Glucuronidation of Polychlorinated Biphenylols and UDP-Glucuronic Acid Concentrations in Channel Catfish Liver and Intestine

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ABSTRACT:

Polychlorinated biphenylols (OH-PCBs) are potentially toxic polychlorinated biphenyl metabolites that can be eliminated by glucuronidation, catalyzed by UDP-glucuronosyltransferases (UGTs). OH-PCBs with a 3,5-dichloro-4-hydroxy substitution pattern have been detected in blood from humans and wildlife, suggesting slow elimination. In this study we assessed the glucuronidation of 4-OH-PCBs with zero, one, or two chlorine atoms flanking the 4-hydroxyl group and zero to four chlorine atoms in the aphenolic ring in microsomes from channel catfish liver and proximal intestine. Product formation was quantitated with [14C]UDP-glucuronic acid (UDPGA). Physiological concentrations of UDPGA were measured in preparations of liver and intestine. When the OH-PCB concentrations were varied in the presence of saturating concentrations of UDPGA concentrations, glucuronidation Vmax values were higher in hepatic than in intestinal microsomes (0.40–3.4 and 0.12–0.78 nmol/min/mg of protein, respectively), whereas the Km values were generally lower for intestine (0.042–0.47 mM) than for liver (0.11–1.64 mM). In both tissues Vmax values with 3,5-dichloro-4-OH-PCBs were lower than with the corresponding 3-chloro-4-OH-PCBs. Varying the UDPGA concentrations in the presence of saturating concentrations of OH-PCB showed that the Km for UDPGA was lower in intestine (27 μM) than in liver (690 μM). The measured concentration of UDPGA in catfish liver (246–377 nmol/g) was lower than the Km for UDPGA, suggesting that in vivo rates of glucuronidation may be suboptimal, whereas in intestine the measured UDPGA concentration (71–258 nmol/g) was higher than the Km for UDPGA. Although liver has a greater glucuronidation capacity than proximal intestine, the properties of intestinal UGTs in channel catfish enable them to efficiently glucuronidate low concentrations of OH-PCBs.

Polychlorinated biphenyls (PCBs) are ubiquitous environmental contaminants, found at all trophic levels around the globe (Erickson, 2001). Biotransformation is an important influence on PCB toxicokinetics in animals and depends on congener structure and the animal’s metabolic capacity. Polychlorinated biphenylols (OH-PCBs) are products of cytochrome P450-dependent hydroxylation of PCBs (James, 2001). Although OH-PCBs are more water-soluble than their parent molecules, they are still lipophilic enough to be orally absorbed and distribute to tissues (Sinjari and Darnerud, 1998). Several OH-PCBs have been detected in the plasma and tissues of animals, including catfish (Li et al., 2003), and humans (Sandau et al., 2002). Plasma OH-PCBs probably arise from recent dietary exposure, biotransformation of PCBs, and perhaps enterohepatic cycling of OH-PCB conjugates. Laboratory studies demonstrated that rats given PCBs excreted OH-PCBs in milk, as evidenced by the presence of OH-PCBs in the stomach contents of nursing pups (Fielden et al., 2001). OH-PCBs may contribute to the recognized toxic effects of PCBs such as endocrine disruption, tumor promotion, and neurological dysfunction (Machala et al., 2004; Meerts et al., 2004; Arulmozhiraja et al., 2005).

The expected pathways of elimination of these toxic metabolites are glucuronidation and sulfonation, yet few studies have examined the conjugation of OH-PCBs (Tampal et al., 2002; Daidoji et al., 2005; Sacco and James, 2005; Wang et al., 2006) particularly by glucuronidation, which is normally a higher capacity pathway than sulfonation. Glucuronidation is catalyzed by a family of microsomal enzymes, the UDP-glucuronosyltransferases (UGTs), and requires the cosubstrate uridine 5'-diphosphoglucuronic acid (UDPGA). UGTs are expressed in the liver and extrahepatic tissues (Daidoji et al., 2005).

The various chlorine and hydroxyl substitution patterns possible on the biphenyl structure may lead to significant differences in glucuronidation kinetics. One possible explanation for the slow elimination of certain OH-PCBs is that they are poor substrates for glucuronidation. The efficiency of glucuronidation of several mostly para-OH-
PCBs by rat liver microsomes varied widely, and substitution of chlorine atoms at the meta- and para-positions on the aphenolic ring lowered $V_{\text{max}}$ (Tampal et al., 2002). In rats, liver microsomes exhibited higher $V_{\text{max}}$ values and enzyme efficiencies for glucuronidation of OH-PCBs than the large and small intestine, lung, kidney, or testis (Daidoji et al., 2005). An exception was that compared with the liver, the rat intestine had similar or higher efficiencies of glucuronidation for two of the substances studied, 4'-OH-CB121 and 4'-OH-CB159.

Most of the persistent OH-PCBs found in human plasma are hydroxylated at the para-position and meta-chlorinated on either side of the phenolic group. The remaining substitution pattern on both rings is highly variable (Bergman et al., 1994). A hydroxyl group in the para-position with two flanking chlorine atoms is associated with inhibition of sulfortransferases (Kester et al., 2000; Wang et al., 2005) and high affinity for the major transport protein for thyroid hormone, transthrethin (Lans et al., 1993).

The other variable that can influence the rate of glucuronidation of a xenobiotic is the tissue concentration of UDPGA. When in vitro studies are conducted, it is common practice to use saturating concentrations of UDPGA, so that maximal UGT activity will be observed. Thus, millimolar concentrations of UDPGA are usually used when in vitro studies are conducted, it is common practice to use saturating concentrations of UDPGA to be used and linearity with protein and time. Duplicate tubes were used for each measurement. The incubation mixture consisted of 0.1 M Tris-Cl buffer (pH 7.6), 5 mM MgCl$_2$, 50 μg of Brij-58, 100 μg of catfish intestinal or hepatic microsomal protein, varying concentrations of [14C]UDPGA, and varying concentrations of OH-PCB substrate in a total reaction volume of 0.1 ml. Blanks contained all components except the OH-PCB. The OH-PCBs were added to tubes from methanol solutions, and the solvent was removed under nitrogen. Mixtures of microsomal protein and Brij-58 were added to the dried substrate, vortex-mixed, and left on ice for 30 min. Subsequently, the buffer, MgCl$_2$, and water were added. After a preincubation of 3 min at 35°C, the optimum temperature for catfish UGT activity, [14C]UDPGA was added to initiate the reaction, which was terminated after a 30-min incubation by the addition of a 1:1 mixture of 2.5% acetic acid and 1.0 ml of PIC-A and 0.3 ml of water. The ion pair of the glucuronide product was extracted into ethyl acetate, 2 × 1.5 ml. For each tube, duplicate portions of the combined ethyl acetate phases were counted for quantitation of glucuronide conjugate, and results were averaged.

The extraction assay was validated with three substrates, 4’OH-CB69, 4’OH-CB112, and 4’OH-CB121. For each of these substrates and for incubation of microsomes with [14C]UDPGA but no substrate, portions of the ethyl acetate extracts were evaporated to dryness and taken up in 0.1 ml of ethanol. For incubations with the OH-PCBs additional residues from the dried ethyl acetate extracts were taken up in 0.1 ml of Trit-C buffer (pH 6) or 0.1 ml of Trit-C buffer (pH 6) containing 7500 units of β-glucuronidase. The tubes with Trit buffer were incubated at 37°C for 16 h; then 0.1 ml of ethanol was added. Samples of each (0.04 ml) as well as samples of the aqueous phase from the incubations (0.005 ml) were applied to the adsorbent phase of LK5DF plates (Whatman, Florham Park, NY). Plates were developed in a solvent system containing 1-butanol-acetone-acetic acid-ammonium hydroxide-water (50:25:9:0.75:15 by volume). Dried plates were placed in a Packard Instant Image to visualize and quantitate the radioactivity by electronic autoradiography.

**Materials and Methods**

**Chemicals.** A total of 18 substrates were used in this study (Fig. 1). The nomenclature of the OH-PCBs as PCB metabolites is based on the recommendations of Maervoet et al. (2004). The following substrates (catalog nos. in parentheses) were purchased from AccuStandard (New Haven, CT): 4'-OH-CB1 (1002N), 4'-OH-CB2 (1003N), 4'-OH-CB9 (2003N), 4'-OH-CB14 (2004N), 4'-OH-CB18 (3004N), 4'-OH-CB69 (4008N), 4'-OH-CB72 (4009N), 4'-OH-CB106 (5005N), 4'-OH-CB112 (5006N), 4'-OH-CB121 (5007N), 4'-OH-CB159 (6001N), and 4'-OH-CB165 (6002N). The compounds 4'-OH-CB1, 4'-OH-CB35, 4'-OH-CB39, 4'-OH-CB68, and 4'-OH-CB79 were synthesized by Suzuki coupling as described previously (Lehmler and Robertson, 2001). The 4-hydroxy biphenyl (4'-OH-BP) was purchased from Sigma-Aldrich (St. Louis, MO). [14C]UDPGA (196 μCi/μmol) was obtained from PerkinElmer Life and Analytical Sciences (Boston, MA). The [14C]UDPGA was diluted with unlabeled UDPGA to a specific activity of 1.5 to 5 μCi/μmol for use in enzyme assays. Tetrabutylammonium hydrogen sulfate (PIC-A) was obtained from Waters (Milford, MA). Other reagents were the highest grade available from Fisher Scientific (Atlanta, GA) and Sigma-Aldrich.

**Animals.** Adult channel catfish (*Ictalurus punctatus*) aged approximately 2.5 years with body weight of 2.1 to 3.7 kg were used for study of OH-PCB glucuronidation. Adult catfish aged approximately 1.5 years with body weight of 0.75 to 1.7 kg were used for measurement of UDPGA concentrations. All fish were kept in flowing well water and fed a fish chow diet (Silvercup, Murray, UT). Care and treatment of the animals were conducted as per the guidelines of the University of Florida Institutional Animal Care and Use Committee. The microsomal fractions were obtained from liver and proximal intestinal mucosa using a procedure described previously (James et al., 1997) and stored in aliquots at −80°C until analysis. Protein determination was carried out by the method of Lowry et al. (1951) using bovine serum albumin as a protein standard.

**Glucuronidation Assay.** A radiochemical ion-pair extraction method was used to investigate the glucuronidation of the 4'-OH-PCBs and 4'-OH-BP (Wang et al., 2004). Substrate consumption did not exceed 10%. Initial experiments determined the saturating concentrations of UDPGA to use and linearity with protein and time. Duplicate tubes were used for each measurement. The incubation mixture consisted of 0.1 M Tris-Cl buffer (pH 7.6), 5 mM MgCl$_2$, 50 μg of Brij-58, 100 μg of catfish intestinal or hepatic microsomal protein, varying concentrations of [14C]UDPGA, and varying concentrations of OH-PCB substrate in a total reaction volume of 0.1 ml. Blanks contained all components except the OH-PCB. The OH-PCBs were added to tubes from methanol solutions, and the solvent was removed under nitrogen. Mixtures of microsomal protein and Brij-58 were added to the dried substrate, vortex-mixed, and left on ice for 30 min. Subsequently, the buffer, MgCl$_2$, and water were added. After a preincubation of 3 min at 35°C, the optimum temperature for catfish UGT activity, [14C]UDPGA was added to initiate the reaction, which was terminated after a 30-min incubation by the addition of a 1:1 mixture of 2.5% acetic acid and 1.0 ml of PIC-A and 0.3 ml of water. The ion pair of the glucuronide product was extracted into ethyl acetate, 2 × 1.5 ml. For each tube, duplicate portions of the combined ethyl acetate phases were counted for quantitation of glucuronide conjugate, and results were averaged.

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**Measurement of UDPGA.** Liver tissue or intestinal mucosa (0.1 g) was mechanically homogenized with 0.4 ml of 0.3 M monobasic ammonium phosphate. The tube containing the homogenate was placed in a boiling water bath for 1 min and then removed and cooled, and the contents were rehomogenized. The tube was centrifuged at 600g for 20 min, and the cloudy supernatant was transferred into a 1.5-ml microcentrifuge tube and centrifuged for 15 min at 16,000g. The supernatant was then filtered by centrifugation in 0.45 μm polycarbonate filters (Corning Life Sciences, Corning, NY). An aliquot, 50 μl of this sample was analyzed by anion-exchange HPLC [model 2300 pump (ISCO, Lincoln, NE)] with a Dynamax UV absorbance detector (Rainin Instruments, Woburn, MA)]. HPLC conditions were an isocratic mobile phase of 0.14 M ammonium phosphate buffer (pH 3.0) at 1 ml/min and a Zorbax SAX column (stationary phase (column size 4.6 mm i.d. × 250 mm, 5 μM; Agilent Technologies, Palo Alto, CA) with UV detection at 260 nm.

**Physicochemical Parameters.** For each OH-PCB and 4'-OH-BP, the $pK_a$ of the phenolic group was calculated using SPARC software (Hilal et al., 1995). Other structural properties, such as log P, dihedral angle, and molecular size,
were calculated with software [ACD/ILab (Advanced Chemistry Development, Toronto, ON, Canada) or ChemDraw 3D (CambridgeSoft, Cambridge, MA)].

Data Analysis. Duplicate values for the rate of conjugate formation at each substrate concentration were used to calculate kinetic parameters using Prism (version 4.0; GraphPad Software, Inc., San Diego, CA). Equations used to fit the data were the Michaelis-Menten equation for one-site binding and the Hill plot for positive cooperativity. The best fit to each equation was selected as the results for each data set. Relationships between structural features and kinetic parameters were analyzed by linear or nonlinear regression, and statistical differences were examined by analysis of variance with post hoc tests using Prism 4.0.

Results
Validation of Assay Method. The tetrabutylammonium ion pairs of the glucuronide conjugates of 4'-OH-CB69, 4'-OH-CB112, and 4'-OH-CB121 were shown by TLC analysis and autoradiography to be completely extracted into ethyl acetate, with no conjugate detected in the aqueous phase. Results for 4'-OH-CB121 are shown in Fig. 2. The glucuronide conjugate of 4'-OH-CB121 had an \( R_f \) of 0.74, glucuronic acid released by hydrolysis had an \( R_f \) of 0.28, and unreacted UDPGA did not move from the origin. TLC analysis of the ethyl acetate extracts (Fig. 2, lanes 1 and 2) showed that no [14C]UDPGA was extracted into ethyl acetate and that incubation of microsomes with [14C]UDPGA did not reveal detectable conjugates. TLC analysis of the aqueous phases (Fig. 2, lanes 5 and 6) showed that no glucuronide conjugate remained in the aqueous phase. Figure 2 (lanes 3 and 4) also shows that the glucuronide of 4'-OH-CB121 was hydrolyzed by \( \beta \)-glucuronidase but not by incubation in buffer alone. Similar results were obtained with the other substrates tested.

With all substrates, assay duplicates showed less than 2% difference from the mean. Although no conjugate can be observed in lane 2 of Fig. 2, a small amount of radioactivity was extracted from incubations that contained microsomes but no substrate, and rates of glucuronidation were corrected for this background. Incubations with no substrate and no microsomes showed no detectable counts in the ethyl acetate extracts.

Kinetics of UDPGA. The kinetic parameters with varying of the concentration of UDPGA were determined for the glucuronidation of three OH-PCBs (Table 1). The apparent \( K_m \) values for UDPGA were more than 10-fold higher in the liver than in the intestine. The concentrations of UDPGA used for studies of OH-PCB substrate kinetics were such that maximal rates were measured and were 1.5 mM for liver microsomes and 0.2 mM for proximal intestinal microsomes.

Aglycone Kinetics. The glucuronidation of most of the OH-PCBs tested followed Michaelis-Menten kinetics in hepatic and intestinal microsomes. Figure 3A shows representative results with hepatic microsomes from four individual catfish for 4'-OH-CB159. In the case of the glucuronidation of 4'-OH-CB18 and 4'-OH-CB112 by

![Fig. 1. Structures of polychlorinated biphenylols used as substrates for glucuronidation in microsomes from channel catfish liver and proximal intestine.](image-url)
From incubation of hepatic microsomes with [14C]UDPGA and 4-OH-CB121 or no substrate, as described under Materials and Methods. Arrows indicate the origin, front, and bands corresponding to the glucuronide conjugate, glucuronic acid, and UDPGA. The ethyl acetate extracts were evaporated to dryness under nitrogen, and the residues were reconstituted in ethanol or Tris buffer. Lane 1, ethyl acetate extract of an incubation with 4'-OH-CB121, showing the glucuronide conjugate; lane 2, ethyl acetate extract from an incubation with no substrate; lane 3, β-glucuronidase-treated ethyl acetate extract from an incubation with 4'-OH-CB121, showing hydrolysis of the glucuronide conjugate to [14C]glucuronic acid; lane 4, ethyl acetate extract from an incubation with 4'-OH-CB121, subsequently incubated with buffer, showing stability of the conjugate; lane 5, aqueous phase from an incubation with 4'-OH-CB121, showing UDPGA but no glucuronide conjugate; and lane 6, aqueous phase from an incubation with no substrate.

![TLC radiochromatograms](image)

**Fig. 2.** Validation of the extraction method for assay of OH-PCB glucuronidation. The figure shows TLC radiochromatograms of the ethyl acetate extract and aqueous phase from incubation of hepatic microsomes with [14C]UDPGA and 4'-OH-CB121 or no substrate. Arrows indicate the origin, front, and bands corresponding to the glucuronide conjugate, glucuronic acid, and UDPGA. The ethyl acetate extracts were evaporated to dryness under nitrogen, and the residues were reconstituted in ethanol or Tris buffer. Lane 1, ethyl acetate extract of an incubation with 4'-OH-CB121, showing the glucuronide conjugate; lane 2, ethyl acetate extract from an incubation with no substrate; lane 3, β-glucuronidase-treated ethyl acetate extract from an incubation with 4'-OH-CB121, showing hydrolysis of the glucuronide conjugate to [14C]glucuronic acid; lane 4, ethyl acetate extract from an incubation with 4'-OH-CB121, subsequently incubated with buffer, showing stability of the conjugate; lane 5, aqueous phase from an incubation with 4'-OH-CB121, showing UDPGA but no glucuronide conjugate; and lane 6, aqueous phase from an incubation with no substrate.

**TABLE 1**

**Kinetic parameters for the cosubstrate UDPGA in the glucuronidation of three OH-PCBs**

<table>
<thead>
<tr>
<th>OH-PCB Concentration</th>
<th>V_max (Apparent) μM</th>
<th>k_m (Apparent) μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4'-OH-CB35</td>
<td>500</td>
<td>0.87 ± 0.20</td>
</tr>
<tr>
<td>4'-OH-CB72</td>
<td>500</td>
<td>0.60 ± 0.10</td>
</tr>
<tr>
<td>4'-OH-CB69</td>
<td>200</td>
<td>0.20 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>697 ± 246</td>
<td>684 ± 323</td>
</tr>
<tr>
<td></td>
<td>697 ± 246</td>
<td>684 ± 323</td>
</tr>
<tr>
<td></td>
<td>27 ± 14</td>
<td></td>
</tr>
</tbody>
</table>

Proximal intestine and 4'-OH-CB35 by liver, the data showed positive cooperativity and fitted the Hill plot. Results for glucuronidation of 4'-OH-CB112 by proximal intestinal microsomes are shown in Fig. 3B. The estimated apparent maximal rate of glucuronidation of polychlorinated biphenyls by channel catfish ranged from 124 to 784 pmol/min/mg for proximal intestine and 317 to 2838 pmol/min/mg for the liver (Table 2). Compared with the other substrates tested, 4'-OH-CB18 and 4'-OH-CB69 were very efficiently glucuronidated by the intestine; these substrates were less efficiently glucuronidated by liver microsomes. The efficiencies of glucuronidation of 4'-OH-CB3, 4'-OH-CB9, 4'-OH-CB79, 4'-OH-CB106, and 4'-OH-CB112 were similar in intestinal and hepatic microsomes, whereas the other OH-PCBs studied exhibited higher rates of clearance in liver than in intestine (Table 2). As has been found previously in studies of fish, there was considerable interindividual variability in the kinetic parameters.

**Fig. 3.** Representative kinetic plots of the glucuronidation of OH-PCBs in four individual channel catfish. A, Michaelis-Menten plot for 4'-OH-CB159 by hepatic microsomes. B, Hill plot for 4'-OH-CB112 by proximal intestinal microsomes.

The uncorrected microsomal protein yield was 4.7 ± 0.5 mg/g (mean ± S.E.M., n = 9) in proximal intestine and 12.4 ± 1.5 mg/g in liver (n = 4). The total microsomal protein per organ was significantly higher in liver (442 ± 54 mg in whole liver) than in proximal intestine (32 ± 6 mg in intestinal mucosa). Because there is an order of magnitude more microsomal protein in the total liver than in the proximal intestinal mucosa, then under optimal in vitro assay conditions the liver should be quantitatively the major organ of glucuronidation of all the OH-PCBs studied in the channel catfish.

With the exceptions of 4-OH-CB1, 4-OH-CB2, and 4'-OH-CB165, K_m values for individual OH-PCBs were different in the liver than in the intestine. For most of the OH-PCBs studied, the K_m values for intestinal catfish UGTs (K_m range: 15–572 μM) were lower than for liver UGTs (K_m range: 57–1643 μM). V_max values were significantly higher in liver than in intestine (p < 0.01). The V_max values of OH-PCB glucuronidation in liver and intestine were correlated with each other (r^2 = 0.36), as shown in Fig. 4. This relationship did not exist for K_m values.

**Structural Influences and Physicochemical Properties.** Five structurally related OH-PCB pairs were studied, in which the chlorine substitution pattern in the aphenolic ring was constant, whereas the phenolic ring had one or two chlorine atoms flanking the hydroxyl
compared for liver and intestine. This analysis showed that in liver, the mean values for glucuronidation of OH-PCBs were significantly decreased upon addition of a second chlorine (Cl) atom flanking the phenolic moiety ($p < 0.01$) (Fig. 5).

The effect of position and number of chlorine substituents in the aphenolic ring on the glucuronidation of OH-PCBs was also investigated. No significant differences in $K_m$ and $V_{\text{max}}$ values were observed with the absence or presence of specific chlorine substituents on the aphenolic ring. Weak negative correlations were found between $K_m$ values in intestine and parameters related to molecular size and lipophilicity. Similar weak negative correlations were found between $V_{\text{max}}$ values in liver and the same parameters (data not shown).

The kinetic parameters of the OH-PCBs studied were grouped according to whether there were zero, one, or two chlorine atoms flanking the OH group, and the mean values for $K_m$ and $V_{\text{max}}$ were compared for liver and intestine. This analysis showed that in liver, both $V_{\text{max}}$ and $K_m$ values were higher for OH-PCBs with one chlorine atom flanking the OH group than for those with zero or two flanking chlorine atoms (Table 3). In intestine, OH-PCBs with two chlorine atoms flanking the OH group showed lower $V_{\text{max}}$ values than those with zero or one. It is known that chlorine atoms adjacent to the phenolic OH group affect the $pK_a$ of chlorophenols and chlorobiphenyls. Plots of $pK_a$ versus $V_{\text{max}}$ for each OH-PCB and including the nonchlorinated 4-OH-BP revealed a trend for higher $V_{\text{max}}$ values with compounds that have $pK_a$ values between 7 and 8 in liver and between 7 and 8.5 in intestine (Fig. 6).

**UDPGLA Concentration in Liver and Intestine.** The HPLC...
TABLE 3

Comparison of composite kinetic parameters for the glucuronidation of OH-PCBs grouped according to the number of chlorine atoms flanking the phenolic group

Results shown are mean ± S.E. In liver, n = 16 for zero flanking chlorine atoms, n = 20 for one flanking chlorine atoms, and n = 24 for two flanking chlorine atoms. In intestine, n = 16 for zero flanking chlorine atoms, n = 20 for one flanking chlorine atoms, and n = 32 for two flanking chlorine atoms.

<table>
<thead>
<tr>
<th>Organ and Parameter</th>
<th>Flanking Chlorines</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Liver</td>
<td>V_max (apparent) (pmol/min/mg)</td>
</tr>
<tr>
<td></td>
<td>776 ± 128^a,b</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Intestine</td>
<td>501 ± 46^d</td>
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<td></td>
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</table>

For any one parameter, values with the same superscript letter are not significantly different from one another. Those with different superscript letters are significantly different, P < 0.01.

Discussion

For OH-PCBs, as for other xenobiotics with one phenolic group and no other polar substituents (Wang et al., 2004), adding PIC-A in the assay stop solution and then extracting the conjugate ion pair into ethyl acetate, is a rapid, sensitive method for measuring rates of glucuronidation of suitable substrates. Microsomal glucuronidation V_max values of the 4-OH-PCBs studied and 4-OH-BP were lower in proximal intestine than in liver, and, for most compounds, K_m values were also lower in intestine than liver, suggesting that intestinal UGTs have higher affinity for OH-PCB substrates than liver. The total capacity of the liver for glucuronidation of OH-PCBs is much greater than that of the intestine when the total content of microosomal protein in these two organs is considered. The yield of microsomal protein from liver was twice that of the intestine, possibly because of the decreased amount of endoplasmic reticulum in enterocytes relative to hepatocytes (DePierre et al., 1987). The greater size of the liver meant that the total amount of microsomal protein in liver was more than 10 times that in the proximal intestine. Thus, the overall capacity of the liver under optimal conditions for glucuronidation is considerably higher than that of the intestine. However, biotransformation in the intestine is important for orally ingested xenobiotics, and the greater efficiency of the intestinal UGTs at low concentrations of OH-PCBs suggests that they play an important role in protecting the organism from these potentially toxic compounds.

These results suggest that liver and proximal intestine have different UGT isofrom profiles, with the intestine possessing one or more isofroms that display greater efficiency than those of the liver for OH-PCBs. We have cloned several UGT isofroms from catfish liver and intestine whose characteristics support this view (Sacco, 2006). UGT isofroms responsible for efficient OH-PCB glucuronidation may be catfish enzymes analogous to rat UGT1A1, UGT1A6, and UGT2B1 (Daidoji et al., 2005).

Of the substrates studied, 4’-OH-CB18 was glucuronidated most readily in the channel catfish intestine and liver, with efficiencies of 47 and 20.8 µl/min/mg of protein, respectively. The poorest substrates were 4-OH-CB14 in the intestine, with an efficiency of 0.7 µl/min/mg of protein and 4’-OH-CB3, 4'-OH-CB9, and 4’-OH-CB112 in the liver all with efficiencies of 1.4 µl/min/mg of protein. Nine of the compounds studied in catfish were studied in rat liver (Tampal et al., 2002; Daidoji et al., 2005), namely 4’-OH-CB3, 4-OH-CB14, 4-OH-
CB39, 4'-OH-CB35, 4'-OH-CB68, 4'-OH-CB69, 4'-OH-CB79, 4'-OH-CB121, and 4'-OH-CB159. There was no correlation in \( V_{\text{max}} \), \( K_m \), or enzyme efficiency for these compounds between catfish liver and rat liver or between catfish intestine and rat liver (\( p > 0.05 \)). All of these compounds were glucuronidated most efficiently in rat liver microsomes, with a mean 24-fold higher efficiency than in catfish liver (range of 5- to 55-fold higher in rat) and 47-fold higher efficiency than in catfish intestine (range of 7- to 173-fold higher in rat liver). Although the greater efficiencies of glucuronidation in rat than in catfish may be ascribed to differences in UGT isoform type, properties, and level of expression, this is an important difference, because glucuronidation is likely to be the major pathway for elimination of OH-PCBs. The relatively poor glucuronidation efficiencies in catfish liver and intestine suggest that both bioavailability and elimination half-lives of OH-PCBs will be higher than those in rat, leading to greater exposure of catfish to potentially toxic OH-PCBs.

Because microsomes contain a heterogeneous population of UGTs exhibiting different levels of expression and activity, it was not the major objective of this study to attempt to predict the effect of molecular structure and physiochemical parameters on the glucuronidation of OH-PCBs, which is better achieved using individual isoforms. However, effects observed at a microsomal level are likely to reflect processes that occur in the intact organism, wherein multiple UGTs contribute to substrate glucuronidation. Recognizing structural features that affect \( K_m \) and \( V_{\text{max}} \) may help to further delineate the different toxicokinetics of OH-PCBs.

OH-PCBs with two chlorine atoms flanking the OH group exhibited lower \( V_{\text{max}} \) values than compounds with one flanking chlorine in catfish liver and proximal intestine (Fig. 5). For example, whereas 4'-OH-CB35 was a good substrate for glucuronidation, addition of a second chlorine flanking the –OH group as in 4'-OH-CB79 resulted in a greater decrease in \( V_{\text{max}} \) than the addition of two adjacent chlorine substituents on the aphenolic ring as in 4'-OH-CB106. The effect of a second flanking chlorine atom on \( V_{\text{max}} \) is interesting from a toxicological standpoint as almost all of the OH-PCBs identified in human plasma feature a 4-hydroxy-3,5-dichloro pattern (Fängström et al., 2002; Hovander et al., 2002; Sandau et al., 2002). It is possible that one reason for the persistence of these OH-PCBs may be slow glucuronidation because of this structural arrangement.

Further examination of the effect of chlorine substitution adjacent to the 4-hydroxy group revealed the pattern that \( V_{\text{max}} \) was highest in liver for compounds with one adjacent chlorine atom, compared with zero or two, whereas in intestine, compounds with two chlorines flanking the hydroxyl group had the lowest \( V_{\text{max}} \) values (Table 3). The physicochemical property most strongly influenced by the presence of adjacent chlorine atoms is the acidity of the phenolic group. The estimated \( pK_a \) values for OH-PCBs with two flanking chlorine substituents were significantly lower than for similar molecules with one flanking chlorine, and those with one flanking chlorine had lower \( pK_a \) values than those with no flanking chlorines (Hilal et al., 1995; Tampal et al., 2002). In catfish, \( V_{\text{max}} \) values for glucuronidation were highest if the estimated \( pK_a \) of the OH-PCB was in the range of 7 to 8 for liver microsomes and 7 to 8.5 for proximal intestinal microsomes (Fig. 6). At physiological pH and the assay pH, substrates with \( pK_a \) values in this range will have approximately half the molecules of OH-PCB in the ionized form, whereas those with lower or higher \( pK_a \) values will have greater or fewer proportionately as the anion. Ease of protonation/deprotonation is likely to influence the interaction of the hydroxyl group with a critical histidine residue in the aglycone binding site that is thought to form part of a charge-relay system important in transfer of the glucuronide (Radominska-Pandya et al., 2005; Locuson and Tracy, 2007). Lipophilicity and molecular size may also influence kinetic properties, but in this study, only weak associations with these parameters were observed.

Catfish liver UDPGA concentrations were similar to those reported previously for mammals, such as humans (279 \( \mu \)M), rats (400 \( \mu \)M), and guinea pigs (413 \( \mu \)M) (Cappiello et al., 1991; Goon and Klaassen, 1992; Yamamura et al., 2000) and higher than those we found from catfish sacrificed in the month of May (133 \( \mu \)M) (James et al., 2008). The results reported for trout (116 \( \mu \)M) and carp (21 \( \mu \)M) liver (Zhirovik et al., 1975) are lower than the levels measured in this study (Fig. 6). Intestinal UDPGA concentrations in the catfish were in the range reported for rat intestine (121 ± 5 \( \mu \)M) (Goon and Klaassen, 1992) but higher than that reported for humans (19 ± 5 \( \mu \)M) (Cappiello et al., 1991) and lower than that reported for guinea pigs (322 ± 5 \( \mu \)M) (Zhirovik et al., 1975). These differences may be species-related or influenced by nutritional or physiological changes influenced by season. Another possible contributor to the discrepancy is that different analytical techniques were used. The values for trout and carp (Zhirovik et al., 1975) were determined from liver homogenized in perchloric acid to solubilize the nucleotides. This process may have led to hydrolysis of UDPGA to UDP and glucuronic acid, which has been shown to increase with decreased pH (Bedford et al., 2003).

Hepatic UDPGA concentrations in channel catfish taken in the month of November (this study) were in the range of 246 to 371 \( \mu \)M. The UDPGA \( K_m \) values obtained for hepatic glucuronidation were 684 and 697 \( \mu \)M for 4'-OH-CB72 and 4'-OH-CB35, respectively (Table 1). This result means that, in vivo, hepatic glucuronidation would proceed at a suboptimal rate both for 4'-OH-CB72 and 4'-OH-CB35. In the intestine, UDPGA concentrations ranged from 71 to 255 \( \mu \)M, higher than the UDPGA \( K_m \) of 27 \( \mu \)M found for 4'-OH-CB69 in proximal intestinal microsomes (Table 1). The UGT isoforms in the intestine responsible for OH-PCB glucuronidation appear to work optimally at much lower concentrations of UDPGA than in liver and thus are well equipped to catalyze the conjugation of low concentrations of OH-PCBs.

In summary, the different \( K_m \) values estimated for glucuronidation of a series of 4-OH-, 4-OH-3-chloro-, and 4-OH-3,5-dichloro-PCBs suggest differences in the UGT isozyme profile of catfish liver and proximal intestine. The rates of maximal glucuronidation for all substrates tested were higher for the liver than for the intestine and were optimal for substrates whose \( pK_a \) values favored transfer of the phenolic proton. The \( V_{\text{max}} \) values for both hepatic and intestinal glucuronidation were lower in compounds with two chlorine atoms flanking the phenolic group, an arrangement typical of OH-PCBs that persist in organisms. The \( K_m \) for UDPGA in catfish liver was more than double the measured hepatic concentration of UDPGA, suggesting that glucuronidation in the intact liver will proceed more slowly than was measured in vitro in the presence of excess UDPGA. In the intestine, however, the measured concentrations of UDPGA were 4 times higher than the UDPGA \( K_m \) value, suggesting that enzyme efficiencies measured in vitro will approximate the expected in vivo rate of glucuronidation of OH-PCBs.

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References

