Polychlorinated biphenyls (PCBs) are highly toxic environmental contaminants that can cause irreversible damage in humans and wildlife. The mechanism of toxicity is not clear, but biotransformation products such as hydroxy PCBs (OH-PCBs) are a major concern. Efforts to elucidate the metabolism of PCBs and their metabolites, however, have paid little attention to the structure of the compound to be eliminated. The objectives of this study were to clarify organ tissue distribution of the glucuronidation activities toward OH-PCBs and to determine the UDP-glucuronosyltransferase (UGT) isoforms responsible for glucuronidation in relation to the OH-PCB structure. 2,4,6-Trichlorobiphenyl and 2,3,4,5-tetrachlorobiphenyl were incubated in primary culture of rat hepatocytes, and the metabolites were identified by HPLC. Organ tissue glucuronidation activities toward 10 OH-PCBs were investigated by reactions of microsomes prepared from brain, liver, small and large intestine, lung, kidney, and testis tissues. To determine substrate specificity of the isoforms toward the OH-PCBs, rat UGT isoforms UGT1A1, UGT1A3, UGT1A5, UGT1A6, UGT1A7, UGT2B1, UGT2B3, and UGT2B12 were expressed in yeast strain AH22. Glucuronidation of the PCBs was found to be contingent on their hydroxylation. The organ tissues had strong glucuronidation activities toward the OH-PCBs tested; and most OH-PCBs were glucuronidated by UGT1A1, UGT1A6, and UGT2B1, all of which were substrate-specific. In conclusion, glucuronidation activities of UGT1A1, UGT1A6, and UGT2B1 toward OH-PCBs is relative to expression of the isoforms in each tissue, and glucuronidation intensity of the isoforms is relative to the structure of the OH-PCB to be glucuronidated.

Polychlorinated biphenyls (PCBs) are highly toxic environmental contaminants that accumulate in lipid-rich body tissues of humans and wildlife through the food chain, exerting a detrimental impact on health. PCBs have adverse effects on neurodevelopment, such as hydroxylation by a diverse system of drug-metabolizing enzymes, i.e., the cytochrome P-450. Such as hydroxylation by a diverse system of drug-metabolizing enzymes, i.e., the cytochrome P-450, and this interaction can lead to either estrogenic or antiestrogenic activity (Connor et al., 1997; Andersson et al., 1999). In turtles, estrogenicity of OH-PCBs has been reported to induce reverse alteration of sex differentiation (Bergeron et al., 1994).

Although PCBs are ubiquitous in the environment, the mechanisms behind their adverse effects in the body are not fully understood. The toxicity may be linked partially to their biotransformation products, such as hydroxy-PCBs, as a result of hydroxylation by a diverse system of drug-metabolizing enzymes, i.e., the cytochrome P-450. Monoxygenases (Matsusue et al., 1996; Letcher et al., 1999). Lans et al. (1994) have linked the neurodevelopment effects of the toxin to the binding of OH-PCBs to thyroid hormone transport protein transthyretin. If the hydroxyl compound is para to the phenyl-phenyl bond and has adjacent chlorine atoms, the structure resembles that of the prohormone thyroxin (T4) (Rickenbacher et al., 1986). This structural resemblance allows OH-PCB to bind with high affinity to the transthyretins (Lans et al., 1993). In rats, OH-PCB decreases circulating levels of thyroid hormones through competitive binding to the transthyretins, thus disrupting the transport of thyroid hormone and retinol (vitamin A) (Brouwer and van den Berg, 1986). This is the presumed mechanism by which OH-PCBs are selectively retained in plasma and could conceivably be the mechanism behind PCB toxicity (Bergman et al., 1994) in the body. Not only can OH-PCB bind the protein to transport thyroxin but also to genes having a thyroxin binding site. Miyazaki et al. (2004), using reporter assay, reported that OH-PCB binds directly to the thyroid hormone responsive element, and this would disrupt expression of the downstream genes regulated by T3.

OH-PCB toxicity eludes easy evaluation in the animal body because the rate of OH-PCB metabolism differs according to the organ tissues, thus causing nonuniformity in overall OH-PCB concentrations in the body. Moreover, OH-PCBs themselves are composed of diversely structured isomers, which may affect metabolism of the compounds. In decades of research, however, such structural considerations have been largely neglected. To elucidate the mechanism governing adverse effects on the target tissues, it is expedient to...
clarify the metabolic system of the OH-PCBs before their arrival at target tissues.

The hydroxy metabolites of PCBs could be converted to either glucuronide conjugate or sulfate conjugate, mainly in the liver, and then excreted in the bile or urine. Glucuronidation formation for lower chlorinated PCB congeners has been reported in vivo and in vitro then excreted in the bile or urine. Glucuronide formation for lower glucuronide conjugate or sulfate conjugate, mainly in the liver, and target tissues.

To elucidate the metabolism, tissue distribution, accumulation and excretion of PCBs, we focused on organ-tissue distribution of the glucuronidation activities toward 10 OH-PCBs and on the possible organ-tissue specificity of UGT isoforms responsible for the glucuronidation reactions.

Materials and Methods

Chemicals. Cholic acid was purchased from Nissui Pharmaceutical Co., Ltd. (Tokyo, Japan) and then purified further and converted to its sodium salt. UDP-glucuronic acid was obtained from Nakarai Pharmaceutical Co., Ltd. (Tokyo, Japan) and then purified further and converted to its sodium salt.

The hydroxy PCBs could be converted to either glucuronide conjugate or sulfate conjugate, mainly in the liver, and then excreted in the bile or urine. Glucuronidation formation for lower chlorinated PCB congeners has been reported in vivo and in vitro (Norback et al., 1981; Schnellmann et al., 1984). In rat liver microsomes, glucuronidation efficiency \( V_{\text{max}}/K_m \) seems to be relative to the substitution of chlorine atoms on the hydroxy-PCBs, as suggested in a study by Tampal et al. (2002). We hypothesized that the glucuronidation efficiency of each organ tissue toward the OH-PCBs depends on whether the structure of the given OH-PCB has affinity for UDG glucuronyltransferase (UGT) isoforms expressed in the tissue.

UGT is an intracellular membrane glycoprotein that catalyzes glucuronidation of bilirubin and steroids as well as xenobiotic compounds, including carcinogens (Dutton, 1980). UGTs have been classified into two major subfamilies, i.e., UGT1 and UGT2, on the basis of similarity in amino acid sequence (Burchell et al., 1991). In the UGT1 family, all mRNAs are produced from a single gene locus by individual promoters, followed by alternative splicing (Emi et al., 1995). The UGT1A transcripts contain individual amino-terminal domains followed by common carboxyl-terminal domains. Isoforms belonging to the UGT1 family primarily conjugate bilirubin or exogenous phenolic compounds (Bock et al., 1979; King et al., 2000). In contrast, UGT2 family members are encoded by individual genes characterized by six exons each (Mackenzie et al., 1997). The UGT2A subfamily consists of a single olfactory-specific isoform, and the UGT2B subfamily in the rat consists of six members: UGT2B1, UGT2B2, UGT2B3, UGT2B6, UGT2B8, and UGT2B12. Isoforms belonging to the UGT2 family conjugate steroids or bulky phenols (Bock et al., 1979; King et al., 2000; Turgeon et al., 2001).

The present study was conducted to determine the explicit UGT isoforms governing the elimination pathways of hydroxylated PCBs and to clarify the possible relationship between the OH-PCB structure and glucuronidation. To elucidate the metabolism, tissue distribution, accumulation and excretion of PCBs, we focused on organ-tissue distribution of the glucuronidation activities toward 10 OH-PCBs and on the possible organ-tissue specificity of UGT isoforms responsible for the glucuronidation reactions.

### Table 1

<table>
<thead>
<tr>
<th>IUPAC Name</th>
<th>Common Name</th>
<th>Structure</th>
</tr>
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<tbody>
<tr>
<td>2,4,6-Trichlorobiphenyl</td>
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</tr>
<tr>
<td>2,3,4,5-Tetrachlorobiphenyl</td>
<td>PCB61</td>
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<td>4-Hydroxy-biphenyl</td>
<td>4-HB</td>
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</table>
solution, and the resulting solution was filtered through stainless mesh to dissociate the hepatocytes. The hepatocytes were resuspended in Hanks’ buffer solution and isolated by slow-speed centrifugation (50g for 3 min; three times). Cell viability was determined by trypan blue staining.

**Incubation of Hepatocytes with PCBs and OH-PCBs.** Six-well tissue culture plates (Asahi Techno Glass Co., Ltd., Chiba, Japan) were coated with Matrigel (BD Biosciences, Bedford, MA), and the wells were prepared with Williams’ medium E (Invitrogen, Carlsbad, CA) supplemented with final concentrations of 5% fetal bovine serum (Asahi Techno Glass Co., Ltd.), 1 μM insulin, 1 μM dexamethazone, and 2 μM glucose. Approximately 10^6 cells were seeded into each well and cultured for 4 h. The monolayers were rinsed once, the culture medium was replaced with insulin-free and dexamethazone-free Williams’ medium E, and the cells were cultured in the medium for 1 day.

The cultured cells were then separated into four batches for incubation with PCB30, PCB61, OH-PCB30, or OH-PCB61 (0.05 mM each) in the medium at 37°C (2 ml) aerated with 5% CO2 and 95% air. Both the PCB30 batch and PCB61 batch were incubated for 12 and 24 h, and both the OH-PCB30 batch and the OH-PCB61 batch for 24 h. When PCB30 and OH-PCB30 were taken up by the culture plate. When PCB61 and OH-PCB61 were added independently to the media, about half (60%) of the total added PCB30 (100 nmol) but none of the OH-PCB30 was taken up by the culture plate. The amount of each PCB derivative absorbed by the hepatocytes was calculated by subtracting the amount of each PCB derivative taken up by the culture plate from the total amount of PCB added to the culture medium.

**High-Performance Liquid Chromatography.** In preparation for HPLC assay for the PCB metabolites, the medium was removed from the culture dish, the monolayer was rinsed with saline, and the hepatocytes were harvested into the medium (100 g for 10 min at 9000 g). After the reactions of microsomes, the resultant reaction solutions (40 g) were centrifuged for 3 min; three times). The supernatants were filtered through a disposable disk filter (HLC-DISK3; Kanto Co., Ltd., Tokyo, Japan), and the filtered samples were injected into the HPLC system (UV-8020, PD0820, SD8022, and CO8020; Tosoh, Tokyo, Japan) equipped with Tosoh TSK-gel 80TS C18 reverse-phase column (4.6 mm × 30 cm). The column temperature was maintained at 40°C and the samples were eluted on a constant flow rate of 1 ml/min.

PCB30 metabolites were eluted in two steps: first, with linear gradient of 24 to 85% methanol and 0.06 to 0.012% acetic acid ammonium for 30 min, and finally with 85% methanol and 0.012% acetic acid ammonium for 20 min. PCB61 metabolites were eluted with linear gradient of 24 to 100% methanol and 0.06 to 0% acetic acid ammonium for 50 min. Detection of the eluted metabolites was made for the PCB metabolite absorption at 222 nm. Hydroxy-PCBs were quantified by standard compound. PCB-glucuronide and PCB-sulfate were quantified according to the amount of the deconjugated hydroxy-PCB remaining after treatment of the reaction products as explained below.

**Metabolite Verification.** After the reactions of microsomes, the resultant medium (100 μl) was mixed with β-glucuronidase (2.5 mg/ml) in 0.5 M acetate buffer solution (20 μl; pH 4.5) and incubated for 30 min at 37°C (Shibata et al., 2002). A separate aliquot of the medium (100 μl) was mixed with sulfatase (type VIII; 4 mg/ml) in 0.5 M acetate buffer solution (50 μl; pH 4.5) and incubated for 9 at 37°C. Acetonitrile was added to the reaction solutions (40 μl for β-glucuronidase reaction, and 50 μl for sulfatase reaction), and each mixture was boiled for 5 min. These mixtures were centrifuged for 10 min at 9000g in room temperature. The supernatant was filtered through a disposable disk filter (HLC-DISK3; Kanto Co., Ltd., Tokyo, Japan) and analyzed by HPLC as described above. The apparent Km values were determined by Lineweaver-Burk plot, Michaelis-Menten plots of the kinetics of the reaction were shown in supplemental data A and B.

**UGT Enzyme Analysis.** The microsomes of each organ tissue were activated with a final concentration of 0.05% sodium cholate. UGT activity was determined by incubation (0.5–2 h) of the microsomes of each organ tissue at 37°C in 0.1 M Tris-HCl buffer (200 μl; pH 7.4) containing lysophosphatidylcholine (50 μg), MgCl2 (5 mM), hydroxy-PCB (0.5 mM each), and UDP-glucuronic acid (3 mM). Linearity of the enzyme reactions was confirmed during incubation (data not shown).

Reaction products were eluted by HPLC with acetonitrile solutions. The 4-hydroxy-biphenyl products were eluted with the solution of 57% acetone/63% water/0.1% acetic acid; HPCB3001N and HPCB3003N with the solution in a ratio of 50:50:0.1%; HPCB3006N, HPCB4001N, HPCB4004N, HPCB4008N, and HPCB5007N with the solution in a ratio of 60:40:0.1%; and finally, HPCB4007N, HPCB5005N, and HPCB6001N with the solution in a ratio of 68:32:0.1%.

**Kinetic Analysis.** Kinetic parameters were determined according to the enzymatic activity against eight concentrations of hydroxy-PCB (0.006–0.5 mM) added to 200 μl of 0.1 M Tris-HCl buffer, pH 7.4, containing 50 μg of lysophosphatidylcholine, and final concentrations of 5 mM MgCl2, and 3 mM UDP-glucuronic acid. The reaction products were determined by HPLC analysis using acetonitrile elution buffer as described above. The apparent Km values were determined by Lineweaver-Burk plot, Michaelis-Menten plots of the liver kinetics toward six representative hydroxy-PCBs are shown in supplemental data A and B.

**UGT1A cDNA Preparation and Amplification.** Total RNA was isolated from 0.2 g of each organ tissue preparation by applying TRIzol reagent (Invitrogen), and single-strand cDNA was synthesized from the total RNA by Rever Tra Ace (Toyobo Co., Ltd., Osaka, Japan). The coding regions of the respective cDNA species were amplified by PCR oligonucleotide primers and Taq polymerase (Takara Co., Ltd., Shiga, Japan). RT-PCR was used with exon-1 primers specific for each member of the rat UGT1A sequence and with one antisense primer specific for the common exon-2 sequence (Table 2). UGT primers were designed to amplify different lengths of cDNA UGT1A1, 945 base pairs (bp); UGT1A2, 692 bp; UGT1A3, 681 bp; UGT1A5, 655 bp; UGT1A6, 794 bp; and UGT1A7, 755 bp.

After preliminary incubation of the reaction mixture at 94°C (2 min), amplification of the UGT1A first-exons was conducted in a three-cycle protocol repeated 35 times, i.e., UGT1A1, UGT1A2, UGT1A3, UGT1A5, UGT1A6, and UGT1A7 were amplified, first at 94°C for 30 s; second, at 55°C for 30 s; and third, at 72°C for 1 min. Upon the 35th repetition, elongation followed at 72°C for 1 min.

**UGT2B cDNA Preparation and Amplification.** UGT2B cDNA was prepared in a manner similar to that for the UGT1A cDNA. For recognizing a position within exon 1, RT-PCR was used with six exon-1-specific sense primers, each possessing an SpeI site (Table 2). For recognizing a position within exon 5, RT-PCR was used with six antisense primers, each possessing...
an Spel site (Table 2). The primers allowed for annealing the beginning of exon 1 and the terminal of exon 5.

After the reaction mixture was incubated at 94°C for 2 min, the UGT2B isoforms were amplified in their full lengths with KOD plus polymerase (Toyobo Co., Ltd.) in a three-cycle protocol repeated 35 times, i.e., UGT2B1 at 94°C for 15 s, at 52°C for 30 s, and at 68°C for 90 s; and UGT2B12 at 94°C for 15 s, at 50°C for 30 s, and at 68°C for 90 s. The individual sense (F) primers and antisense (R) primers were used for amplification of the UGT1A family and UGT2B family.

Expression of UGT Isoforms. cDNAs of the rat UDP-glucuronosyltransferase isoforms UGT1A1, 1A3, 1A5, 1A6, 1A7, 2B1, 2B3, 2B12 obtained by RT-PCR were expressed in yeast AH22 cells according to slight modification of the method described previously (Yokota et al., 1999). Briefly, UGT2B3 was inserted into the yeast expression vector pGYR (Shinkyo et al., 2002), whereas the other seven isoforms were used with the vector pAH5 (Yokota et al., 1999). Protein concentration was determined by the method of Lowry et al. (1951) with bovine serum albumin as the standard. A UGT isoform expressed by yeast microsomes was used for glucuronidation of hydroxy-PCBs, and the resultant glucuronide conjugate was determined by HPLC using acetonitrile elution buffer as described above.

Immunoblot Analysis. Microsomes and other protein samples were subjected to SDS-PAGE through 10% acrylamide gel. The resulting polypeptide bands were transferred to nitrocellulose membranes, and polyclonal antibodies against the UGT isoforms (antibodies generously provided by Dr. S. Ishihoro, Toyama Prefectural University, Toyama, Japan) were used for detecting the immunoreactive bands. Anti-UGT1A1 and anti-UGT2B1 antibodies can recognize the C-terminal region, i.e., Glu112GluGluValGlu215 and Asp95CRK-TANMGKKEEK99 (Ishihoro et al., 1995; Ishihoro et al., 1997). For anti-UGT1A antibody, the staining intensity of each band was calculated by NIH Image, and specific enzyme activities of the expressed UGT1A subfamily (UGT1A1, 1A3, 1A5, 1A6, and 1A7) were corrected with UGT1A1 as standard.

Results

PCB30 and PCB61 Hydroxylation and Glucuronidation. For PCB30 and PCB61, HPLC chromatograms disclosed three main metabolites, i.e., 4-OH-PCB, glucuronide conjugate and sulfate conjugate (Fig. 1). PCB30 and PCB61 entered the hepatocytes within 12-h incubation, and large amounts of their metabolites were excreted into the respective culture media within 24-h incubation (Figs. 2 and 3). Of the PCB30 absorbed by the hepatocytes, 14.1% was excreted into the culture medium as glucuronide conjugate and 9.0% was excreted into the culture medium as sulfate conjugate (Fig. 2). When OH-PCB30 was added to the culture medium, the amount of excreted glucuronide conjugate increased dramatically to 38.1%, whereas the excreted sulfate conjugate showed a slight increase to 10.2% (Fig. 2). Of the PCB61 absorbed by the hepatocytes, 46.7% was excreted into the medium as glucuronide conjugate and 5.5% as sulfate conjugate (Fig. 3). When OH-PCB61 was added to the culture medium, the amounts of excreted glucuronide conjugate (46.6%) and sulfate conjugate (6.2%) were comparable with those found in the medium after addition of PCB61 (Fig. 3). For both PCB30 and PCB61, the glucuronide conjugate was the predominant metabolite, but OH-PCB was negligible in the hepatocytes as well as in the medium (Figs. 2 and 3). This suggests that glucuronidation of the PCBs was contingent on PCB oxidation.

Also in liver microsomes incubated in the presence of NADPH and UDP-glucuronic acid, glucuronidation was evident as a tandem reaction to oxidation. Upon incubation of either PCB30 or PCB61 with the
liver microsomes in the presence of NADPH, 4-OH-PCB occurred in the incubation buffer in 1 h (Fig. 4). Upon addition of NADPH and UDP-glucuronic acid, the hydroxy-metabolite disappeared and the glucuronide conjugate was present (Fig. 4), as in the primary cultured cells (Figs. 1–3). This interchange between the dissipation of hydroxy-metabolite and the emergence of glucuronide conjugate indicates that the OH-PCB produced by cytochrome P450 was conjugated immediately by UDP-glucuronosyltransferase.

**UGT Activity toward 10 OH-PCBs.** The liver, small and large intestines, lung, kidney, and testis differed markedly in their reactions toward the 10 OH-PCBs tested and toward 4-hydroxy-biphenyl, as evidenced by a wide range of UGT activities including $V_{\text{max}}$, $K_m$ values, and intrinsic clearance ($V_{\text{max}}/K_m$) for the enzyme reactions (Fig. 5). Liver microsomes had high levels of UGT activity toward the 10 OH-PCBs and toward 4-hydroxy-biphenyl, and microsomes from the small and large intestines showed marked activity toward the 4-OH-PCBs having substitution of chlorine atoms on one or both sides of the hydroxy residue, i.e., HPCB5005N, HPCB5007N, and HPCB6001N (Fig. 5). The kidney and testis had low levels of UGT activity, but all 10 OH-PCBs were nevertheless glucuronidated in spite of the low activity. 4-OH-PCB30 and 4-OH-PCB61, however, were glucuronidated solely by the liver microsomes (Fig. 5).

**Substrate Specificities of UGT Isoforms.** All the UGT isoforms except UGT2B8 were expressed in the liver, although in various levels of intensity, as shown by the amplified cDNAs (Fig. 6). UGT1A1 was expressed in all but the brain and lung, UGT1A6 was expressed in all the organ tissues tested, and UGT1A7 in all but the brain and kidney (Fig. 6). Except for UGT2B8 and UGT2B12, the isoforms belonging to the UGT2B subfamily were expressed solely in the liver. UGT2B8 was not expressed in hepatic or in extrahepatic tissue. UGT2B12 was expressed not only in the liver but also in the kidney (Fig. 6). The isoforms expressed in brain, liver, lung, small and large intestines, kidney, and testis, i.e., UGT1A1, UGT1A3, UGT1A5, UGT1A6, UGT1A7, UGT2B1, UGT2B3 and UGT2B12, were also expressed in yeast AH22 cells, as shown by immunoblotting analysis (Fig. 7).

The various UGT isoforms displayed vastly different characteristics toward the 10 OH-PCBs and toward 4-hydroxy-biphenyl, as evidenced by the intrinsic clearance ($V_{\text{max}}/K_m$) calculated from the $V_{\text{max}}$ and $K_m$ values (Fig. 8). UGT2B3 and UGT2B12, which are responsible for the glucuronidation of testosterone and 4-methylumbelliferone, respectively, showed no activity toward any of the OH-PCBs.
towards 2-hydroxy-2,3,5-trichlorobiphenyl (HPCB4001N). On the preference for the OH-PCB with substitution of hydroxy residues in (HPCB5005N, HPCB5007N, and HPCB6001N). UGT1A6 showed a chlorine atoms on one or both sides of the hydroxy position tested. UGT1A1 glucuronidated the OH-PCB with substitutions of UDP-glucuronic acid (chromatogram A), with NADPH (chromatogram B) or without either NADPH or UDP-glucuronic acid (chromatogram C). Arrows indicate glucuronidated PCB (peak a, 6.3 min on A; 9.1 min on B), 4-hydroxy-PCB (peak b, 19.5 min on A; 23.6 min on B) and parent PCB (peak c, 28.6 min on A; 35.7 min on B).

This study had five main findings. First, PCB glucuronidation was contingent on hydroxylation of the parent PCB (OH-PCB). Second, the liver as well as extrahepatic organ tissues had strong glucuronidation activities toward the 10 OH-PCBs tested. Third, UGT1A6, and UGT2B1 were the isoforms responsible for glucuronidation of most of the OH-PCBs. Fourth, each of the isoforms UGT1A1, UGT1A6, and UGT2B1 had unique substrate specificity to certain organ tissues. Finally, glucuronidation intensity of these three UGT isoforms was relative to the structure of each OH-PCB compound to be eliminated.

**PCB Hydroxylation, a Prerequisite to OH-PCB Glucuronidation.** Our study bears out that, in rats, elimination of environmental PCBs from the body depends on the parent PCB, because intense glucuronidation is contingent on oxidation. PCB30 and PCB61 are metabolized to 4-OH-PCB30 and 4-OH-PCB61, respectively. Because these two OH-PCBs are especially potent in estrogenic activity (Carlson and Williams, 2001), which is known to cause irreversible damage in humans and wildlife, these compounds were selected as the focus of the present investigation. Both 4-OH-PCB30 and 4-OH-PCB61 are conjugated mainly by glucuronic acid and are also manifested as sulfate conjugate, as demonstrated by our primary cultured hepatocytes. Furthermore, the glucuronide conjugate is formed in the presence of NADPH and UDP-glucuronic acid, as shown by the microsomal assays. Because of impending harm from the hydroxylated PCBs, intense glucuronidation is necessary but, paradoxically, the hydroxylation itself is a prerequisite in the mechanism of their glucuronidation.

In spite of their indispensable role in glucuronidation, however, OH-PCBs inhibit sulfation, which is one of the important elimination pathways. In rats, Schuur et al. (1998a,b) showed that OH-PCBs inhibit a sulfotransferase isoform responsible for sulfation toward thyroxin. In renal excretion of the estrogenic steroid 17β-estradiol, human estrogen sulfotransferase (hEST), a metabolic enzyme expressed in cytosol, catalyzes sulfation of the steroid (Coughtrie et al., 1998). Curiously, however, certain OH-PCBs can inhibit the activity of hEST (Kester et al., 2000), even though the IC_{50} values of many OH-PCBs are approximately 40- to 50-fold lower than the K_{m} value of 17β-estradiol sulfotransferase (Kester et al., 1999). This phenomenon may be explained by the crystal structure of hEST containing OH-PCB, which is a structure allowing OH-PCBs to bind to the enzyme (Shvetsov et al., 2003).

OH-PCB can bind not only to the metabolic enzyme hEST but also to genes having a thyroxin binding site. Miyazaki et al. (2004), in a study using reporter assay, reported that OH-PCBs bind directly to the thyroid hormone responsive element. In our study, however, the OH-PCBs were glucuronidated in the organ tissues, thus suggesting it is strongly unlikely that the glucuronide conjugate would bind to the thyroid hormone responsive element, because the PCB structure would change. In support of this view, we found that, in primary cultured cells of rat liver, PCBs are metabolized mainly to the glucuronide conjugate. This result is in line with work documenting the fate of glucuronide conjugate in vivo (Anderson et al., 1977; Norback et al., 1981). In rat in vivo, the glucuronide conjugate of PCBs is excreted in the urine and feces and is the main metabolite of PCBs (Anderson et al., 1977), and in rhesus monkey the glucuronide conjugate is eliminated in biliary excretion (Norback et al., 1981).

The lipophilic nature of PCBs enables their intracellular invasion into the endoplasmic reticulum. Accordingly, in the present study UGTs, which are expressed in the endoplasmic reticulum, immediately converted the OH-PCBs to glucuronic acid conjugate, as shown by OH-PCB reaction with liver microsomes in the presence of NADPH and UDP-glucuronic acid. In our primary cultured hepatocytes as well, conjugation of the OH-PCBs with glucuronic acid was contingent on PCB hydroxylation. These results support the findings by Oberg et al. (2002) that in rats given a single oral dose of PCBs, the PCBs accumulate in large amounts in the liver immediately after uptake through the gastrointestinal tract and then recede drastically. Together with a growing body of research, our study has clarified that elimination of PCBs from the body depends on their hydroxylation and, in turn, on glucuronidation of the OH-PCB.

![HPLC chromatograms of PCB30 metabolites (A) and PCB 61 metabolites (B) in rat liver microsomes. Reaction was done with NADPH and UDP-glucuronic acid (chromatogram A), with NADPH (chromatogram B) or without either NADPH or UDP-glucuronic acid (chromatogram C). Arrows indicate glucuronidated PCB (peak a, 6.3 min on A; 9.1 min on B), 4-hydroxy-PCB (peak b, 19.5 min on A; 23.6 min on B) and parent PCB (peak c, 28.6 min on A; 35.7 min on B).](image-url)
Organ Tissues and UGT Isoforms Associated with OH-PCB Glucuronidation. In conjunction with the literature (Anderson et al., 1977; Norback et al., 1981; Oberg et al., 2002), our results indicate that hepatic as well as extrahepatic organ tissues can glucuronidate OH-PCB and that, as we suspected, the UGT isoforms are the isoforms largely responsible for the OH-PCB glucuronidation. UGT1A1, UGT1A6, and UGT2B1 are the isoforms predominantly governing OH-PCB glucuronidation, as disclosed by our tests on 10 OH-PCBs.

OH-PCB Glucuronidation Relative to Organ Tissue Specificity. Each of the isoforms UGT1A1, UGT1A6, and UGT2B1 has specificity toward certain organ tissues in the body, as demonstrated by this study. Depending on the organ tissue and the compound to be glucuronidated, generally UGT2B1 has the strongest glucuronidation activity, followed by UGT1A6; but UGT1A1 is able to compensate for its low activity because of the wide range of specificity for the various tissues.

Organ-Tissue Specificity of UGT1A1. In this study, UGT1A1 could glucuronidate HPCB5005N, HPCB5007N, and HPCB6001N. Coincidentally, these three compounds (HPCB5005N, HPCB5007N, and HPCB6001N) are all characterized by substitution of chlorine atoms on one or both sides of the 4-hydroxy position (Table 1). UGT1A1 activities toward these compounds are comparable in the rat liver, small and large intestines, kidney, and testis but different in the lung, as borne out by the $V_{\text{max}}/K_m$ values in this study. $V_{\text{max}}$ values toward HPCB5005N, HPCB5007N, and HPCB6001N in lung tissue, which does not express the isoform UGT1A1, were lower than the $V_{\text{max}}$ values in liver, intestines, kidney and testis, where UGT1A1 is ex-
pressed. Similarly, $K_m$ values toward HPCB5007N and HPCB6001N were the same for liver, intestines, kidney, and testis but different for the lung. The $V_{max}$ and $K_m$ values toward HPCB5005N, HPCB5007N and HPCB6001N in liver, intestines, kidney, and testis indicate that glucuronidation of these three compounds is mediated by the isoform UGT1A1 in the liver, intestine, kidney, and testis but not in the lung.

**Organ-Tissue Specificity of UGT1A6.** With the sole exception of the brain tissue, all other organ tissues in our study (liver, small and large intestines, lung, kidney, and testis) showed UGT activity toward the 4-hydroxy compounds HPCB3001N, HPCB3003N, and HPCB4001N. The substrate specificities of liver, small and large intestines, kidney, and testis toward HPCB3001N, HPCB3003N, and HPCB4001N are consistent with the tissue specificity of expressions of UGT1A6, the sole UGT isoform found to be expressed in every one of the organ tissues tested. UGT1A6, however, shows a preference for OH-PCBs having substitutions of hydroxy residues in either the ortho- or meta-position, such as HPCB4001N. Notably, UGT activity toward HPCB4001N was detectable in microsomes from the brain (data not shown), whereas the $K_m$ values for the reaction with HPCB4001N were the same in all organ tissues other than the brain (i.e., liver, small and large intestines, lung, kidney, and testis). Plausibly, then, HPCB4001N glucuronidation can be mediated by UGT1A6 in all the rat organ tissues tested in this study.

**Organ-Tissue Specificity of UGT2B1.** Of the UGT2B family of isoenzymes, which are generally associated with glucuronidating bulky phenols such as 4-hydroxy-biphenyl (Bock et al., 1979), UGT2B1 was able to glucuronidate all the 4-OH-PCBs tested in our study except for HPCB6001N. Liver tissue demonstrated intense glucuronidation activity toward 4-OH-PCB30 (HPCB3006N), HPCB4008N, and 4-hy-
and curiously, all these compounds possess either a single substitution or no substitution of chlorine atoms on the sides of the 4-hydroxyl residue. Moreover, the hepatic activity toward these three compounds (HPCB3006N, HPCB4008N, and 4-hydroxy-biphenyl) is consistent with that demonstrated by UGT2B1. In the liver, the $K_m$ values toward HPCB3006N and HPCB4008N were the same as the value for UGT2B1, indicating that glucuronidation toward HPCB3006N and HPCB4008N is mediated by UGT2B1 expressed in the liver. In extrahepatic tissues expressing UGT1A1, however, glucuronidation toward HPCB4008N would be mediated by UGT1A1, given that UGT1A1 is able to glucuronidate a compound if the compound possesses the substitution of chlorine atoms on the sides of the hydroxy position, as in the case of HPCB4008N. Conversely, it is plausible that the reason HPCB3006N eludes glucuronidation by extrahepatic tissues is that HPCB3006N lacks the substitution of chlorine atoms on the sides of the hydroxy position, thus precluding glucuronidation by UGT1A1.

On the other hand, the UGT2B1 glucuronidation activity toward HPCB5007N is weak. In spite of sharing a structure similar to that of HPCB3006N (4-OH-PCB30) and HPCB4008N, the compound HPCB5007N substitutes chlorine atoms on both sides of the 4-hydroxy residues. The weak UGT2B1 glucuronidation activity toward HPCB5007N, therefore, would indicate that the UGT2B1 activity site is obstructed by the chlorine atoms on dual sides of the 4-hydroxy residues.

Although UGT2B1 has intense glucuronidation activity toward the nonplanar PCBs such as HPCB3006N (4-OH-PCB30) and HPCB4008N, the activity of the isoform is weak toward the planar OH-PCBs such as HPCB4007N and HPCB5005N, having a single chlorine atom at the 2' position. Thus, UGT2B1 is rendered incapable of accommodating a planar compound.

**Role of the OH-PCB Structure.** This study has borne out that although glucuronidation activity toward OH-PCB in the respective organ tissues depends on the intensity of the tissue-specific expressions of UGT1A1, UGT1A6, and UGT2B1, the glucuronidation by these isoforms is either enhanced or precluded according to the structure of the compound to be eliminated.

Sandau et al. (2000), studying xenobiotic PCBs in organ tissues from humans, reported that 20% of total PCB in human blood consists
of hydroxy metabolites, predominantly 4-OH-PCB109, 4-OH-PCB146, and 4-OH-PCB187. Of these compounds, Tampal et al. (2002) have shown that, in rat liver microsomes, 4-OH-PCB146 and 4-OH-PCB187 are glucuronidated to a less extent than other OH-PCBs. In the light of our present results, we surmise that the poor glucuronidation of 4-OH-PCB146 and 4-OH-PCB187 reported by Tampal et al. (2002) could be attributed to the structure shared by these two OH-PCBs. Given that glucuronidation intensity of the UGT isoforms in our study was relative to the structure of the compound to be glucuronidated, it is plausible that the OH-PCB structure impedes or precludes UGT2B1 glucuronidation activity toward those two OH-PCBs in spite of the intense glucuronidation activity generally exhibited by UGT2B1 in the liver.

We strongly suspect that the reason the three major hydroxy metabolites accumulating in human blood (4-OH-PCB109, 4-OH-PCB146, and 4-OH-PCB187) elude glucuronidation by UGT2B1 in the liver microsomes is that whereas these three OH-PCBs have substitutions of chlorine atoms on both sides of the 4-hydroxyl residue, the UGT2B1 preference is allied with compounds having either no substitution or a single substitution of a chlorine atom on the sides of the hydroxy position, as indicated by our present results. Because UGT1A1 favors 4-hydroxy-PCBs having substitutions of chlorine atoms on one or both sides of the hydroxy position, 4-OH-PCB109, 4-OH-PCB146, and 4-OH-PCB187 would likely be glucuronidated by UGT1A1 expressed in liver, small and large intestines, lung, kidney and testis, in spite of the weak glucuronidation activity associated with UGT1A1.

Although most OH-PCBs in this study were highly vulnerable to the intense glucuronidation activities of UGT1A1, UGT1A6, and UGT2B1, the compound HPCB4007N remained persistent and was only marginally glucuronidated by either UGT1A1 or UGT2B1. Given that the liver microsomes alone had robust glucuronidation activity toward HPCB4007N, on conjecture we propose that UGT isoform(s) other than those used in our study may be at work toward glucuronidating the toxic planar OH-PCB HPCB4007N in the liver. Further studies are warranted.

A limitation of the present work was that a few of the other known UGT isoforms were not expressed in our yeast AH22 cells, leaving that fraction of the total gamut of UGT isoforms remaining to be tested for tissue specificity and glucuronidation activity in relation to the OH-PCB structures.

Conclusions

Results of this study support our hypothesis that in rat organ tissues the glucuronidation efficiency toward OH-PCB is dependent on whether the UGT isoforms expressed in each given tissue show a preference for the structure of the target OH-PCB. Providing new insight into the mechanism behind PCB toxicity in the body, this study shows that fraction of the total gamut of UGT isoforms remaining to be tested for tissue specificity and glucuronidation activity in relation to the OH-PCB structures.

References


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