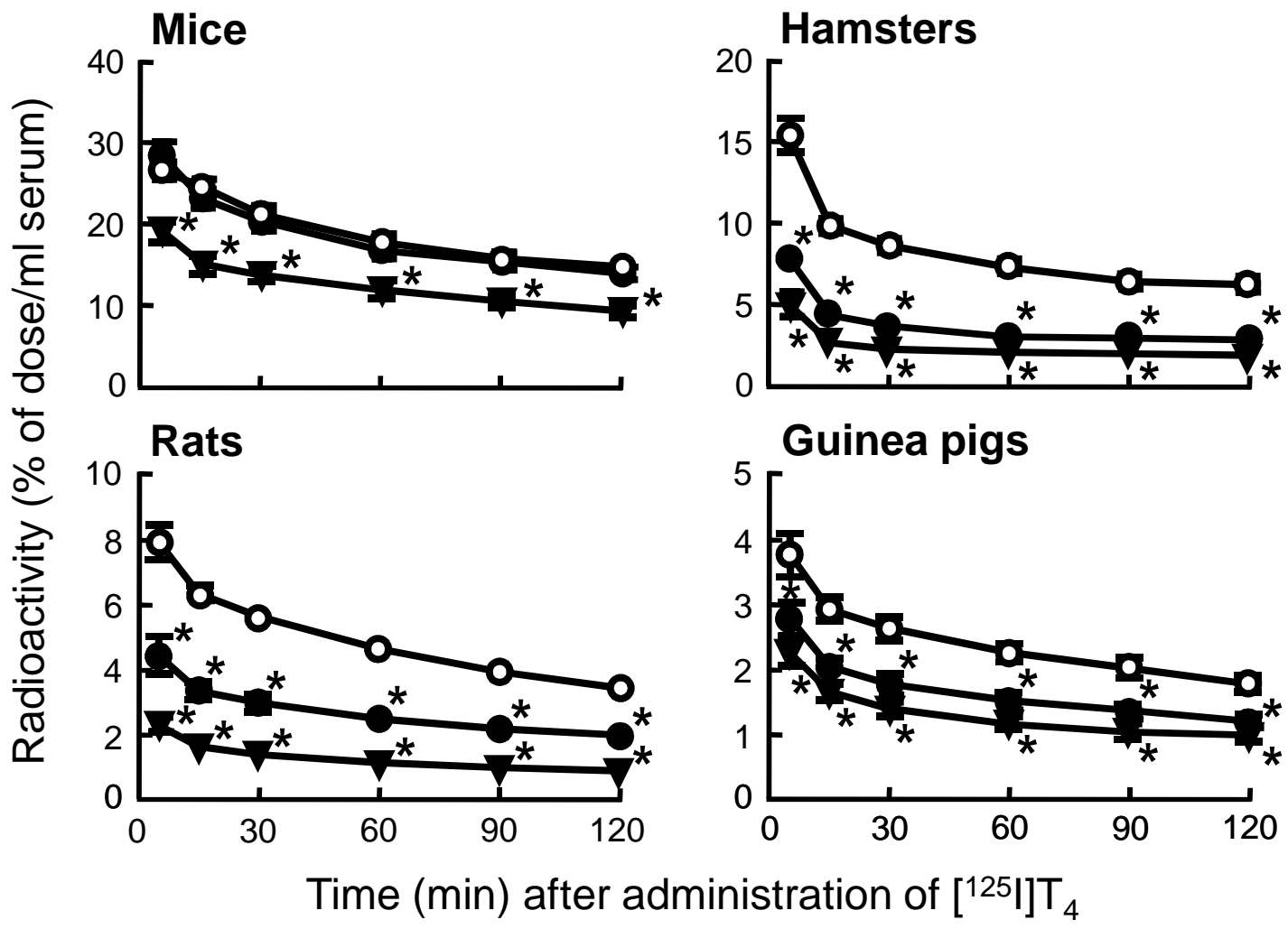


Fig. 6

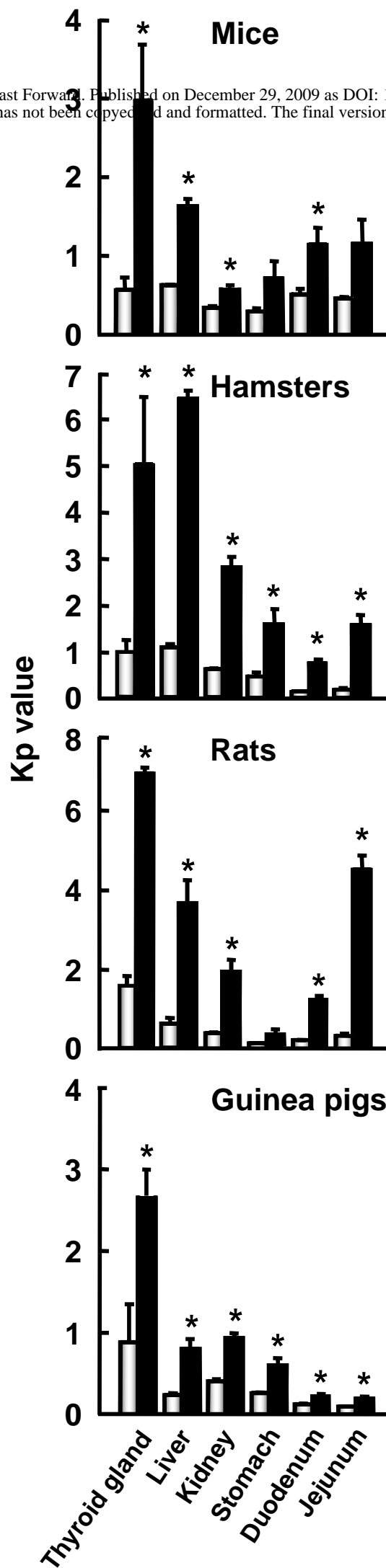


Fig. 7

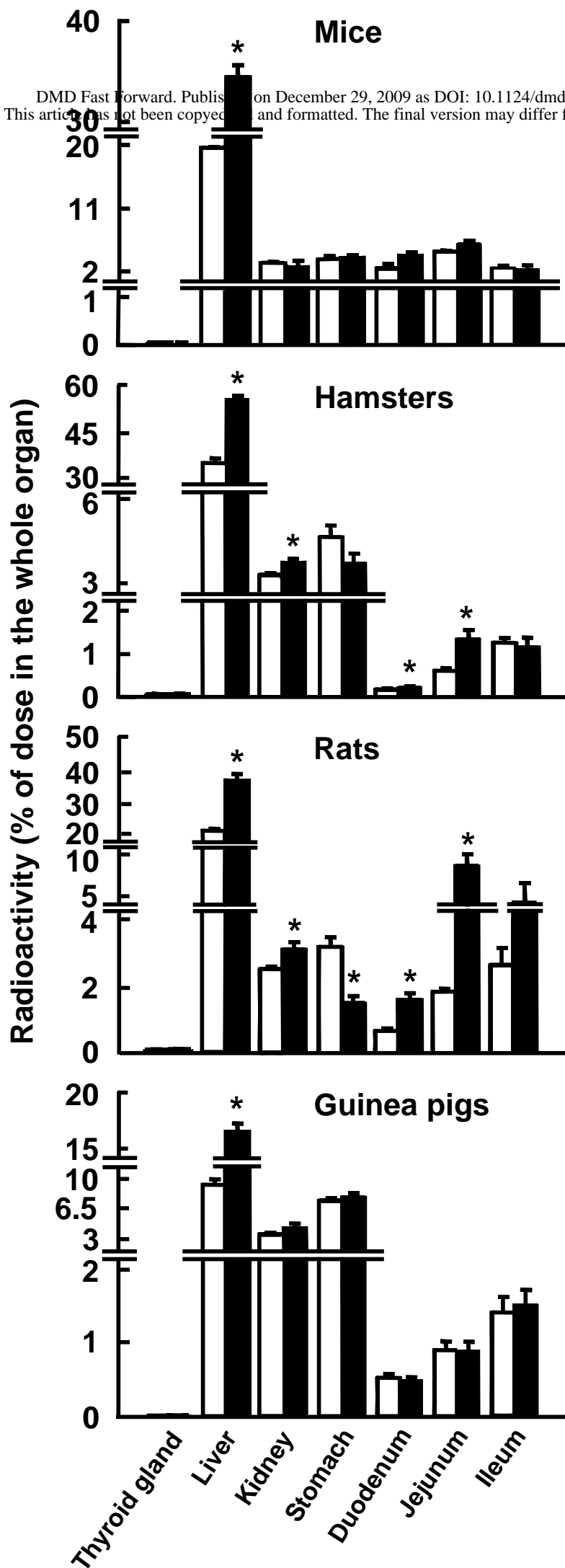


Fig. 8

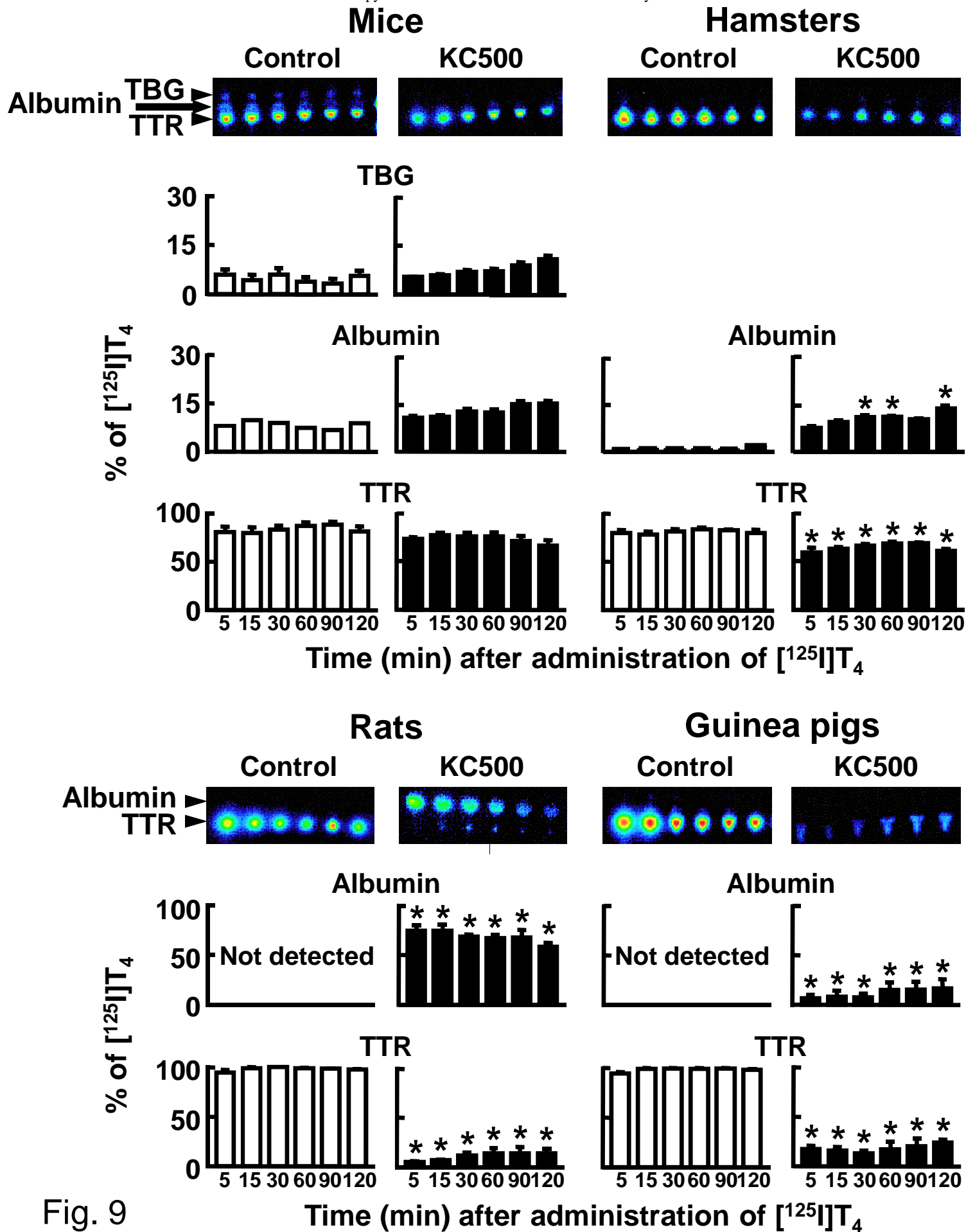


Fig. 9