Sulfonation is a major phase II biotransformation reaction. In this study, we found that several polychlorobiphenylols (OH-PCBs) inhibited the sulfonation of 3-hydroxybenzo[a]pyrene (3-OH-BaP) by human liver cytosol and some cDNA-expressed sulfotransferases. At concentrations > 0.15 µM, 3-OH-BaP inhibited its own sulfonation in cytosol fractions that were geno-
typed for SULT1A1 variants, as well as with expressed SULT1A1*1, SULT1A1*2, and SULT1E1, but not with SULT1A3 or SULT1B1. The inhibition fit a two-substrate kinetic model. We exam-
inec the effects of OH-PCBs on the sulfonation of 0.1 or 1.0 µM 3-OH-BaP, noninhibitory and inhibitory substrate concentrations, respectively. At the lower 3-OH-BaP concentration, OH-PCBs with a 3-chloro-4-hydroxy substitution pattern were more potent inhibitors of cyto-
solic sulfotransferase activity [with concentrations that produced 50% inhibition (IC50) between 0.33 and 1.1 µM] than were OH-PCBs with a 3,5-dichloro-4-hydroxy substitution pattern, which had IC50 values from 1.3 to 6.7 µM. We found similar results with expressed SULT1A1*1 and SULT1A1*2. The OH-PCBs were considerably less potent inhibitors when assay tubes contained 1.0 µM 3-OH-BaP. The inhibition mechanism was noncompetitive, and our results suggested that the OH-PCBs competed with 3-OH-BaP at an inhibitory site on the enzyme. The OH-PCBs tested inhibited sulfonation of 3-OH-BaP by SULT1E1, but the order of inhibitory potency was different than for SULT1A1. SULT1E1 inhibitory potency correlated with the dihedral angle of the OH-PCBs. The OH-PCBs tested were generally poor inhibitors of SULT1A3- and SULT1B1-dependent activity with 3-OH-BaP. These findings demonstrate an interaction between potentially toxic hydroxylated metabolites of PCBs and polycyclic aromatic hydro-
carbons, which could result in reduced clearance by sulfonation. **Key words:** 3-hydroxy-
benzo[a]pyrene, human liver cytosol, inhibition of sulfonation, polychlorobiphenylols, SULT1A1*1, SULT1A1*2, SULT1E1. *Environ Health Perspect* 113:680–687 (2005). doi:10.1289/ehp.7837 available via [http://dx.doi.org](http://dx.doi.org) ([Online 24 February 2005])

Polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) are two classes of environmentally prevalent pollutants. PAHs are formed through the combustion of fossil fuels and the burning of organic materi-
als (Dipple 1985). PCBs were first produced industrially in the middle of the last century for their desirable dielectric properties (Erickson 2001) and remain in the environ-
ment because of their continued use, because of their release from waste sites, and because many congeners are slowly degraded. The more lipophilic PAHs and PCBs are often found in the same environmental samples, such as soils and sediments, and are bio-
transformed in animals by similar pathways (James 2001).

Of the PAHs, benzo[a]pyrene (BaP) is a well-studied chemical carcinogen, which is metabolized by cytochrome P450 (CYP) to a variety of products (Dipple 1985). These include 3-hydroxybenzo[a]pyrene (3-OH-BaP), a major metabolite of BaP in humans and animals, which has estrogenic properties and binds to hemoglobin (Charles et al. 2000; Sugihara and James 2003). Hydroxylated PAH metabolites such as 3-OH-BaP are sub-
strates for glucuronidation and sulfonation, catalyzed by one or more of the UDP-glucu-
ronosyltransferases and 3’-phosphoadenosine 5’-phosphosulfate (PAPS)-dependent sulfotransferases (SULTs), respectively (James et al. 2001). Sulfonation is considered a detoxification pathway for 3-OH-BaP.

PCBs have several metabolites of toxicology importance, including polychlorobi-
phe nyls (OH-PCBs), which are formed in vitro from CYP-dependent mono-oxygena-
tion of PCBs (James 2001). Although they are slightly more hydrophobic than are the parent PCBs, several OH-PCBs are elimi-
nated slowly (Klasson-Wehler et al. 1993). Some people who are highly exposed to PCBs through the diet typically have OH-PCBs in their blood, some bound to plasma proteins (Guvenius et al. 2003; Sandau et al. 2000). Several OH-PCB congeners interact with components of the endocrine system, poten-
tially interfering with thyroid hormone and estrogen function (Lans et al. 1993; Safe 1994; Schuur et al. 1998). Although the OH-PCBs have low affinities for both α and β estrogen receptors, some OH-PCBs are strikingly potent inhibitors of human estrogen sulfotransferase (SULT1E1), with sub-
nanomolar concentrations that produced 50% inhibition (IC50) (Kester et al. 2000). This suggests that OH-PCBs may be indirectly estrogenic by increasing estradiol bioavailability in target tissues. As well as possibly causing toxicity by inhibiting the sulfo-
nation of hormones, several OH-PCBs inhibited the sulfonation and glucuronidation of the PAH metabolite 3-OH-BaP in channel catfish intestine (van den Hurk et al. 2002).

Sulfonation is an important phase II conjugation pathway for the detoxification of xenobiotics as well as the modulation of endogenous compounds such as thyroid hor-
mones, steroids, and neurotransmitters (Coughrie et al. 1998). One or more mem-
bers of a superfamily of cytosolic SULT enzymes catalyze these reactions (Blanchard et al. 2004). SULT1A1, SULT1B1, and SULT1E1 are the major phenol sulfotrans-
ferases expressed in human liver, with SULT1A1 (also known as ST1A3) found at the highest concentration (Honma et al. 2002). SULT1A3 is expressed in the gut but is present in very low concentrations in adult human liver (Richard et al. 2001). Genetic polymorphisms are known for SULT1A: a G<sup>358→A</sup> transition leading to an Arg<sup>213→His</sup> exchange in the protein was observed with a frequency of 33.2% in Caucasian subjects, 8% in Chinese, and 29.4% in African Americans (Carlini et al. 2001). SULT1A1*His (SULT1A1*2) was a less thermostable protein than SULT1A1*Arg (SULT1A1*1), and some authors have reported that the SULT1A1*2 variant is less catalytically active (Ozawa et al. 1998; RAFTOGIANIS et al. 1997).
Materials and Methods

Materials. The structures of the OH-PCBs used in this study are shown in Figure 1. In naming these OH-PCBs, we followed the recommendation of Maervoet et al. (2004) to name them as metabolites of PCBS, referring back to the Ballschmiter and Zell numbering system for PCBS (Ballschmiter and Zell 1980). The 6'-OH-CB35 (A1), 4'-OH-CB35 (B1), 4'-OH-CB36 (B2), 4'-OH-CB79 (C1), and 4'-OH-CB36 (C2) were synthesized by Suzuki coupling as described previously (Bauer et al. 1995; Lehmler and Robertson 2001). We verified the structures of each of these OH-PCBs by 1H and 13C-nuclear magnetic resonance spectroscopy, Fourier transform infrared spectroscopy, and gas chromatography–mass spectrometry (GC-MS). We found that each OH-PCB was > 99% pure by GC-MS analysis (Mass Spectrometry Facility, University of Kentucky, Lexington, KY), combustion analysis (Atlantic Microlab, Atlanta, GA), and thin-layer chromatography. The 4'-OH-CB69 (B3), 4'-OH-CB106 (B4), 4'-OH-CB112 (B5), 4'-OH-CB121 (C3), 4'-OH-CB159 (C4), 4'-OH-CB165 (C5), and 4'-OH-CB72 (C6) were purchased from AccuStandard (New Haven, CT). S.S. Singer (University of Dayton, Dayton, OH) supplied the PAPS. We purchased 35S-PAPS, 3.05 µCi/nmol (99.1% pure), from PerkinElmer Life Science (Boston, MA). Benzo[a]pyrene-3-sulfate (BaP-3-SO4) and 3-hydroxybenzo[a]pyrene (3-OH-BaP) were purchased from the NCI Chemical Carcinogen Reference Standard Repository (Midwest Research Institute, Kansas City, MO). We obtained Haell from Fisher Scientific (Atlanta, GA) and Taq DNA polymerase, along with other polymerase chain reaction (PCR) reagents, from Promega (Madison, WI). Integrated DNA Technologies (Coralville, IA) supplied primers for use in genotyping. We purchased the highest available grade of other reagents from Fisher Scientific (Atlanta, GA) and Sigma Chemical Company (St. Louis, MO).

Physicochemical properties of the OH-PCBs. We calculated the structural characteristics of dihedral angle, molecular volume, molecular surface area, pKa, log P, and log D at pH 7.0 with MM2* using GB/SA water solvent continuum as implemented by MacroModel 5.0 (Schrödinger, Portland, OR) and described previously by Tampal et al. (2002).

Cytosolic preparations. F.P. Guengerich (Vanderbilt University) kindly donated the samples of human liver, which were procured from organ donors (Guengerich 1995). We prepared liver cytosolic fractions from four livers by standard methods and stored aliquots at −80°C until use (Wang et al. 2004). We used three or four of these cytosol fractions in each experiment.

SULT1A1 genotype determination. We used a genomic DNA isolation kit (EASY-DNA; Invitrogen, Carlsbad, CA) to extract genomic DNA from samples of the individual human livers used in this study. We used a published method to detect the SULT1A1 polymorphism status of each liver (Nowell et al. 2000; Ozawa et al. 1998). Amplified DNA fragments were digested with HaeII, and the fragments were resolved on 2% (weight/volume) agarose gels. Fragments from individuals homozygous for SULT1A1*1 exhibited two bands, visualized by ultraviolet transillumination, whereas DNA fragments from individuals homozygous for SULT1A1*2 were not cleaved by this enzyme and exhibited one band.

Expression and partial purification of SULT isozymes. The expression of human SULT1A1*, SULT1A3, SULT1B1, and SULT1E1 in Escherichia coli has been described previously (Dajani et al. 1998; Wang et al. 1998). We grew E. coli cells containing the respective sulfotransferase genes as described previously (Falany et al. 1990, 1994), and prepared 100,000g supernatant fractions for use in inhibition studies and for partial purification of the SULT enzymes. We purchased expressed SULT1A1*2 cytosolic extract from PanVera (Madison, WI) and used it as supplied.

The 100,000g supernatant fractions of SULT1A1*, SULT1A3, SULT1B1, and SULT1E1 were partially purified by chromatographic methods (Falany et al. 1990, 1994). After the last step, a 3'-phosphoadenosine

Figure 1. Structures of the hydroxylated PCBs used in this study. Type A, hydroxy without a flanking chlorine atom; type B, para-hydroxy with one flanking chlorine atom; type C, para-hydroxy with two flanking chlorine atoms.
5'-phosphate (PAP)-agarose affinity column, we dialyzed the fractions eluted with PAP with three changes of buffer to remove PAP before the assay of SULT activity with 3-OH-BaP as substrate. We analyzed active fractions by SDS-PAGE (Laemmli 1970) to assess the purity of each SULT enzyme. We stained the gels with Coomassie R-250 reagent and determined the percentage of protein present as each respective SULT enzyme by scanning densitometry.

**Kinetic analysis of 3-OH-BaP sulfonation.** We determined SULT activity with 3-OH-BaP as substrate by a fluorimetric assay of BaP-3-SO4 product formation, as described previously (Wang et al. 2004). We ensured that the formation of BaP-3-SO4 was linear previously (Wang et al. 2004). We determined SULT activity with 3-OH-BaP as substrate by a fluorimetric assay of BaP-3-SO4 product formation, as described in “Materials and Methods.”

We determined the percentage of protein present as each respective SULT enzyme by scanning densitometry. We examined the kinetics of sulfonation in three liver cytosol fractions by systematically varying the concentration of 3-OH-BaP or PAPS. When the variable substrate was 3-OH-BaP, we used 12 concentrations in the range from 0.035 to 2.00 µM, and the concentration of PAPS was kept constant at 10 µM. When we varied PAPS, we used 7 concentrations from 0.157 to 10.0 µM and kept the concentration of 3-OH-BaP constant at 0.100 µM.

We determined the kinetic parameters for 3-OH-BaP sulfonation by partially purified preparations of the cDNA-expressed SULT isozymes under incubation conditions similar to those used for liver cytosol. For SULT1A1*1 and -1A1*2, we used seven substrate concentrations in the range from 5 to 100 nM; for SULT1E1 we used six 3-OH-BaP concentrations from 15.6 to 1,000 nM; and for SULT1A3 and 1B1 we used seven concentrations of 3-OH-BaP from 0.25 to 5.0 µM.

**Inhibition of SULT activity by OH-PCBs.** To assess inhibition of 3-OH-BaP SULT activity, we prepared stock solutions of OH-PCBs in dimethyl sulfoxide (DMSO) and added aliquots to incubation mixtures such that the final concentration of OH-PCB was in the range of 0.01–200 µM and the DMSO concentration did not exceed 0.5% (vol/vol). For each OH-PCB, we examined the concentration dependence of inhibition with three liver cytosol fractions, as well as with cytosol fractions from the E. coli expressing SULT1A1*1, SULT1A3, SULT1B1, and SULT1E1, and the purchased SF-9 cytosol fraction (PanVera, Madison, WI) containing SULT1A1*2. For studies with HL cytosol, SULT1A1*1, and SULT1E1, we examined two concentrations of 3-OH-BaP, 0.1 µM and 1.0 µM. For studies with SULT1A1*2, we examined only 0.1 µM 3-OH-BaP, a concentration that did not elicit substrate inhibition. For studies with SULT1B1, we used only 1.0 µM 3-OH-BaP because this enzyme had very low activity at 0.1 µM 3-OH-BaP and did not exhibit substrate inhibition. Examination of the effect of 50 µM concentrations of several OH-PCBs on the activity of SULT1A3, measured with 1.0 µM 3-OH-BaP, revealed little inhibition, so no further concentrations were studied.

**Kinetics of inhibition.** To study the type of inhibition produced by OH-PCBs, we used 4'-OH-CB112 (B5) as a model inhibitor. We prepared four sets of assay tubes containing HL cytosol and varying amounts of 3-OH-BaP from 35 to 150 nM: one set (control) contained no 4'-OH-CB112; the other sets contained 0.25 µM, 0.5 µM, or 1.0 µM 4'-OH-CB112.

**Data analysis.** We calculated the enzyme kinetic parameters from studies with variable concentrations of 3-OH-BaP using nonlinear regression analysis and GraphPad 4.0 software (GraphPad Software, San Diego, CA). We selected the built-in Michaelis-Menten equation for most analyses. Where we found evidence of 3-OH-BaP substrate inhibition, we fit the data into an equation derived from a two-substrate model (Zhang et al. 1998):

\[
V = V_{max}[1 + (V_i/S)/V_iK_i] + (1 + K_{ii}/S + S/K_i).
\]

This equation denoted the constant for binding of the first substrate (S) molecule as \(K_{ii}\) and the second substrate molecule as \(K_i\). \(V_i\) is the maximum rate for the noninhibitory substrate concentration range, and \(V_{max}\) is the minimum rate in the inhibitory substrate concentration range.

We calculated the effects of OH-PCBs on 3-OH-BaP SULT activity as percentage inhibition compared with the controls without an inhibitor. We obtained IC_{50} values by fitting log OH-PCB concentration and percent control activity to a sigmoidal curve. We examined the relationships between IC_{50} and physicochemical properties of the OH-PCBs by linear correlation analysis. We calculated the inhibitory constant (\(K_i\)) from the kinetic studies with 4'-OH-CB112 by means of Dixon plots and plots of \(K_i/V_{max}\) against inhibitor concentration (Cornish-Bowden 1995).
Results

SULT1A1 genotype of the liver donors. We found that the HL cytosols used were from individuals with different SULT1A1 genotypes, as determined by PCR amplification of the region of the SULT1A1 gene flanking the polymorphic base pair. The G to A mutation in SULT1A1 removed the restriction site for the endonuclease HaeII. As shown in Figure 2, an individual homozygous for the SULT1A1*2 allele did not have the HaeII restriction site, and the PCR product was not cleaved (lane 1). The PCR product from the individual homozygous for SULT1A1*1 showed complete cleavage by HaeII, generating two fragments of approximately 100 and 181 bp (lane 3). Enzymatic digestion of the PCR product from the heterozygote (SULT1A1*1/*2) generated one band of 281 bp and the two fragments of 100 and 181 bp (lane 2). Thus, the individual liver designated HL 1 was homozygous for the SULT1A1*1 allele, HL 2 was heterozygous, and HL 3 was homozygous for the SULT1A1*2 allele.

Sulfonation of 3-OH-BaP by HL cytosol and expressed human SULT isoforms. Initial studies of the sulfonation of 3-OH-BaP by HL cytosol revealed that concentrations of 3-OH-BaP > 0.15 µM resulted in a decrease in activity. To find a saturating concentration of PAPS, we conducted incubations in the presence of 0.1 µM 3-OH-BaP and varying concentrations of PAPS. The data fit the Michaelis-Menten equation, with an apparent $K_m$ of 0.56 ± 0.09 µM and a $V_{max}$ of 48 ± 2 pmol/min/mg protein (mean ± SD; n = 3). The dependence of activity upon PAPS concentration in expressed human SULT1A1*2, in the presence of 0.1 µM 3-OH-BaP, also followed Michaelis-Menten kinetics. The apparent $K_m$ was 0.32 µM, and $V_{max}$ was 684 pmol/min/mg protein. As shown in Figure 3, cytosol and the expressed enzyme were saturated by a PAPS concentration of 10 µM, and we used this concentration in subsequent studies.

We conducted detailed studies of the effect of a range of 3-OH-BaP concentrations up to 2 µM on reaction rates with HL cytosol and expressed human SULT1A1*2. We obtained preliminary estimates of the kinetic constants $K_m$ and $V_1$ by fitting the initial rates of sulfonation at concentrations < 0.15 µM 3-OH-BaP to the Michaelis-Menten equation. We then obtained the values of $K_m$ and $V_1$ through constraining $K_m$ using the equation of Zhang et al. (1998). We also analyzed data by constraining $V_1$, but a better fit was found when constraining $K_m$. Figure 4A shows how the data fit this equation for three individual HL cytosols. Kinetic studies with expressed SULT1A1*2 revealed substrate inhibition with the single enzyme (Figure 4B). Table 1 shows values for $K_m$, $K_i$, $V_1$, and $V_2$ for each HL cytosol and the expressed SULT1A1*2. The expressed enzyme showed a lower value for $K_m$ (0.022 µM) and $K_i$ (0.16 µM) than did any of the HL cytosols.

Table 1 presents the IC50 values of 3-OH-BaP inhibition curves from selected OH-PCBs with 1.0 µM 3-OH-BaP. The 4-OH-PCBs with one (B group) or two (C group) flanking chlorine substituents inhibited HL cytosolic 3-OH-BaP sulfotransferase activity in a concentration-dependent manner. Figure 5A shows inhibition curves from selected OH-PCBs in the presence of 0.1 µM 3-OH-BaP, and Figure 5B shows the same compounds studied with 1.0 µM 3-OH-BaP. Table 3 presents the IC50 values of 3-OH-BaP sulfotransferase activity with all the tested compounds, each at two concentrations of 3-OH-BaP. Compounds B1–B5 with the 3-chloro-4-hydroxy substitution pattern were potent inhibitors, with IC50 values ranging from 0.33 to 1.08 µM, when activity was measured with 0.1 µM 3-OH-BaP. The OH-PCBs with two chlorine atoms flanking the hydroxy group (C1–C6) were less potent inhibitors under these conditions (IC50, 1.31–6.71 µM; Table 3). The single 6-OH-PCB studied, A1, was a very weak inhibitor, with an IC50 of > 100 µM (Figure 5). When activity was
measured with 1 µM 3-OH-BaP, a concentration at which substrate inhibition occurred, the measured IC_{50} values showed lower inhibitory potencies for all OH-PCBs, but especially so for the C group compounds, whose IC_{50} values ranged from 3 to 58.7 µM (Table 3).

**Inhibition of 3-OH-BaP sulfonation by OH-PCBs with cDNA-expressed SULTs.** For SULT1A1*, Figure 6A shows inhibition curves with selected OH-PCBs using 0.1 µM 3-OH-BaP, whereas Figure 6B shows results with a substrate concentration of 1.0 µM 3-OH-BaP. We found that 6′-OH-CB35 (A1) was a poor inhibitor of 3-OH-BaP sulfonation under both conditions of substrate concentration. When using 0.1 µM 3-OH-BaP, type B compounds (B1–B5) showed IC_{50} values ranging from 0.77 to 1.31 µM, whereas type C compounds (C1–C6) exhibited IC_{50} from 2.16 to 6.65 µM (Table 3). When using 1.0 µM 3-OH-BaP, the inhibitory potencies of the OH-PCBs were dramatically reduced. The IC_{50} values for type B OH-PCBs were reduced to 10.3–67.5 µM, and for type C OH-PCBs were 33.8 to >100 µM (Table 3).

For SULT1A1*2, the IC_{50} of 6′-OH-CB35 (A1) was >100 µM, as shown in Table 3. At 0.1 µM 3-OH-BaP, the IC_{50} ranged from 0.54 to 1.48 µM for type B (B1–B5) compounds and from 1.67 to 6.52 µM for type C compounds (C1–C6). When using 1.0 µM 3-OH-BaP, type B compounds (B1–B5) showed IC_{50} values ranging from 3 to 58.7 µM (Table 3). We used 1.0 µM 3-OH-BaP as substrate, there was a 3- to 5-fold reduction in inhibitory potency, and the order of potency remained as it was with 0.1 µM 3-OH-BaP.

**Structure–activity relationships.** For HL cytosol, expressed SULT1A1*, SULT1A1*, and SULT1E1, we investigated the relationship between inhibitory potency, measured at 0.1 µM 3-OH-BaP, and each of several physicochemical properties of the 4-OH-PCBs. For HL cytosol, SULT1A1*, and SULT1E1, we investigated the relation-ship between dihedral angle, molecular surface area, molecular surface volume, log D at pH 7.0, or pK_{a}. The IC_{50} values with SULT1E1 showed a significant (p < 0.001) linear correlation with dihedral angle, as shown in Figure 8. No other significant correlations were found.

| Compound no. | Compound     | Log D at pH 7.0 | Dihedral angle (°) | IC_{50} (µM) |
|--------------|--------------|-----------------|-------------------|-------------|
| A1           | 6′-OH-CB35   | 4.7             | 50                | >100        |
| B1           | 4′-OH-CB35   | 4.8             | 39                | >100        |
| B2           | 4′-OH-CB56   | 4.8             | 39                | >100        |
| B3           | 4′-OH-CB69   | 5.1             | 72                | 0.37±0.04   |
| B4           | 4′-OH-CB106  | 5.2             | 60                | 0.37±0.04   |
| B5           | 4′-OH-CB112  | 5.2             | 78                | 1.08±0.12   |
| C1           | 4′-OH-CB79   | 4.5             | 38                | 6.71±0.91   |
| C2           | 4′-OH-CB36   | 4.2             | 38                | 2.30±0.45   |
| C3           | 4′-OH-CB121  | 4.7             | 72                | 3.95±0.23   |
| C4           | 4′-OH-CB159  | 4.7             | 78                | 1.31±0.14   |
| C5           | 4′-OH-CB165  | 4.6             | 57                | 2.87±0.09   |
| C6           | 4′-OH-CB72   | 4.5             | 57                | 1.72±0.21   |

*Values for HL cytosol are the means ± SDs of three livers, tested in duplicate; results for expressed SULT enzymes are the means of duplicate determinations.

For SULT1E1, compound A1 (6′-OH-CB35) was a poor inhibitor of 3-OH-BaP sulfonation at either of the substrate concentrations studied (Table 3). When using 0.1 µM 3-OH-BaP, OH-PCBs with no or one ortho-substituted chlorine (B1, B2, B4, C1, C2, C4, and C6) were potent inhibitors of 3-OH-BaP sulfonation, with IC_{50} values between 0.24 and 1.3 µM (Table 3). The OH-PCBs with two ortho-substituted chlorine atoms (B3, B5, C3, and C5) were less potent inhibitors, with IC_{50} values of 4.87–7.98 µM (Table 3). When we used 1.0 µM 3-OH-BaP as substrate, there was a 3- to 5-fold reduction in inhibitory potency, and the order of potency remained as it was with 0.1 µM 3-OH-BaP.

**Table 3. In vitro inhibition of 3-OH-BaP sulfotransferase activity by the tested OH-PCBs using HL cytosol and cDNA-expressed sulfotransferases at 0.1 and 1.0 µM substrate concentration.**

**Figure 6.** Inhibition of 3-OH-BaP sulfotransferase in SULT1A1*1 by OH-PCBs. (A) 0.1 µM 3-OH-BaP. (B) 1.0 µM 3-OH-BaP. 3-OH-BaP sulfotransferase activity is given as percentage of control. Data given are the mean ± SD of three experiments. Structures of the tested OH-PCBs tested are shown in Figure 1.

**Figure 7.** Inhibition of 3-OH-BaP sulfotransferase activity with SULT1A3 by OH-PCBs shown in Figure 1, each at 50 µM.
Kinetics of 3-OH-BaP sulfotransferase inhibition by 4’-OH-CB112. We investigated the type of inhibition of 3-OH-BaP sulfonation using HL cytosol. Figure 9A shows that 4’-OH-CB112 (B5) reduced sulfotransferase activities at all the tested 3-OH-BaP concentrations in a concentration-dependent manner. The kinetic constants showed a steady reduction in $V_{\text{max}}$ with increasing concentration of 4’-OH-CB112, but little change in $K_m$, indicating a noncompetitive type of inhibition (Table 4). Figure 9B shows a plot of apparent $K_m/V_{\text{max}}$ versus the concentration of 4’-OH-CB112, which indicated a $K_i$ value for 4’-OH-CB112 of 0.52 ± 0.14 µM.

Discussion

The major human metabolite of BaP, 3-OH-BaP, was very readily sulfonated in HL cytosol, especially at concentrations < 0.15 µM. We observed substrate inhibition in HL cytosol and with SULT1A1 and SULT1E1, but not with SULT1A3 or SULT1B1. We studied the kinetics of substrate inhibition in liver cytosol and SULT1A1*2 and found that they fit a two-substrate model proposed for the sulfonation of estradiol by SULT1E1. This model suggested that SULT1E1 could bind two molecules of estradiol per molecule of enzyme, one at a preferred site for sulfonation and the other at an allosteric site associated with substrate inhibition (Zhang et al. 1998). Our results suggest a similar scenario for the interaction of 3-OH-BaP with SULT in HL cytosol and SULT1A1. The $K_m$ values for each of the three tested HL cytosol fractions (48–51 nM), SULT1A1*2 (22 nM), and SULT1A1*1 (18 nM) were low, indicating that 3-OH-BaP has a very high affinity for human SULT1A1. The $K_i$ values were about 10-fold higher. The 3-OH-BaP was not, however, specific for SULT1A1 but was a substrate for the other human phenol sulfotransferases studied. In particular SULT1E1 showed a high affinity for 3-OH-BaP, with a $K_m$ of 50 nM. A related compound, 1-hydroxyxyprene, also had a very low $K_m$ with SULT1A1 (8 nM) and SULT1E1 (21 nM) but a higher $K_m$ with SULT1A3 (0.8 µM) (Ma et al. 2003). When we calculated 3-OH-BaP clearance values ($V_{\text{max}}/K_m$) for the four partially purified SULT isoforms, the highest value was found for SULT1A1 (Table 2). Thus, 3-OH-BaP was a selective but not specific substrate for SULT1A1. Other investigators showed that the SULT1B1 protein in liver cytosol was about one-fourth that of SULT1A1 (Honma et al. 2002). The present study showed that expressed SULT1B1 had a 40-fold higher $K_m$ value (2.0 µM) than found in HL cytosol (0.05 µM), so it is not likely to contribute much to HL cytosolic sulfonation of 3-OH-BaP at 0.1 µM substrate concentration (Table 2). Although SULT1A3 had activity with 3-OH-BaP, it is expressed at very low levels in the adult liver (Richard et al. 2001) and is unlikely to contribute much to 3-OH-BaP sulfonation in human liver. Because $K_m$ values for 3-OH-BaP in HL cytosol were similar to those of purified SULT1A1 and SULT1E1, and others have shown that SULT1A1 is expressed in liver at approximately 14-fold higher concentrations than SULT1E1 (Honma et al. 2002), we conclude that the observed activity with 3-OH-BaP in HL cytosol is catalyzed largely by SULT1A1. Differing structural features for inhibition of SULT1A1 and SULT1E1 by OH-PCBs further support our conclusion that, in HL cytosol, activity with 3-OH-BaP is due primarily to SULT1A1. By chance, the three HL cytosol fractions we used in these studies were from individuals with different SULT1A1 genotypes. One was SULT1A1*1 homozygous, a second was heterozygous for SULT1A1*1/*2, and the third was SULT1A1*2 homozygous. Kinetic analysis showed little difference among the three cytosol fractions for $V_i$, which was 121 pmol/min/mg for the homozygous SULT1A1*1 liver and 94 pmol/min/mg protein for the SULT1A1*2 liver (Table 1); however, the small size of our sample precludes a more detailed analysis of genotype effects on 3-OH-BaP sulfonation activities.

In previous studies, we showed that OH-PCBs inhibited 3-OH-BaP sulfonation in catfish intestinal cytosol (van den Hurk et al. 2002) and that a compound structurally related to OH-PCBs, 2,4,4’-trichloro-2’-hydroxydiphenyl ether (triclosan), inhibited sulfonation and glucuronidation of 3-OH-BaP and other substrates in HL cytosol and with SULT1A1, SULT1B1, and SULT1E1 (Wang et al. 2004). Here we demonstrated that a set of 4-OH-PCBs inhibited SULT activity with 3-OH-BaP, the major metabolite of another pollutant chemical, BaP, in HL cytosol as well as with cDNA-expressed SULTs. In HL cytosol, all the 4-OH-PCBs examined inhibited the sulfonation of 3-OH-BaP. Under incubation conditions in which the 3-OH-BaP substrate did not cause substrate inhibition (0.1 µM 3-OH-BaP), compounds with one chlorine atom adjacent to the OH group (B1–B5) were more potent inhibitors of sulfonation than were compounds in type C, with chlorine atoms flanking the OH group on each side. We observed very similar results for potency of inhibition and order of inhibitory potency with all three liver cytosol fractions and the two allelic variants of expressed SULT1A1. When incubated with 1.0 µM 3-OH-BaP, a concentration that produced substrate inhibition in liver cytosol and with both SULT1A1 variants, the OH-PCBs were considerably less potent inhibitors in cytosol and even more so with the expressed SULT1A1*1 and SULT1A1*2 enzymes (Table 3 and data not shown). The effect of substrate concentration on the
significant linear correlation between inhibitory coplanar conformation. We found a preferentially adopt coplanar conformation of biphenyls with less than one substituted chlorine atoms. Substituted sulfonation than were those with two substitutions were more potent as inhibitors of 3-OH-BaP. The structure–potency requirement for a xenobiotic SULT1E1 substrate, 3-OH-BaP.

SULT1A3 metabolized 3-OH-BaP with a very high Vmax. Although its preferred substrates are reported to be catecholamines and other monocyclic phenols containing hydroxyl bond donors (Dajani et al. 1998). Interestingly, 50 µM OH-PCBs caused little or no inhibition of this enzyme, thereby showing that the inhibitory interaction was enzyme selective. SULT1B1, the thyroid hormone sulfotransferase, catalyzed the sulfonation of 3-OH-BaP; however, OH-PCBs that were potent inhibitors of SULT1A1 were only weak inhibitors of the SULT1B1-catalyzed reaction. In contrast to results with the other enzymes, compound A1 (6′-OH-CB35) was a fairly potent inhibitor of SULT1B1 (Table 3).

Previously, ortho-, meta-, and para-hydroxylated PCBs were found to inhibit thyroid hormone sulfonation (Schuur et al. 1998). The meta-hydroxylated PCB, 3-OH-2,3′,4′,4′,5′-pentachlorobiphenyl (3-OH-CB118), was the most potent inhibitor of thyroid hormone sulfonation in male rat liver cytosol, followed by two para-hydroxylated PCBs. The ortho-hydroxylated PCB had the lowest potency among the four OH-PCBs studied. However, with 3-OH-BaP as substrate, the ortho-OH-PCB, 6′-OH-CB35, was a more potent inhibitor than those with para-OH groups, which suggested that the inhibitory interaction with SULT1B1 was substrate dependent.

Because several OH-PCBs have been detected in human blood and are presumably also present in liver and other tissues, it is important to understand their biologic activities. Some OH-PCBs interact with components of thyroid hormone and estrogen hormone systems (Kester et al. 2000; Klassen-Wehler et al. 1993; Schuur et al. 1998; Sinjari and Darnerud 1998). Our finding that OH-PCBs inhibited the sulfonation of 3-OH-BaP in HL suggests another aspect of the toxicology of OH-PCBs. The interaction with phenol sulfotransferase may be of toxicologic importance because sulfonation is a major pathway of xenobiotic biotransformation (Glatt 2002). Sulfonation is particularly important at low concentrations of hydroxylated xenobiotics, such as may be encountered from environmental exposure to pollutants that require CYP-dependent biotransformation to introduce a hydroxyl group before their elimination. Formation of sulfate conjugates of phenolic xenobiotics usually decreases their toxicity, so inhibition of this pathway may lead to prolonged exposure to the parent compound, a shift to an alternative phase II conjugation pathway, glucuronidation, or to further CYP-dependent metabolism. Both 3-OH-BaP and BaP-3-glucuronide bind to hemoglobin (Sugihara and James 2003), a potentially toxic interaction. Further CYP-dependent biotransformation of 3-OH-BaP may lead to more toxic metabolites such as 3-OH-BaP-7,8-dihydrodiol-9,10-oxide (Glatt et al. 1987; Ribeiro et al. 1986). On the other hand, xenobiotics that are activated by sulfonation, such as 2-hydroxyamino-1-methyl-6-phenylimidazido[4,5-b]pyridine (Ozawa et al. 1998), may be rendered less toxic in the presence of inhibitors of sulfonation.

Our findings may be placed in the context of the structures of OH-PCBs that have been reported in human blood. All OH-PCB metabolites identified in blood have the hydroxy group in a para- or meta-position, with chlorine atoms on vicinal carbon atoms (Hovander et al. 2002; Sandau et al. 2000, 2002; Sjödin et al. 2000). The para-OH-PCBs found in blood are likely to fall into the type C OH-PCBs examined in this study. Although these were generally less potent as inhibitors of SULT1A1 than the type B OH-PCBs, it is possible that the concentrations of these OH-PCBs may reach inhibitory levels in tissues of highly exposed people or animals. Sjödin et al. (2000) reported total measured OH-PCB concentrations of up to 6 µM in blood lipids, whereas Sandau et al. (2000) reported whole blood concentrations up to 30 nM. Tissue concentrations have not been reported but they may be higher than blood levels. Type B OH-PCBs with the 3-chloro-4-hydroxy substitution pattern do not appear to be persistent in blood; however, of the 209 PCB congeners, 19 have a 3-chloro substitution in one of the phenyl rings, which can be biotransformed to type B OH-PCBs. If type B OH-PCBs are formed in people, their high potency as inhibitors of 3-OH-BaP sulfonation may cause increased toxicity in people who are coexposed to PAH and PCBs.

**Conclusion**

We found that several OH-PCBs, especially those with a 3-chloro-4-hydroxy substitution...
pattern in the phenolic ring, inhibited the sulfonation of 3-OH-BaP in cytosol and with SULT1A1 at submicromolar concentrations. Some OH-PCBs with no or one ortho chlorine were potent inhibitors of 3-OH-BaP sulfonation with SULT1E1. SULT1B1- and SULT1A1-catalyzed sulfonation of 3-OH-BaP was less sensitive to inhibition by OH-PCBs.

The inhibitory interaction of OH-PCBs with SULT1A1 and SULT1E1 may have consequences for the biotransformation and toxicity of phenolic xenobiotics.

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