Hydroxylated Polychlorinated Biphenyls as Inhibitors of the Sulfation and Glucuronidation of 3-Hydroxy-Benzo[a]pyrene

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Polychlorinated biphenyls (PCBs) can be metabolized by cytochromes P450 to hydroxylated biotransformation products. In mammalian studies, some of the hydroxylated products have been shown to be strong inhibitors of steroid sulfotransferases. As a part of ongoing research into the bioavailability of environmental pollutants in catfish intestine, we investigated the effects of a series of hydroxylated PCBs (OH-PCBs) on two conjugating enzymes, phenol-type sulfotransferase and glucuronosyltransferase. We incubated cytosolic and microsomal samples prepared from intestinal mucosa with 3-hydroxy-benzo[a]pyrene and appropriate cosubstrates and measured the effect of OH-PCBs on the formation of BaP-3-glucuronide and BaP-3-sulfate. We used PCBs with 4, 5, and 6 chlorine substitutions and the phenolic group in the *ortho*, *meta*, and *para* positions. OH-PCBs with the phenolic group in the *ortho* position were weak inhibitors of sulfotransferase; the median inhibitory concentration (IC_{50}) ranged from 330 to 526 μM. When the phenol group was in the *meta* or *para* position, the IC_{50} was much lower (17.8–44.3 μM). The OH-PCBs were more potent inhibitors of glucuronosyltransferase, with IC_{50}s ranging from 1.2 to 36.4 μM. The position of the phenolic group was not related to the inhibitory potency: the two weakest inhibitors of sulfotransferase, with the phenolic group in the *ortho* position, were 100 times more potent as inhibitors of glucuronosyltransferase. Inhibition of glucuronosyltransferase by low concentrations of OH-PCBs has not been reported before and may have important consequences for the bioavailability, bioaccumulation, and toxicity of other phenolic environmental contaminants. *Key words:* biotransformation, enzyme inhibition, polychlorinated biphenyls, sulfotransferase, UDP-glucuronosyltransferase, xenobiotics. *Environ Health Perspect* 110:343–348 (2002). [Online 1 March 2002]
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Polychlorinated biphenyls (PCBs) are considered important environmental contaminants (*1*). Even though the production of PCBs was banned in 1976 in the United States, there is still great concern about the potential toxic effects of PCBs that have been released into the environment. Recent investigations showed that human populations with a high fish consumption have considerably higher concentrations of PCBs and PCB metabolites in their tissues than control populations (*2–4*).

The lipophilic character of PCBs enables these compounds to bioaccumulate in adipose tissues. Depending on the chlorine substitution pattern, PCBs are more or less easily oxygenated by cytochromes P450, of which CYP1A and CYP2B are considered the most important isozymes to catalyze this reaction (*5*). Oxygenation of PCBs may occur by direct insertion, giving a hydroxylated metabolite (OH-PCB), or by epoxide formation. The epoxide may rearrange to give an OH-PCB or react with glutathione to yield a mercapturic acid or a methyl sulfone conjugate. Methyl sulfone-PCBs can covalently bind to proteins, cause induction of cytochrome P450 enzymes, or exert endocrine-related effects (*5*).

Most metabolized PCBs are excreted through urine and bile (*6*). Some OH-PCBs accumulate in blood plasma. These metabolites have a chlorine substitution pattern that resembles the iodine substitution on thyroxine, a thyroid hormone, which enables them to bind to the thyroxine transport protein. This apparently causes reduced blood plasma thyroid levels, which has been linked to impaired fetal development in pregnant individuals (*7*). PCBs and methylsulfone PCBs also induce uridine diphosphate-glucuronosyltransferase (UGT), including the form that glucuronidates thyroxine (*8*). Schuur et al. (*9,10*) described several OH-PCBs that inhibit thyroxine sulfation, the major regulatory pathway for this thyroid hormone. More recently, several OH-PCBs were found to be very potent inhibitors of estrogen sulfotransferase (EST), which may explain the estrogenic effect of PCBs (*11*).

PCBs are often found together with polynuclear aromatic hydrocarbons (PAHs) in contaminated aquatic environments. Aquatic organisms, and especially bottom dwellers like channel catfish (*Ictalurus punctatus*), are prone to exposure to these compounds, either through their diet or through uptake over the gills (*12*). Intestinal absorption is an important route for uptake and first-pass metabolism of lipophilic environmental toxicants in fish (*13*).

One of the most widely studied PAHs is benzo[a]pyrene (BaP), which after oxygenation by cytochromes P450 can form toxic and carcinogenic metabolites. In previous work we demonstrated that intestinal mucosa of catfish has inducible CYP1A activity and that phenolic metabolites of BaP are easily conjugated by phenol-type sulfotransferases (SULT) and UGT (*14–16*). These phase II conjugation reactions are important detoxification pathways for phenolic metabolites of PAHs, which may otherwise be further oxidized to more toxic quinones and diol-epoxides (*17–19*).

Doi et al. (*20*) demonstrated that a model coplanar PCB [3,3′,4,4′-tetrachlorobiphenyl (TCB)] was slowly metabolized to OH-PCB in catfish intestine (*20*). This raised the question of the inhibitory effect of OH-PCBs on the conjugation of hydroxylated BaP. In this study we investigated the effect of a set of OH-PCBs on the sulfation and glucuronidation of 3-OH-BaP in subcellular fractions of channel catfish intestinal mucosa. The OH-PCBs selected for this study were potential metabolites of the toxic coplanar congener 3,3′,4,4′-TCB and of selected *ortho*-chlorinated tetra, penta, and hexachlorobiphenyls found to persist in the environment (*21*). We compared inhibition characteristics of the OH-PCBs for both enzymes and investigated inhibition kinetics.

Materials and Methods

Chemicals. 4-Hydroxy-2′,3,5,5′-TCB (4′-OH-CB 72) and 4-hydroxy-2′,3,3′,5,5′,6′-hexachlorobiphenyl (4′-OH-CB 165) were purchased from AccuStandard, Inc. (New Haven, CT). The other OH-PCBs were

synthesized as described below. 3′-Phospho-adenosine-5′-phosphosulfate (PAPS) was obtained from S.S. Singer (University of Dayton, Dayton, OH), and uridine 5′-diphosphoglucuronic acid (UDPGA) was obtained from Sigma (St. Louis, MO).

3-Hydroxy-BaP, BaP-3-sulfate, and BaP-3-β-D-glucopyranosuronic acid were purchased from Midwest Research Institute (Kansas City, MO) through the Chemical Carcinogen Reference Standard Repository of the National Cancer Institute. Other reagents

were the highest grade available from Fisher Scientific (Atlanta, GA) and Sigma.

Synthesis and structural verification of OH-PCBs. PCBs and PCB metabolites were anticipated to be human carcinogens and were therefore handled in an appropriate manner. 2-Hydroxy-3,3′,4,4′-TCB (2-OH-CB 77), 5-hydroxy-3,3′,4,4′-TCB (5-OH-CB 77), and 4-hydroxy-2,3,3′,4′-TCB (4-OH-CB 56) were synthesized by the Cadogan coupling (*22,23*) of 3,4-dichloro-aniline with 2,3-dichloroanisole and subsequent demethylation of the methoxy PCBs with boron tribromide. The structures of 2-OH-CB 77 and 4-OH-CB 56 were assigned by ^{1}H nuclear Overhauser effect (NOE) nuclear magnetic resonance (NMR) experiments. 4′-Hydroxy-3,3′,5-TCB (4′-OH-CB 78) was synthesized by coupling of 4-amino-2,6-dichlorophenol and 1,2-dichlorobenzene. The reaction mixture was methylated with dimethyl sulfate. 4-Methoxy-3,3′,4′,5-TCB was isolated by flash column chromatography on silica gel with petroleum ether:dichloromethane [8.5:1.5 (v/v)] and petroleum ether:iso-propylether (9.75:0.25 v/v). The OH-PCB was obtained by demethylation with boron tribromide. 6-Hydroxy-3,3′,4,4′-TCB (6-OH-CB 77) was synthesized from
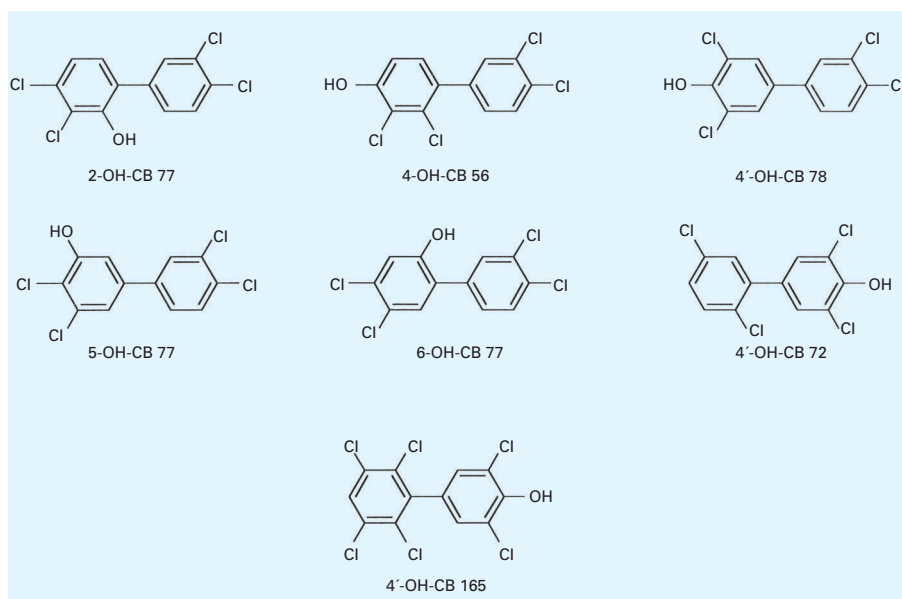

Figure 1. Structures and acronyms for OH-PCBs investigated for their inhibiting capacity of phenol-type SULT and UGT.

Table 1. Analytical data for methoxy PCBs.

PCB	mp	^{1}H-NMR	MS (m/z) (relative intensity, %)
2-Methoxy-3,3′,4,4′-TCB	79°C	(CD$_2$Cl$_2$, 200 MHz) δ 3.53 (s, -OCH$_3$, 3H), 7.21 (d, J = 8.4 Hz, H6), 7.34 (d, J = 8.4 Hz, H5), 7.43 (d, J = 2.1 Hz, d, J = 8.4, H6′), 7.54 (d, J = 8.4 Hz, H5′), 7.68 (d, J = 2.1 Hz, H2′)	320 (78, C$_{13}$H$_8$Cl$_4$O·+), 305 (36, M-CH$_3$), 277 (29, M-CO-CH$_3$), 207 (26, M-CO-CH$_3$-Cl$_2$)
5-Methoxy-3,3′,4,4′-TCB	146°C	(CD$_2$Cl$_2$, 200 MHz) δ 3.97 (s, -OCH$_3$, 3H), 7.01 (d, J = 2.0 Hz, H6), 7.29 (d, J = 2.0 Hz, H2), 7.43 (d, J = 2.2 Hz, d, J = 8.4, H6′), 7.56 (d, J = 8.4 Hz, H5′), 7.69 (d, J = 2.2 Hz, H2′)	320 (77, C$_{13}$H$_8$Cl$_4$O·+), 277 (29, M-CO-CH$_3$), 207 (22, M-CO-CH$_3$-Cl$_2$)
4-Methoxy-2,3,3′,4′-TCB	168°C	(CD$_2$Cl$_2$, 200 MHz) δ 3.94 (s, -OCH$_3$, 3H), 6.96 (d, J = 8.7 Hz, H5), 7.22 (d, J = 8.7 Hz, H6), 7.27 (d, J = 2.0 Hz, d, J = 8.4, H6′), 7.50 (d, J = 2.0 Hz, H2′), 7.52 (d, J = 8.4 Hz, H5′)	320 (64, C$_{13}$H$_8$Cl$_4$O·+), 270 (100, M-Cl-CH$_3$), 207 (25, M-CO-CH$_3$-Cl$_2$)
4-Methoxy-3,3′,4′,5-TCB	122–123°C	(CD$_2$Cl$_2$, 200 MHz) δ 3.92 (s, -OCH$_3$, 3H), 7.39 (d, J = 2.2 Hz, d, J = 8.4, H6′), 7.52 (s, H2 and H6), 7.54 (d, J = 8.4 Hz, H5′), 7.65 (d, J = 2.2 Hz, H2′)	320 (76, C$_{13}$H$_8$Cl$_4$O·+), 305 (61, M-CH$_3$), 277 (24, M-CO-CH$_3$), 207 (26, M-CO-CH$_3$-Cl$_2$)
6-Methoxy-3,3′,4,4′-TCB	139°C	(CD$_2$Cl$_2$, 200 MHz) δ 3.79 (s, -OCH$_3$, 3H), 7.08 (s, H2), 7.32 (d, J = 2.0 Hz, d, J = 8.4, H6′), 7.35 (s, H5), 7.48 (d, J = 8.4 Hz, H5′), 7.58 (d, J = 2.0 Hz, H2′)	320 (76, C$_{13}$H$_8$Cl$_4$O·+), 270 (36, M-Cl-CH$_3$), 207 (20, M-CO-CH$_3$-Cl$_2$)

Abbreviations: δ, chemical shift (ppm); d, doublet; J, coupling constant; s, singlet.

Table 2. Analytical data for OH-PCBs.

PCB	mp	^{1}H-NMR	MS (m/z) (relative intensity, %)
2-OH-CB 77	177°C	(CD$_2$Cl$_2$, 200 MHz) δ 5.99 (s, -OH, 1H), 7.13 (d, J = 8.4 Hz, H6), 7.18 (d, J = 8.4 Hz, H5), 7.39 (d, J = 2.1 Hz, d, J = 8.4, H6′), 7.52 (d, J = 8.4 Hz, H5′), 7.65 (d, J = 2.1 Hz, H2′)	306 (53, C$_{12}$H$_6$Cl$_4$O·+), 207 (100, M-99)
5-OH-CB 77	169°C	(CD$_2$Cl$_2$, 200 MHz) δ 5.86 (s, -OH, 1H), 7.12 (d, J = 2.0 Hz, H6), 7.25 (d, J = 2.0 Hz, H2), 7.38 (d, J = 2.2 Hz, d, J = 8.4, H6′), 7.52 (d, J = 8.4 Hz, H5′), 7.64 (d, J = 2.2 Hz, H2′)	306 (80, C$_{12}$H$_6$Cl$_4$O·+), 207 (16, M-99)
4-OH-CB 56	163°C	(CDCl$_3$, 400 MHz) δ 5.85 (s, -OH, 1H), 7.01 (d, J = 8.6 Hz, H6), 7.15 (d, J = 8.6 Hz, H5), 7.24 (d, J = 2.0 Hz, d, J = 8.4, H6′), 7.50 (d, J = 2.0 Hz, H2′), 7.50 (d, J = 8.4 Hz, H5′)	306 (74, C$_{12}$H$_6$Cl$_4$O·+), 207 (29, M-99)
4′-OH-CB 78	191°C	(CDCl$_3$, 400 MHz) δ 5.94 (s, -OH, 1H), 7.33 (d, J = 2.0 Hz, d, J = 8.4, H6′), 7.45 (s, H2 and H6), 7.50 (d, J = 8.4 Hz, H5′), 7.58 (d, J = 2.2 Hz, H2′)	306 (74, C$_{12}$H$_6$Cl$_4$O·+), 207 (61, M-99)
6-OH-CB 77	135°C	(CDCl$_3$, 400 MHz) δ 5.23 (s, -OH, 1H), 7.08 (s, H2), 7.31 (d, J = 2.0 Hz, d, J = 8.4, H6′), 7.31 (s, H5), 7.55 (d, J = 8.4 Hz, H5′), 7.57 (d, J = 2.0 Hz, H2′)	306 (74, C$_{12}$H$_6$Cl$_4$O·+), 236 (54, M-Cl$_2$)

Abbreviations: δ, chemical shift (ppm); d, doublet; J, coupling constant; s, singlet.

3,4-dichloroaniline and 3,4-dichlorophenol in an analogous manner. The structures of the OH-PCBs used in this study are shown in Figure 1.

We characterized all OH-PCBs by ^{1}H NMR and mass spectrometry (MS) and we observed their melting points (mp). The ^{1}H NMR spectra were recorded on a Varian Gemini 200 or a Varian INOVA 400 (Varian Inc., Walnut Creek, CA) by using CD_2Cl_2 or $CDCl_3$ (Cambridge Isotope Laboratories, Inc., Andover, MA) as a solvent and tetramethylsilane as an internal standard. ^{1}H NOE NMR experiments were performed on a Bruker AM-400 (Bruker NMR, Billerica, MA). Gas chromatography (GC)–MS analyses were performed in the mass spectrometry facility of the University of Kentucky (Lexington, KY). The purity of all compounds was analyzed with a Hewlett Packard 5890 A gas chromatograph equipped with an HP-1 (methyl silicone gum) column (Hewlett Packard, Avondale, PA). The following conditions were used for the GC analysis: injector: 200°C; flame ionization detector: 320°C; starting temperature: 130°C; final temperature: 310°C; heating rate: 8°/min. The purity was > 99% based on relative peak area. The analytical data for methoxy PCBs are shown in Table 1 and those for the OH-PCBs are shown in Table 2.

Animals. We used cytosol and microsomes of four channel catfish (*Ictaluris punctatus*) in this study (two females and two males) with weights ranging from 1,664 to 2,016 g. All fish were kept in flowing well water and fed regular fish chow diet (Silvercup, Murray, UT). Care and treatment of the animals was conducted according to the guidelines of the University of Florida Institutional Animal Care and Use Committee. Cytosol and microsomes from intestinal mucosa were prepared as described previously (*24*).

Sulfation assay. The reaction mixture for measuring the sulfation of 3-OH-BaP consisted of 0.4% bovine serum albumin (BSA), 50 mM Tris (pH 7.0), 20 µM PAPS, 25 µg cytosolic protein, 1 µM 3-OH-BaP, and varying concentrations of inhibitor in a total reaction volume of 500 µL. All inhibitors used in this study were dissolved in dimethylsulfoxide (DMSO). DMSO concentration in the final reaction mix was kept below 0.5% and did not affect the enzymatic reaction or the fluorescence of the sulfation product. The substrate (3-OH-BaP in methanol) was blown dry in the final reaction tube. PAPS was added to this tube. The enzyme, in buffer with BSA, was preincubated with the inhibitor for 5 min at 35°C. The entire mix was then transferred to the tube with substrate and PAPS and incubated for 10 min. Reaction was stopped with 2 mL ice-cold methanol. Precipitated protein was pelleted

by centrifugation; 2 mL of the clear solution was then mixed with 0.5 mL sodium hydroxide (1 N) and fluorescence of $BaP-3-SO_4$ was measured at the optimal excitation/emission wavelengths of 294/415 nm (*14*).

Glucuronidation assay. The reaction mixture for measuring the glucuronidation of 3-OH-BaP consisted of 0.1 M Tris-HCl buffer (pH 7.6), 5 mM $MgCl_2$, 200 µM UDPGA, 50 µg microsomal protein, 1 µg 3-OH-BaP, and Lubrol PX (0.5 mg/mg protein; Sigma). The assay procedure was identical to the sulfation assay described above, except the final incubation time was 30 min and the fluorescence of BaP-3-glucuronic acid was measured at excitation/emission wavelengths of 300/421 nm (*25*).

To investigate the inhibition kinetics, we changed the reaction conditions by lowering the substrate concentration to < 50 nM and using a protein concentration of 10 µg/mL reaction mix and a 2-min incubation time to avoid substrate inhibition and to keep the reaction in the linear range.

Data analysis. Inhibition data are presented as percentage of inhibition compared with the controls without inhibitor. Each inhibitor was used with cytosol and microsomes of three different individuals. Each inhibitor concentration was tested in duplicate. Fifty percent inhibition concentrations (IC_{50}) were obtained from regression analysis through the linear part of the inhibition curves and are presented as mean ± SD from a pool of three individual fish. Significant differences between IC_{50} values were obtained by one-way analysis of variance, followed by Tukey's test. Inhibition kinetics were retrieved from double reciprocal (Lineweaver-Burke) and Eadie-Hofstee plots.

Results

Inhibition of sulfation. We calculated inhibition of the sulfation of 3-OH-BaP by six different PCB metabolites as the percentage of control SULT activity without inhibitor. Control activity (mean ± SD) was 0.98 ± 0.21 nmol/min/mg cytosolic protein. Inhibition

curves for the PCB metabolites show two clearly separated groups with different inhibition potential (Figure 2). The more potent inhibitors reduced the sulfation of 3-OH-BaP to around 10% of the control activity at 100 µM inhibitor. The less potent inhibitors were tested up to concentrations of 500 and 1,000 µM, but solubility of the PCB metabolites became a limiting factor at these high concentrations. The IC_{50} values for the two poor inhibitors (330 µM for 6-OH-CB 77 and 526 µM for 2-OH-CB 77) were significantly different from each other and from the four more potent inhibitors (Figure 3; *p* < 0.001 for all comparisons). There were no significant differences in IC_{50} values among the four potent inhibitors, which ranged from 17.8 to 44.3 µM (*p* > 0.05 for all comparisons).

From these data it appears that the position of the hydroxyl group is of crucial importance for the inhibitory potency of the OH-PCBs tested in this study. When the hydroxyl group was in the 2 or 6 position (*ortho*), the PCB metabolite was far less potent than when the hydroxyl group was in the 4 or 5 position (*meta* and *para*). There was no apparent difference between the 4 and 5 position (4′-OH-CB 78 and 5-OH-CB 77) or between the number of chlorine substitutions on the opposite ring when the hydroxyl group was in the 4 position (4′-OH-CB 78 and 4′-OH-CB 165). Even though the planar metabolites (5-OH-CB 77 and 4′-OH-CB 78) seemed to be slightly more potent inhibitors than the nonplanar compounds (Figure 3), these differences were not statistically significant (*p* > 0.05).

Kinetics of sulfation inhibition. To determine the type of inhibition, we incubated a concentration series of 3-OH-BaP with increasing amounts of one of the OH-PCBs with the hydroxyl group in the *ortho* position, sided by two chlorine substitutions (4′-OH-CB 72). The OH-PCBs with this configuration appeared to be the most potent inhibitors. The IC_{50} for this congener was
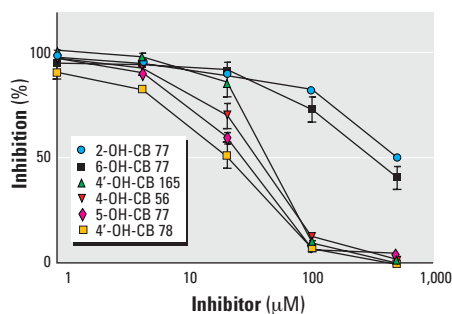

Figure 2. Inhibition of 3-OH-BaP sulfation by different OH-PCBs in channel catfish intestinal cytosol, presented as the percentage reduction of the control rate. Error bars represent SD.
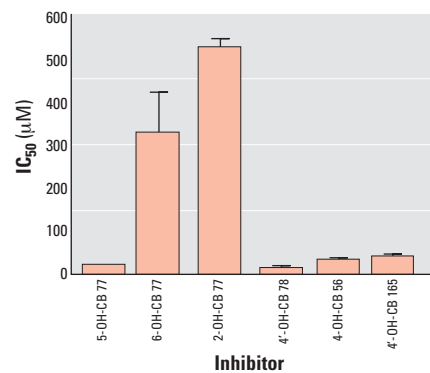

Figure 3. IC_{50} values for the inhibition of 3-OH-BaP sulfation by different OH-PCBs in channel catfish intestinal cytosol. Error bars represent SD.

46.6 ± 2.2 μM (mean ± SD) and was very consistent among the seven different substrate concentrations (Figure 4). However, a double reciprocal (Lineweaver-Burke) plot of these data resulted in nonlinear regresssion lines, which was confirmed by plotting the data in an Eadie-Hofstee plot. The observed nonlinear inhibition kinetics obscure the actual type of inhibition.

Inhibition of glucuronidation. We calculated inhibition of the glucuronidation of 3-OH-BaP by PCB metabolites as the percentage of control UGT activity without inhibitor, as described above for sulfation. Control activity was 0.16 ± 0.04 nmol/min/mg microsomal protein (mean ± SD). The inhibition curves show that the PCB metabolites were much more potent inhibitors of glucuronidation than of sulfation (Figure 5). The IC_{50}s ranged from 1.17 to 36.4 μM (Figure 6). The differences were most pronounced for 6-OH-CB 77 and 2-OH-CB 77, the metabolites with the hydroxyl group in the *ortho* position. These metabolites were almost two orders of magnitude more potent as inhibitors of glucuronidation than of sulfation (4.3 and 6.9 μM vs. 330 and 526 μM). On the other hand, the hexachlorobiphenyl metabolite had an almost identical IC_{50} for both enzymes.

Structural differences between the PCB metabolites investigated here seem to be less predictable for inhibition of glucuronidation than they are for inhibition of sulfation. The importance of a hydroxyl group in the *meta* or *para* position does not hold for inhibition of UGT as it does for SULT. However, for UGT the highest chlorine-saturated metabolite was the least potent inhibitor (36.4 μM for 4′-OH-CB 165, significantly higher than all other metabolites, $p < 0.001$), whereas for SULT there appeared to be no relationship between chlorine substitution and inhibition.

Kinetics of glucuronidation inhibition. The kinetics of the inhibition of 3-OH-BaP glucuronidation by 4′-OH-CB 72 were measured at lower substrate and protein concentrations to avoid substrate inhibition. Under these conditions the IC_{50} for this inhibitor was around 1 μM, with almost complete inhibition at 5 μM (Figure 7). These observations are consistent with the data from previous experiments obtained at much higher substrate concentrations (Figure 5). However, Lineweaver-Burke and Eadie-Hofstee plots revealed that the analytic variation in the data from the treatments with higher inhibitor concentrations was too high to draw reliable conclusions about the type of inhibition.

Discussion

Recently, inhibition of phase II enzymes by OH-PCBs has received considerable attention

(*26*). Kester et al. (*11*) found a number of OH-PCBs to be extremely potent inhibitors of recombinant human EST. IC_{50} values for the most potent compounds were found in the subnanomolar range (0.10–0.20 nM). Strong inhibition of EST by OH-PCBs would result in a reduced removal of estrogen, which may lead to higher estrogen concentrations in target tissues. This may explain the estrogenic effect of OH-PCBs, as reported elsewhere (*27*).

Four of the OH-PCBs investigated here were also tested as inhibitors of EST (*11*), and though the IC_{50}s are about five orders of magnitude lower for EST than for phenol-type SULT, the ranking of the inhibitors is the same, with 4′-OH-CB 78 being the most potent, 4′-OH-CB 165 intermediate, and 2-OH-CB 77 and 6-OH-CB 77 the least potent.

Studies of PCB concentrations in people have shown that metabolites as well as parent PCB are present. Inuit men had mean whole-blood concentrations of 12.9 ng/g wet weight for total PCBs and 1.7 ng/g wet weight for OH-PCBs, corresponding to molar concentrations in whole blood of approximately 0.04 μM for total PCBs and 0.005 μM for OH-PCBs (*3*). Men in Latvia and Sweden who reported high fish consumption had

plasma concentrations of total OH-PCBs ranging from 170 to 2,200 ng/g lipid weight (10–90th percentiles) and of total parent PCB from 1,000 to 5,300 ng/g lipid weight (*4*). This corresponded to OH-PCB concentrations of approximately 0.5–6 μM and PCB concentrations of 2.5–15 μM in plasma lipids. Much higher total PCB concentrations, sometimes as high as 1 mM, are present in top marine predators, especially in fatty tissues (*28,29*). Fish that inhabit contaminated sites accumulate high concentrations of PCB; a marine fish, the scup, collected from a contaminated harbor had body burdens of 272 μg/g dry weight (*30*). Intestinal and hepatic concentrations of OH-PCBs have not been reported in fish-eating people. In animals, the liver is an important site of distribution and the major site of cytochrome P450-dependent biotransformation of PCB (*31–33*). The OH-PCBs formed in the liver may be secreted into blood or bile. One study of the distribution of OH-PCBs between blood and liver of rats treated with selected PCB congeners showed that blood contained 1.7- to 3.5-fold higher concentrations than liver (*33*). With IC_{50} values in the low micromolar range for individual congeners, as found in our studies, negative effects of mixtures of OH-PCBs could be expected in
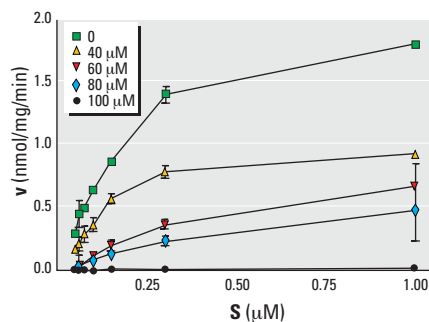

Figure 4. Kinetics of the inhibition of 3-OH-BaP sulfation by 4′-OH-CB 72. Abbreviations: S, substrate concentration; v, reaction rate. Dashed lines connect data points with equal amounts of inhibitor. Error bars represent SD.

Figure 5. Inhibition of 3-OH-BaP glucuronidation by different OH-PCBs in channel catfish intestinal microsomes, presented as the percentage reduction of the control rate. Error bars represent SD.

Figure 6. IC_{50} values of the inhibition of 3-OH-BaP glucuronidation by different OH-PCBs in channel catfish intestinal microsomes. Error bars represent SD.

Figure 7. Kinetics of the inhibition of 3-OH-BaP glucuronidation by 4′-OH-CB 72. Abbreviations: S, substrate concentration; v, reaction rate. Dashed lines connect data points with equal amounts of inhibitor. Error bars represent SD.
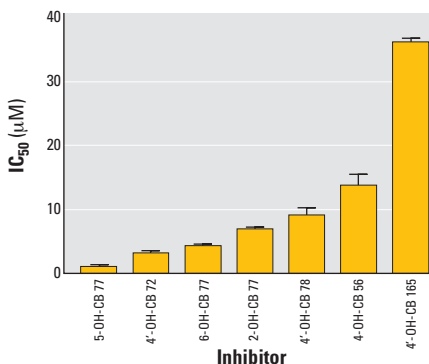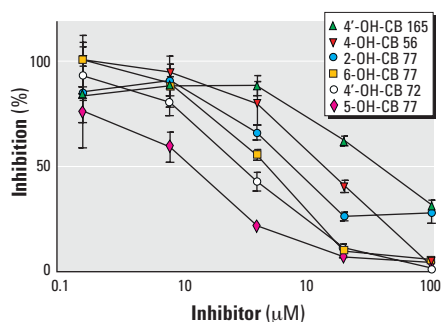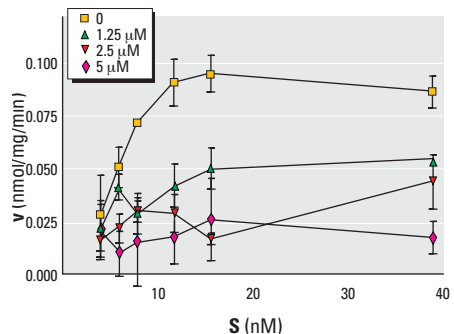
organisms exposed to field situations as described above.

In their studies, Kester et al. (*11*) also found that the *ortho*-hydroxylated compounds were the weakest inhibitors, whereas the *para*-hydroxylated metabolites with two adjacent chlorine substituents were the strongest inhibitors. They concluded that two or three chlorine substitutions in the nonphenolic ring did not affect the inhibition potential, but that four or more chlorine substituents negatively affected the inhibitory potency. This is not confirmed by the results described here, as 4′-OH-CB 165 was a relatively potent inhibitor.

Noncompetitive inhibition is described as the kinetic type of inhibition for EST (*11*). This would be in line with previously reported cooperativity in EST, with the inhibitor binding to an allosteric binding site (*34*). Our data did not allow a distinction between competitive, noncompetitive, or uncompetitive inhibition because of nonlinearity in the double reciprocal and Eadie-Hofstee plots. Nonlinearity can be caused by a number of factors, among which are multiple or partial inhibition, substrate, or product inhibition, as well as by cooperativity (*35*). Given the previous observations of cooperativity in SULT, it will be interesting to further investigate this phenomenon, including the effects of inhibitors.

OH-PCBs have also been tested as inhibitors of thyroid hormone sulfation (*9,10*). IC$_{50}$s ranged from 0.2 to 6.9 μM, with the *meta*- and *para*-hydroxylated metabolites as the most potent inhibitors, whereas the *ortho* hydroxy-substituted PCBs were very weak inhibitors of thyroid SULT activity. This indicates a close resemblance between the active sites of estrogen, thyroid, and phenol-type SULT. There is overlap in the substrate specificity of SULT (*36*). The comparable inhibition activities of OH-PCBs for the different types of SULT only emphasizes the similarities among the isoforms.

To date there have been no published studies of the effect of OH-PCBs on UGTs with endogenous or xenobiotic substrates. Our results show that OH-PCBs are inhibitors of SULT and UGT, but they might also serve as substrates for these enzymes. It has been suggested that sulfate and glucuronosyl conjugates of OH-PCBs would be excreted through bile and urine, with bile as the preferred pathway (*5*). However, actual data for this pathway are not well documented. Evidence for sulfate or glucuronosyl conjugates of OH-PCBs in bile or urine was sparse [reviewed by Connor et al. (*27*)]. OH-PCBs are only slightly less lipophilic than the parent PCB, and if not conjugated, are likely to be retained in lipid fractions of tissues. Likewise, there are very

few studies describing *in vitro* experiments on the conjugation of OH-PCBs (*27*).

The observed inhibitory effects of OH-PCBs on the phase II conjugation of BaP metabolites has implications concerning the interactions of environmental chemicals. PCBs and PAHs are often found together in contaminated aquatic areas, and bottom-dwelling fish that live in these areas are likely to be exposed to this mixture. Metabolism of PCBs will lead to the formation of a variety of OH-PCBs, some of which may be bound to thyroid hormone transporters in blood plasma. These PCB metabolites resembling thyroxine are also the most potent inhibitors of phenol-type SULT and UGT. Structurally related OH-PCBs were present in rat liver at concentrations similar in order of magnitude to those found in blood (*33*). The persistence and systemic distribution of these OH-PCBs may have important consequences for all regulatory processes that require sulfation or glucuronidation and may also have a deleterious effect on the detoxification of absorbed environmental toxicants such as OH-PAHs and other hydroxylated xenobiotics.

Comparison between the inhibition of SULT and UGT shows that glucuronidation is much more likely to be affected by OH-PCB inhibition than sulfation. From a kinetic standpoint, this is an interesting phenomenon. For many substrates SULTs are high-affinity–low-capacity enzymes that are efficient at low substrate concentrations, whereas UGTs are low-affinity–high-capacity enzymes that take over as a phase II conjugating enzyme when substrate concentrations increase (*37*). This would mean, given the differences in IC$_{50}$s, that the effects of OH-PCBs are likely to become apparent when substrate concentrations increase, possibly leading to a synergistic effect in the accumulation of harmful hydroxylated PAH metabolites.

In conclusion, this study reports for the first time that OH-PCBs have a significant inhibitory effect on phenol-type SULT and UGT in channel catfish intestinal mucosa. Some OH-PCBs inhibit UGT and SULT, but particularly UGT, at environmentally relevant, low micromolar concentrations.

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