ABSTRACT:
Metabolism of polychlorinated dibenzo-p-dioxins by cytochrome P450 (P450) and UDP-glucuronosyltransferase (UGT) was examined using a recombinant enzyme system and human liver microsomes. We analyzed the glucuronidation of 2,3,7-trichlorodibenzo-p-dioxin (2,3,7-triCDD) by rat CYP1A1 expressed in yeast microsomes and human UGT expressed in baculovirus-infected insect cells. Multiple UGT isozymes showed glucuronidation activity toward 8-hydroxy-2,3,7-triCDD (8-OH-2,3,7-triCDD), which was produced by CYP1A1. Of these UGTs, UGT1A1, 1A9, and 2B7, which are constitutively expressed in human livers, showed remarkable activity toward 8-OH-2,3,7-triCDD. The apparent kinetic parameters of glucuronidation, $K_m$ and $k_{cat}$, were estimated to be 0.8 μM and 1.8 min$^{-1}$, respectively, for UGT1A1, 0.8 μM and 1.8 min$^{-1}$, respectively, for UGT1A9, and 3.9 μM and 7.0 min$^{-1}$, respectively, for UGT2B7. In human liver microsomes with NADPH and UDP-glucuronic acid, 2,3,7-triCDD was first converted to 8-OH-2,3,7-triCDD, then further converted to its glucuronide. We compared the ability of 10 human liver microsomes to metabolize 2,3,7-triCDD and observed a significant difference in the glucuronidation of 2,3,7-triCDD that originated from the difference of the P450-dependent hydroxylation of 2,3,7-triCDD.

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POLYCHLORINATED DIBENZO-P-DIOXINS (PCDDs) are well known as environmental contaminants. Of the 75 types of PCDDs, 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-tetraCDD) is known to be the most toxic. In the last two decades, the metabolism of PCDDs has been studied in vivo using experimental animals and in vitro using liver microsomal fractions (Tulp and Hutzinger, 1978; Poiger et al., 1982; Tai et al., 1993; Petrovsky et al., 1997; Huwe et al., 1998; Hu and Bunce, 1999a,b; Hak et al., 2001). Our recent studies revealed that multiple forms of human P450s had high monooxygenase activity toward DD, 1-CDD, 2-CDD, and 2,3-DCDD (Inouye et al., 2002). In addition, CYP1A1, 1A2, and 1B1 showed high activity toward 2,7-DCDD and 2,3,7-triCDD. However, none of the P450s demonstrated activity toward 2,3,7,8-tetraCDD.

In addition to hydroxylated products of PCDD, other metabolites have been identified as glucuronide and sulfate conjugates (Tulp and Hutzinger, 1978; Wroblewski and Olson, 1985). The formation of glucuronides is an indispensable step in the detoxification of these lipophilic compounds, which are transformed into more hydrophilic metabolites and excreted in bile and urine. Glucuronidation of steroids, bile acids, bilirubin, hormones, drugs, and environmental toxicants is catalyzed by UDP-glucuronosyltransferases (UGTs), which have a gene family containing a huge amount of isozymes with different substrate specificity (Mackenzie et al., 1997). The UGTs use UDP-glucuronic acid (UDP-GlcUA) to transfer glucuronic acid to the substrates. Glucuronides can be formed through hydroxyl (alcoholic and phenolic), carboxyl, sulfuryl, carbonyl, and amino (primary, secondary, or tertiary) linkages. UGTs, therefore, accept a lot of agents as substrates for a range of substrate specificities. The glucuronidation of a huge number of compounds has been examined to identify UGT isozymes by using commercially available recombinant human UGT isozymes. The metabolites of PCDD are thought to be conjugated by some UGT isozymes, but human UGT isozymes catalyzing glucuronidation of PCDDs have not been identified.

In this study, we examined the additional metabolism of dioxins by...
the conjugating enzyme UGT following P450-dependent reaction. The metabolism of 8-OH-2,3,7-triCDD by 12 species of human UGTs was investigated by using a recombinant system. Because 8-OH-2,3,7-triCDD is a major metabolite of 2,3,7,8-tetraCDD in mammals (Poiger et al., 1982; Mason and Safe, 1986), metabolism of 8-OH-2,3,7-triCDD seems to be quite important in understanding the metabolism of 2,3,7,8-tetraCDD in mammals. Our previous studies revealed that 2,3,7-triCDD is readily converted into 8-OH-2,3,7-triCDD by P450s in the CYP1 family (Inouye et al., 2002; Sakaki et al., 2002; Shinkyo et al., 2003a). Thus, we examined the metabolism of 2,3,7-triCDD in human liver microsomes instead of 2,3,7,8-tetraDD, which is hardly metabolized. The results obtained from the recombinant systems for P450s and UGTs combined with those obtained from human liver microsomes will help us understand the metabolism of dioxins in the human body.

Materials and Methods

Materials. 2,3,7-triCDD was purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA). NADPH was purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan). UDP-GlcUA and Escherichia coli β-glucuronidase were purchased from Sigma-Aldrich (St. Louis, MO). Endoglycosidase H was purchased from New England Biolabs (Beverly, MA). Deuterized chloroform (CDCl3, 0.035%; tetramethylsilane, 99.8%; NMR analytical grade) was purchased from Merck (Darmstadt, Germany). Rat UGT1A1-specific anti-peptide antibody against a C-terminal peptide common to all UGT1A isozymes, designated anti-UGT1A1, was used for immunostaining of human UGT1A isozymes (Ikushiro et al., 1995, 2002). To prepare human UGT2B-specific anti-peptide antibody, the region of internal sequence (355-367, KWIQQDNLGHPK) in UGT2B7 deduced from cDNA sequence was selected (Jin et al., 1993a). The peptide was synthesized, and anti-peptide antibody against human UGT2B2, designated as anti-UGT2B2, was prepared from the immunized rabbit as described previously (Ikushiro et al., 1995). Human liver microsomes from 10 individual human livers (HH13, H112, HH47, HG95, HH18, HG43, HG74, HK25, HG89, and HG93) were purchased from BD Gentest (Woburn, MA). Recombinant human UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2B7, UGT2B4, UGT2B15, and UGT2B17 expressed in baculovirus-infected insect cells (Supersomes) and control microsomes from insect cells infected with Escherichia coli were purified by amylose-conjugated affinity chromatography. The concentration of the standard proteins (MBP-UGT1AC, 68 kDa; MBP-UGT2B7C, 67 kDa) was estimated by bicinchoninic acid protein assay.

Metabolism of 2,3,7-triCDD by Rat CYP1A1 and Human UGT1A9. Our previous studies (Sakaki et al., 2002; Shinkyo et al., 2003a,b) revealed that rat CYP1A1 had high 8-hydroxylation activity toward 2,3,7-triCDD. Thus, the recombinant yeast microsomes expressing rat CYP1A1 were used to obtain 8-OH-2,3,7-triCDD. The substrate 2,3,7-triCDD was dissolved in acetonitrile. The reaction mixture of 2,3,7-triCDD contained 0.5 mg of protein/ml of human UGT1A9 microsomes, 30 nM rat CYP1A1, 10 μM 2,3,7-triDD, 1 mM NADPH, 1 mM MgCl2, 1% acetone, and 2 mM UDP-GlcUA in 100 mM potassium phosphate buffer, pH 7.4. These reactions were performed at 37°C. Aliquots of the reaction mixture were extracted with 4 volumes of chloroform/methanol (3:1, v/v). The organic phase was recovered and carefully dried in a vacuum evaporator centrifuge (Sakura Seisakuso, Tokyo, Japan) without evaporating the substrates and metabolites. On the other hand, the equal volume of acetone was added to the aqueous phase and dried in a vacuum evaporator centrifuge. The resulting residue was solubilized with acetonitrile and water (1:1) and then applied to HPLC performed on a YMC-Pack ODS-AM [4.6 mm (inner diameter) × 300 mm] (YMC, Kyoto, Japan). A linear gradient of 10 to 100% acetonitrile aqueous solution containing 0.01% trifluoroacetic acid was used as a mobile phase. The flow rate was 1.0 ml/min, and the elution was detected by measuring absorption at 227 nm. The column temperature was maintained at 40°C. Because the metabolites observed in the aqueous phase appeared to be glucuronides, they were treated with β-glucuronidase. The putative glucuronides isolated from HPLC effluents were incubated for 1 h with 0.11 mg/ml β-glucuronidase in 20 mM potassium phosphate buffer, pH 7.4, at 37°C. The reaction was terminated by adding acetonitrile and 0.05 M HCl. The mixture was centrifuged at 10,000g for 15 min, and the supernatant was applied to HPLC.

Metabolism of 8-OH-2,3,7-triCDD by Recombinant Human UGTs. The substrate 8-OH-2,3,7-triCDD, which had been produced by rat CYP1A1 expressed in yeast cells, was dissolved in acetonitrile. The reaction mixture containing 0.05 mg of protein/ml of human liver microsomes, 10 μM 2,3,7-triDD, 1 mM MgCl2, 1% acetone, and 1 mM NADPH in 100 mM potassium phosphate buffer, pH 7.4, was incubated for 1 h at 37°C, and the metabolites were analyzed as described above. The conversion rate of 2,3,7-triCDD to 8-OH-2,3,7-triCDD was examined at the time of 0, 1, 2, 4, 8, 10, 15, 20, 30, and 60 min after the reaction started.

8-OH-2,3,7-triCDD glucuronidation in human liver microsomes in the presence of UDP-GlcUA. 8-OH-2,3,7-triCDD, which had been produced by rat CYP1A1 expressed in yeast cells, was dissolved in acetonitrile. The reaction mixture containing 0.5 mg of protein/ml of human liver microsomes, 2.5 μM 8-OH-2,3,7-triCDD, 1 mM MgCl2, 1% acetone, and 2 mM UDP-GlcUA in 100 mM potassium phosphate buffer, pH 7.4, was incubated for 1 h at 37°C, and the metabolite was analyzed as described above.

2,3,7-triCDD metabolism in human liver microsomes in the presence of NADPH and UDP-GlcUA. The reaction mixture containing 0.5 mg of protein/ml of human liver microsomes, 10 μM 2,3,7-triCDD, 1 mM MgCl2, 2 mM UDP-GlcUA, 1% acetone, and 1 mM NADPH in 100 mM potassium phosphate buffer, pH 7.4, was incubated for 1 h at 37°C, and the metabolites were analyzed as described above. The conversion rate of 2,3,7-triCDD to its glucuronide or 8-OH-2,3,7-triCDD was examined at the time of 0, 2, 4, 8, 10, 15, 20, 30, and 60 min after the reaction started. Metabolites were analyzed as described above.

Kinetic Analyses. The kinetic studies were performed using human liver microsomes (HG89), recombinant human UGT1A1, UGT1A9, and UGT2B7 expressed in baculovirus-infected insect cells. In determining the kinetic parameters, the 8-OH-2,3,7-triCDD concentration ranged from 0 to 10 μM. Kinetic parameters were determined on the basis of fitted curves using KaleidaGraph (Synergy Software, Reading, PA). The equation \( \frac{v}{k_{\text{cat}}} \times \left[ S \right] / \left[ S \right] / \left( K_m + \left[ S \right] \right) \) (eq. 1) was applied for Michaelis-Menten kinetics, where [S]
and \([E]_0\) are the substrate and UGT concentrations, respectively, and \(k_m\) and \(k_{cat}\) represent a Michaelis-Menten and catalytic rate constant, respectively.

**Mass Spectrometric Analysis of the Metabolites.** Isolated metabolites from HPLC effluents were subjected to GC-quadrupole ion-trap mass spectrometric analysis using a Finnigan mat Thermo Quest GCQ plus in the electron ionization mode. Chrompack Cpd-Sil 24 CB-MS (0.32 mm × 30 m) was used as a GC column.

**1H NMR Analysis.** The 500-MHz 1H NMR spectra of authentic standard of 2,3,7-triCDD and its metabolite were measured on a Varian VXR-500 (1H, 499.9 MHz). The standard compound (20 μg) and purified metabolite (20 μg) were dissolved in 300 μl of CDCl₃, with 0.03% tetramethylsilane and transferred into a probe. To obtain two-dimensional correlation spectroscopy spectra of authentic standard, 128 experiments resulting in 1 K data points for a spectral width of 1043 Hz were measured, and the time of domain data was multiplied with a squared sine-bell function.

**Metabolism of Phenacetin in Human Liver Microsomes.** The reaction mixture containing 0.4 mg of protein/mL of human liver microsomes, 0.1 mM phenacetin, 1% methanol, and 1 mM NADPH in 100 mM potassium phosphate buffer, pH 7.4, was incubated for 1 h at 37°C, and the metabolites were analyzed as described by Distlerath et al. (1985).

**Other Methods.** The concentrations of 2,3,7-triCDD and 8-OH-2,3,7-triCDD were estimated by using a molar extinction coefficient of 4.12 × 10⁴ M⁻¹ cm⁻¹ at 277 nm on the basis of spectral analysis. The activity for the formation of 2,3,7-triCDD glucuronide was estimated on the basis of the chromatographic peak area using 2,3,7-triCDD as a standard.

**Results**

**Immunoblot Analysis of the Recombinant UGT Isozymes and Human Liver Microsomes.** Immunoblot analysis using anti-UGT1A and UGT2B antibodies confirmed the expression of UGT isozymes in human hepatic microsomes and the recombinant system (Fig. 1). Anti-UGT1A antibody against a C-terminal peptide common to all rat UGT1A isozymes could cross-react with human UGT1A isozymes in hepatic microsomes and insect microsomes. To detect human UGT2B isozymes, anti-UGT2B antibody against the conserved peptide region in human UGT2B isozymes was developed. The anti-UGT2B cross-reacted with recombinant UGT2B4, 2B7, 2B15, and 2B17 in microsomes. Quantitative immunoblot analysis of UGT1A isozymes and UGT2B7 using the corresponding MBP-UGT fusion proteins enabled the contents of the UGTs in microsomes to be determined. Taken together with the data for relative expression levels of the UGT1A isozymes, the contents of UGT1A1, 1A3, 1A4, 1A6, 1A7, 1A8, 1A9, 1A10, and 2B7 in microsomes were estimated to be 1.7, 0.8, 1.4, 1.6, 1.0, 1.1, 1.0, 1.6, and 0.6 nmol/mg protein, respectively. On the basis of the assumption that the reactivity of the anti-UGT2B toward UGT2B4, 2B15, and 2B17 is similar to that of UGT2B7, the expression levels of UGT2B4, 2B15, and 2B17 were estimated to be 0.3, 0.3, and 0.6 nmol/mg protein, respectively. In addition, the contents of the UGT1A and UGT2B subfamily in the human liver microsomes (HG89) were estimated to be 0.3 and 0.5 nmol/mg protein, respectively.

**Metabolism of 2,3,7-triCDD by Recombinant Rat CYPIA1 and Human UGT1A9.** On the P450- and UGT-dependent metabolism of 2,3,7-triCDD, recombinant rat CYPIA1 expressed in yeast and human UGT1A9 expressed in baculovirus-infected insect cells were used in the presence of NADPH and UDP-GlcUA. The metabolites in the aqueous and organic phase were separately analyzed after extraction with 4 volumes of chloroform/methanol (3:1). As shown in Fig. 2, A and B, the metabolite M1 was detected in the organic phase 30 min after the reaction started. The mass spectrum of M1 showed a molecular ion at m/z 304.0 (M with 2 35Cl and 1 37Cl)⁺ with a relative intensity of 100, or 302.0 (M with three 35Cl)⁺ with a relative intensity of 99, and fragment ions at 267.1 (M-Cl)⁺ with a relative intensity of 31, and 239.1 (M-COCl)⁺ with a relative intensity of 52, suggesting that M1 is 8-OH-2,3,7-triCDD as described previously (Shinkyo et al., 2003a). Moreover, the metabolite M2, which seemed to be 2,3,7-triCDD glucuronide, was detected on the HPLC profile of the aqueous phase. Because M2 was converted to 8-OH-2,3,7-triCDD by β-glucuronidase, we made sure that the metabolite was 2,3,7-triCDD glucuronide as might have been thought. These results suggest that 2,3,7-triCDD is converted to 8-OH-2,3,7-triCDD by rat CYPIA1, and the resulting 8-OH-2,3,7-triCDD is further converted to 2,3,7-triCDD glucuronide by human UGT1A9.

**1H NMR Spectra of Authentic Standard of 2,3,7-triCDD and Its Metabolite.** To confirm that the metabolic M1 is 8-OH-2,3,7-triCDD, we performed NMR analysis of 2,3,7-triCDD and the metabolite M1. The proton chemical shifts and coupling constants assigned by one- and two-dimensional correlation spectroscopy spectra are as follows: authentic standard of 2,3,7-triCDD (in CDCl₃), δ (ppm): 6.786 (1H, d, J = 8.5, H-9), 6.872 (1H, d, J = 2.0, H-6), 6.958 (1H, dd, J = 2.0, 8.5, H-8), 6.958 (1H, s, H-1 or 4), 6.965 (1H, s, H-1 or 4); the metabolite M1 (in CDCl₃), δ (ppm): 6.579 (1H, s, H-9), 6.865 (1H, s, H-6), 6.946 (1H, s, H-1 or 4), 6.965 (1H, s, H-1 or 4). The most pronounced difference between the 1H NMR spectra of the metabolite and 2,3,7-triCDD was the disappearance of the H-8 signal. Also, all of resonances in the metabolite were observed as singlets, and the resonance from H-9 (6.579 ppm) was shifted upfield compared with that of 2,3,7-triCDD (6.786 ppm). These findings indicated that an electron-donating substituent such as a hydroxyl group was introduced to the C-8 position. The resonances from all metabolite protons matched those of the synthetic compound reported previously (Mason and Safe, 1986). Based on GC-MS and NMR analyses, the metabolite M1 was identified as 8-OH-2,3,7-triCDD.

**Metabolism of 8-OH-2,3,7-triCDD by the Recombinant Human UGTs.** To examine the ability of human UGT isozymes to glucuronidate 8-OH-2,3,7-triCDD, 12 recombinant UGTs expressed in baculovirus-infected insect cells (supersomes; BD Gentest) were used. 2,3,7-triCDD glucuronide was detected in the recombinant system containing UGT1A1, UGT1A3, UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2B7, UGT2B15, and UGT2B17, whereas it was not detected in the system containing UGT1A4, UGT1A6, and UGT2B4 (Fig. 3). Glucuronidation activity \([v/\text{E}_0\text{ (minutes }^{-1})]\) was calculated from eq. 1 on the basis of the results of the immunoblot analysis to estimate the ability of each UGT isozyme to produce the glucuronide.

**Kinetic Analysis of Glucuronidation of 8-OH-2,3,7-triCDD by Human UGT1A1, UGT1A9, UGT2B7, and Human Liver Microsomes.** Based on the data shown in Fig. 3 and expression levels of the UGTs in human livers (Tukey and Strassburg, 2000), we assumed that UGT1A1, 1A9, and 2B7 play an important role in the glucuronidation of 8-OH-2,3,7-triCDD in human livers. The initial rates of the glucu-
uronide formation catalyzed by human UGT1A1, UGT1A9, and UGT2B7 were measured at various concentrations of 8-OH-2,3,7-triCDD. The apparent kinetic parameters $K_m$ and $k_{cat}$, calculated from three independent experiments, were 0.8 ± 0.1 μM and 1.8 ± 0.2 min$^{-1}$, respectively, for UGT1A1, 0.8 ± 0.3 μM and 1.8 ± 0.2 min$^{-1}$, respectively, for UGT1A9, and 3.9 ± 0.4 μM and 7.0 ± 0.5 min$^{-1}$, respectively, for UGT2B7. In addition, the initial rate of the formation of 2,3,7-triCDD glucuronide catalyzed by human liver microsomes (HG89) was measured at various concentrations of 8-OH-2,3,7-triCDD. The mean $K_m$ value calculated from two independent experiments was 3.1 μM.

**Metabolism of 2,3,7-triCDD and 8-OH-2,3,7-triCDD in Human Liver Microsomes.** 2,3,7-triCDD hydroxylation activity in human liver microsomes was measured in the presence of NADPH. There is a significant difference in hydroxylation activity among 10 human liver microsomes, as shown in Fig. 4A. Activity range is 15-fold. Our previous study suggests that CYP1A2 is the major enzyme that catalyzes hydroxylation of 2,3,7-triCDD in human livers (Inouye et al., 2002). It is well known that phenacetin $O$-deethylation is specifically metabolized by CYP1A2 (Lisa et al., 1996). Thus, we examined the correlation between 2,3,7-triCDD 8-hydroxylation and phenacetin $O$-deethylation in human liver microsomes. As shown in Fig. 5, good correlation ($r = 0.92$) was observed between two reactions. On the other hand, chlorozoxazone 6-hydroxylation, which is known to be a CYP2E1-specific reaction, showed no correlation with 2,3,7-triCDD 8-hydroxylation (data not shown). These results strongly suggest that CYP1A2 is responsible for 2,3,7-triCDD 8-hydroxylation in human livers. Therefore, the difference in hydroxylation may be attributed to activity of CYP1A2 in individual liver microsomes. In contrast, glucuronidation activity toward 8-OH-2,3,7-triCDD in Fig. 4B showed no significant difference among 10 human liver microsomes. Activity range was 1.9-fold. The ability of 10 human liver microsomes to convert 2,3,7-triCDD to its glucuronide was compared in Fig. 4C. It is noted that the interindividual difference in Fig. 4C is very similar to that in Fig. 4A, which means the difference in the hydroxylation activity directly leads to the difference of the ability to produce the glucuronide from 2,3,7-triCDD.

**Discussion**

In the last two decades, the metabolism of PCDDs has been studied in vivo using experimental animals (Tulp and Hutzinger, 1978; Poiger et al., 1982; Tai et al., 1993; Petroske et al., 1997; Huwe et al., 1998; Hu and Bunce, 1999a,b; Hakk et al., 2001). Major metabolites were hydroxylated products, glucuronide conjugates, and sulfate conjugates. To the best of our knowledge, however, no reports identifying metabolites of PCDDs in humans have been published. Our previous study used a yeast expression system of human P450s to reveal human P450-dependent metabolism of PCDDs (Inouye et al., 2002). This
study examined the sequential metabolism of PCDDs by P450 and human UGTs to predict the metabolism of PCDDs in the human body. The hydroxylated dioxin 8-OH-2,3,7-triCDD was transformed into glucuronides by multiple UGTs, suggesting that the dioxins are collectively metabolized by several UGTs, not by one specific UGT in the human body. A kinetic analysis of the glucuronidation of 8-OH-2,3,7-triCDD by human UGT1A1, UGT1A9, and UGT2B7 showed higher glucuronidation activity compared with the other UGTs. The apparent $K_m$ of UGT1A1 and UGT1A9 was less than $1 \mu M$, which is quite small compared with the values of UGTs in other substrates (Green and Tephly et al., 1996; Jin et al., 1997; Coffman et al., 1998). Thus, 8-OH-2,3,7-triCDD seems to be a good substrate for the UGTs. Judging from the $K_m$ value of the human liver microsomes (HG89), UGT2B7 seems to play a central role in the glucuronidation of 8-OH-2,3,7-triCDD in the human liver. These results are consistent with results showing that UGT2B7 is highly expressed in the human liver (Jin et al., 1997). As reported previously (Jin et al., 1997), small phenolic compounds such as 4-nitrophenol, 1-napthol, and a coumarin derivative 4-methylumbelliferone serve as good substrates for most of the UGT1A isozymes except for UGT1A4. In fact, UGT1A4 showed no detectable activity toward OH-DD (data not shown) or 8-OH-2,3,7-triCDD. Moreover, the slightly larger bulky phenols such as 4-tert-butyl phenol and those that include the naturally occurring anthraquinones and flavanoids were not readily glucuronidated by UGT1A6, although they make excellent substrates for most of the UGT1 proteins (Ebner and Burchell, 1993). As expected, UGT1A6 did not show any glucuronidation activity toward OH-DD (data not shown) or 8-OH-2,3,7-triCDD. High activity of UGT2B7 toward 8-OH-2,3,7-triCDD was not expected because of its substrate specificity (Tukey and Strassburg, 2000). However, our results seem reasonable since UGT2B7 catalyzes benzo(a)pyrene glucuronidation (Jin et al., 1993b).

Studies have shown that 8-OH-2,3,7-triCDD is one of the major metabolites of 2,3,7,8-tetraCDD in the dog and rat (Poiger et al., 1982; Mason and Safe, 1986). If 2,3,7,8-tetraCDD is converted to 8-OH-2,3,7-triCDD in humans, UGT2B7, UGT1A1, and UGT1A9 may play an important role in the glucuronidation of 8-OH-2,3,7-triCDD. Thus, the sequential metabolism of 2,3,7-triCDD through 8-OH-2,3,7-triCDD observed in this study seems to be quite important in understanding the metabolism of 2,3,7,8-tetraCDD. An Ah receptor assay using mouse Hepa I cells revealed that the affinity of 8-OH-2,3,7-triCDD for the Ah receptor was less than 0.01% of 2,3,7,8-tetraCDD and less than 10% of 2,3,7-triCDD (Shinkyo et al., 2003b). Thus, the conversion of 2,3,7,8-tetraCDD and 2,3,7-triCDD into 8-OH-2,3,7-
tricDD was considered to be the first step in detoxification ( xenobiotic phase I). Since 8-OH-2,3,7-triCDD is a strongly hydrophobic compound, the glucuronidation of 8-OH-2,3,7-triCDD ( xenobiotic phase II) is thought to be indispensable to excretion from the human body. The results here combined with our previous studies on the metabolism of PCDDs by human P450s indicate that PCDDs are metabolized and excreted through hydroxylation by P450s and glucuronidation by UGTs; i.e., they are metabolized by a sequential hydroxylation-glucuronidation pathway.

When 2,3,7-triCDD was metabolized in 10 human liver microsomes, 2,3,7-triCDD was first converted into 8-OH-2,3,7-triCDD, probably by CYP1A2, and further converted into its glucuronide. No significant interindividual difference was observed in the glucuronidation of 8-OH-2,3,7-triCDD. This is probably due to the presence of multiple forms of UGT showing activity toward 8-OH-2,3,7-triCDD. However, it should be emphasized that a significant interindividual difference occurs in the metabolism of dioxin. Recently, Chen et al. (2004) indicated that ambient exposure is not the most important contributor to serum concentrations of PCDD/PCDF when compared with other exposure sources, such as dietary intake. They also revealed that higher serum concentrations of PCDD/PCDF were presented in older groups and suggested that older ages are likely to show increased accumulation of adipose tissue and decreased metabolism. Our results suggest that the ability to metabolize PCDD/PCDF might be one of the most important contributors to serum concentrations of PCDD/PCDF.

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