

Potent Inhibition of Estrogen Sulfotransferase by Hydroxylated Metabolites of Polyhalogenated Aromatic Hydrocarbons Reveals Alternative Mechanism for Estrogenic Activity of Endocrine Disrupters

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Polyhalogenated aromatic hydrocarbons (PHAHs), such as polychlorinated dibenzo-*p*-dioxins and dibenzofurans, polybrominated diphenylethers, and bisphenol A derivatives are persistent environmental pollutants, which are capable of interfering with reproductive and endocrine function in birds, fish, reptiles, and mammals. PHAHs exert estrogenic effects that may be mediated in part by their hydroxylated metabolites (PHAH-OHs), the mechanisms of which remain to be identified. PHAH-OHs show low affinity for the ER. Alternatively, they may exert their estrogenic effects by inhibiting E2 metabolism. As sulfation of E2 by estrogen sulfotransferase (SULT1E1) is an important pathway for E2 inactivation, inhibition of SULT1E1 may lead to an increased bioavailability of estrogens in tissues expressing this enzyme. Therefore, we studied the possible inhibition of human SULT1E1 by hy-

droxylated PHAH metabolites and the sulfation of the different compounds by SULT1E1. We found marked inhibition of SULT1E1 by various PHAH-OHs, in particular by compounds with two adjacent halogen substituents around the hydroxyl group that were effective at (sub)nanomolar concentrations. Depending on the structure, the inhibition is primarily competitive or noncompetitive. Most PHAH-OHs are also sulfated by SULT1E1. We also investigated the inhibitory effects of the various PHAH-OHs on E2 sulfation by human liver cytosol and found that the effects were strongly correlated with their inhibitions of recombinant SULT1E1 ($r = 0.922$). Based on these results, we hypothesize that hydroxylated PHAHs exert their estrogenic effects at least in part by inhibiting SULT1E1-catalyzed E2 sulfation. (*J Clin Endocrinol Metab* 87: 1142–1150, 2002)

RECENTLY, MANY STUDIES have been performed on the interaction of environmental chemicals with the endocrine system, which results in reproductive and developmental anomalies in various organisms (1–4). Endocrine-disrupting effects have been observed in adult animals that were exposed *in utero* to polyhalogenated aromatic hydrocarbons (PHAHs) such as polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs) (5–7). Hilakivi-Clarke *et al.* (8) demonstrated in rats that exposure to natural estrogens *in utero* advanced puberty onset and increased breast cancer risk in the offspring; this may also apply to environmental estrogens. In humans, abnormalities in the development of the reproductive tract, reduced sperm counts (3, 9), and increased incidence of germ cell cancer (10) have been related to exposure to endocrine disrupters present in the environ-

ment. Furthermore, several studies have reported increased levels of PCBs, 2,2-bis-(4-chlorophenyl)-1,1,1-trichloroethane and its metabolite 2,2-bis-(4-chlorophenyl)-1,1-dichloroethylene in breast cancer patients (11, 12). However, recent epidemiological research does not support the hypothesis that women exposed to organochlorines such as PCBs, PCDDs, 2,2-bis-(4-chlorophenyl)-1,1,1-trichloroethane, and 2,2-bis-(4-chlorophenyl)-1,1-dichloroethylene have an increased breast cancer risk (13–15).

Hydroxylated metabolites of the PHAHs (PHAH-OHs) may contribute to the aforementioned effects. Hydroxylated metabolites of PCBs (PCB-OHs), PCDDs (PCDD-OHs), and PCDFs (PCDF-OHs) and other organohalogenes have been identified in blood, bile, and urine of animals treated with the parent compound, but also in wildlife samples as well as in environmentally exposed human subjects (16–20). Hydroxylation of PCDDs and PCDFs in mammals preferentially occurs on the lateral (2, 3, 7, 8) positions (18–20). For PCBs, it has been determined that hydroxylated metabolites are partially responsible for the endocrine-disrupting effects. For instance, Crews *et al.* (21) showed that different hydroxylated PCBs altered the sexual differentiation of the turtle.

Abbreviations: BPA, Bisphenol A; -OH, hydroxylated metabolite of; PAPS, 3'-phosphoadenosine-5'-phosphosulfate; PBDE, polybrominated diphenylether; PCB, polychlorinated biphenyl; PCDD, polychlorinated dibenzo-*p*-dioxin; PCDF, polychlorinated dibenzofuran; PHAH, polyhalogenated aromatic hydrocarbon; SULT1E1, estrogen sulfotransferase.

The mechanisms by which PHAH-OHs exert their estrogenic effects are still not understood. Binding affinities for ER α and ER β are relatively low (22, 23). It is possible, however, that PHAH-OHs exert part of their estrogenic effects by increasing the bioavailability of E2 through inhibition of E2 inactivation in target tissues. Sulfation by the specific estrogen sulfotransferase SULT1E1 is an important pathway for the inactivation of E2 (24). The human enzyme has a low K_m value of 4 nM for E2 and is expressed in target tissues such as the endometrium, mammary gland, and testis as well as in the liver (25–29). Recently, we demonstrated potent inhibition of SULT1E1 by hydroxylated PCB metabolites (30), in particular compounds with two adjacent chlorine substituents around the hydroxyl group. Here, we have investigated the potency and type of inhibition of human SULT1E1 by other important PHAH-OHs, such as PCDD-OHs, PCDF-OHs, and hydroxylated polybromodiphenylethers (PBDE-OHs), and by halogenated bisphenol A (BPA) derivatives as well as their sulfation by SULT1E1. To determine the relevance of our findings using recombinant enzyme, we also studied the inhibition of E2 sulfation by native SULT1E1 in human liver cytosol (29). Furthermore, we analyzed the sulfation of the various PHAH-OHs by SULT1E1 and SULT1A1, another phenol sulfotransferase abundantly expressed in human liver (31, 32).

Materials and Methods

Materials

E2 and 3'-phosphoadenosine-5'-phosphosulfate (PAPS) were obtained from Sigma (St. Louis, MO), [3 H]E2 (3.22 MBq/nmol) was obtained from Amersham Pharmacia Biotech (Little Chalfont, UK), and [35 S]PAPS (52.9 MBq/ μ mol) was purchased from NEN Life Science Products (Boston, MA). The sources of the various hydroxylated organohalogenes have been described previously (23, 33, 34). Human SULT1E1 (35) and SULT1A1 (31) were expressed in *Salmonella typhimurium* as previously described (36, 37). Cytosolic fractions were prepared and used without further purification (36, 37). SULT1E1 accounted for 5–7% of the cytosolic proteins. Similar results were obtained using recombinant SULT1E1 expressed in *Escherichia coli* and purified as previously described (38). Normal human liver was obtained at surgical resection of liver tumors, and cytosol was prepared as previously described (39). Approval was obtained from the medical ethical committee of Erasmus University Medical Center.

Estrogen sulfotransferase assay

Estrogen sulfotransferase activity was analyzed by measuring the formation of water-soluble [3 H]E2 sulfate after incubation of 1 nM [3 H]E2 for 30 min at 37 C with recombinant SULT1E1 (0.1 μ g total cytosolic protein/ml) in the presence or absence (blanks) of 50 μ M of the cofactor PAPS in 0.2 ml 0.1 M sodium phosphate (pH 7.2), 2 mM EDTA, and 1 mM dithiothreitol. The reactions were stopped by the addition of 2 ml ice-cold water, and unreacted [3 H]E2 was removed by extraction with 2 ml dichloromethane. Sulfate formation was quantified by liquid scintillation counting of 1 ml of the aqueous phase. Enzymatic sulfation was corrected for background radioactivity estimated in the blanks. Inhibition of E2 sulfation by PHAH-OHs was assessed by addition of 0.01 nM to 10 μ M of these compounds to the reaction mixtures. Kinetic parameters for E2 sulfation were determined by Lineweaver-Burk analysis (40) of the sulfation of varying substrate concentrations. Apparent K_i values for inhibitors were calculated from the change in slope of the Lineweaver-Burk plot in the presence of a fixed inhibitor concentration (40).

Sulfation of PHAH-OHs

The above assay of estrogen sulfotransferase activity is based on the sulfation of limited concentrations of radioactive E2 by excess unlabeled PAPS. As radioactive PHAH-OHs are not available, their sulfation was analyzed in comparison with E2 by determining the transfer of $^{35}\text{SO}_3^-$ from [35 S]PAPS to an excess of unlabeled substrate. Sulfation of PHAH-OHs by SULT1E1 was compared with their sulfation by the human phenol sulfotransferase SULT1A1. Assay mixtures contained 1 μ M PHAH-OH or E2, 0.3 μ M [35 S]PAPS, and 15 μ g (recombinant SULT1E1) or 100 μ g (recombinant SULT1A1) of total cytosolic protein/ml in a total volume of 150 μ l 10 mM potassium phosphate (pH 7.4) and were incubated for 30 min at 37 C. The formation of sulfated products was analyzed using the BaSO $_4$ precipitation method of Foldes and Meek (41) as well as by HPLC. In the former method, unreacted [35 S]PAPS and protein were precipitated by successive addition of 200 μ l 0.1 M barium acetate, 200 μ l 0.1 M barium hydroxide, and 200 μ l 0.1 M zinc sulfate. The presumably soluble sulfated products were quantified by liquid scintillation counting of 500 μ l of the supernatant. Sulfation was corrected for background radioactivity determined in blanks without substrate. For HPLC analysis, the reactions were stopped by the addition of 150 μ l ice-cold methanol. After centrifugation, 100 μ l of the supernatant were injected onto a 4.6 \times 250-mm Symmetry C $_{18}$ column connected to an Alliance HPLC system (Waters Chromatography BV, Etten-Leur, The Netherlands), and eluted with a gradient of acetonitrile in 50 mM triethyl ammonium acetate (pH 6.8) at a flow of 1.0 ml/min. The proportion of acetonitrile was increased linearly from 35% to 65% in 15 min and further to 90% in an additional 10 min. The radioactivity in the eluate was determined using a Radiomatic A-500 flow scintillation detector (Packard, Meriden, CT).

Results

The effects of 0.01 nM to 10 μ M of different classes of PHAH-OHs on the sulfation of 1 nM E2 by SULT1E1 were tested. Table 1 lists the exact structural formulas of the tested compounds, and Figs. 1–4 show their core structures. The concentration-dependent inhibition of SULT1E1 activity by subsets of various types of PHAH-OHs is presented in Fig. 1 (PCDD-OHs), Fig. 2 (PCDF-OHs), Fig. 3 (PBDE-OHs), and Fig. 4 (BPA derivatives). From these concentration-inhibition curves, the concentrations causing 50% inhibition (IC_{50} values) were determined. Table 1 presents the IC_{50} values and relative potencies compared with the cognate substrate E2 for all compounds tested.

Figure 1 shows the results with PCDD-OHs having the hydroxyl group in position 2, and the same 7,8-dichloro substitution pattern in the nonphenolic ring. Increasing potencies were observed as the number of chlorine substituents surrounding the hydroxyl group increased from 0 to 2, with mean IC_{50} values of 300 nM for 2-OH-7,8-DiCDD, 30 nM for 2-OH-3,7,8-TrCDD, and 4 nM for 2-OH-1,3,7,8-TeCDD (Table 1).

Figure 2 presents the concentration-inhibition relationships for PCDF-OHs with the hydroxyl group in the 2 or 3 position, *para* or *meta* to the furan oxygen, respectively. Again, the potency increased with increasing number of chlorine substituents adjacent to the hydroxyl group. Furthermore, 3-OH-PCDFs appeared to be slightly more potent inhibitors than 2-OH-PCDFs possessing comparable chlorine substitution patterns. Of all the PCDF-OHs tested, 3-OH-2,4,7,8,9-PeCDF was the most potent inhibitor of E2 sulfation. With a mean IC_{50} value of as low as 0.18 nM, this compound has a more than 30-fold higher affinity for SULT1E1 than the natural substrate E2 (Table 1).

As demonstrated in Fig. 3, all PBDE-OHs tested were

TABLE 1. Potency of inhibition of human SULT1E1 activity by PHAH-OHs

Compound	Code	IC ₅₀ (nM)	Relative potency
E2		3.8–7.1	1
PCDD-OH			
2-Hydroxy-7,8-dichlorodibenzo-p-dioxin	2-OH-7,8-DiCDD	200–390	0.02
2-Hydroxy-3,7,8-trichlorodibenzo-p-dioxin	2-OH-3,7,8-TrCDD	28–40	0.17
2-Hydroxy-1,3,7,8-tetrachlorodibenzo-p-dioxin	2-OH-1,3,7,8-TeCDD	2.4–6.1	1.4
PCDF-OH			
4-Hydroxy-1,3,6,7-tetrachlorodibenzofuran	4-OH-1,3,6,7-TeCDF	6.6–6.7	0.84
3-Hydroxy-2,6,7,8-tetrachlorodibenzofuran	3-OH-2,6,7,8-TeCDF	5.6–9.1	0.76
3-Hydroxy-2,4,7,8-tetrachlorodibenzofuran	3-OH-2,4,7,8-TeCDF	0.68–2.2	4.0
3-Hydroxy-2,4,7,8,9-pentachlorodibenzofuran	3-OH-2,4,7,8,9-PeCDF	0.16–0.20	31
2-Hydroxy-7,8-dichlorodibenzofuran	2-OH-7,8-DiCDF	230–560	0.02
2-Hydroxy-6,7,8-trichlorodibenzofuran	2-OH-6,7,8-TrCDF	350–800	0.01
2-Hydroxy-1,3,7,8-tetrachlorodibenzofuran	2-OH-1,3,7,8-TeCDF	5.6–6.2	0.97
1-Hydroxy-2,4,7,8-tetrachlorodibenzofuran	1-OH-2,4,7,8-TeCDF	240–280	0.02
PHDE-OH			
4-Hydroxy-2',4',6'-tribromodiphenylether	4-OH-2',4',6'-TrBDE	780 to >1,000	<0.01
4-Hydroxy-2',3,4',6'-tetrabromodiphenylether	4-OH-3,2',4',6'-TeBDE	>1,000	<0.01
4-Hydroxy-2',3,4',5,6'-pentabromodiphenylether	4-OH-3,5,2',4',6'-PeBDE	200–240	0.03
2-Hydroxy-2',4,4'-trichlorodiphenylether	2-OH-4,2',4'-TrCDE	850 to >1,000	<0.01
BPA			
4,4'-Isopropylidenediphenol (bisphenol A)	BPA	>10,000	<0.001
3,3',5,5'-Tetrachlorobisphenol A	3,5,3',5'-TeCBPA	29–53	0.15
3,3',5,5'-Tetrabromobisphenol A	3,5,3',5'-TeBBPA	12–33	0.30

IC₅₀ values are presented as the range of values from two to four experiments. Relative potencies are calculated as ratio of the IC₅₀ value of E2 over that of inhibitor.

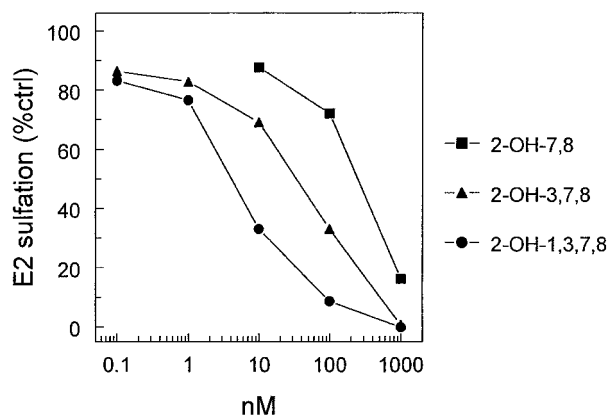
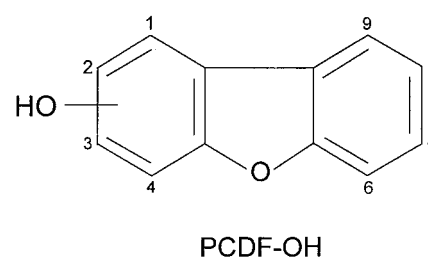
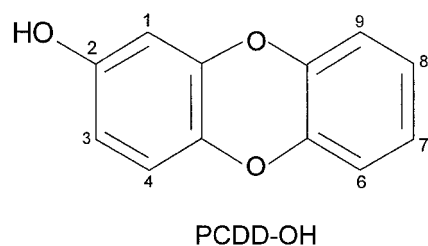


FIG. 1. *Upper part*, Core structure of PCDD-OH. Chlorine substituents are not indicated. *Lower part*, Inhibition of E2 sulfation by recombinant human SULT1E1 by 0.1–1000 nM PCDD-OHs. Reaction conditions: 1 nM [³H]E2, 0.1 μg total cytosolic protein/ml, 50 μM PAPS, and 30-min incubation. Results are the means of two to four experiments; the coefficient of variation was less than 20%.

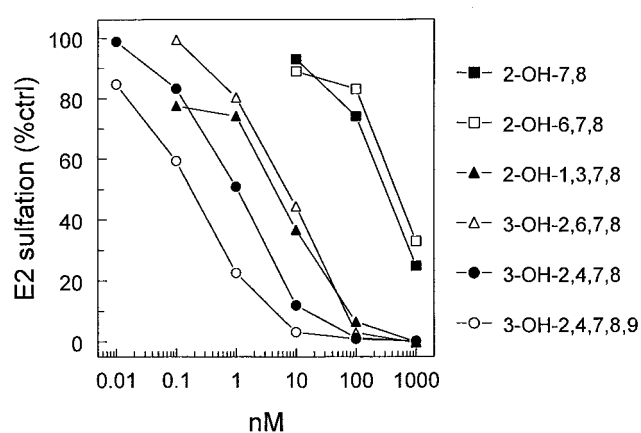
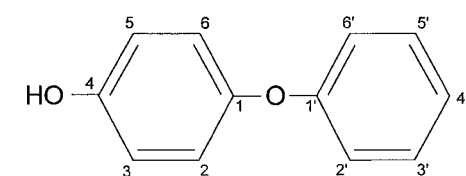


FIG. 2. *Upper part*, Core structure of PCDF-OH. The position of the hydroxyl group varies between the different PCDF-OHs. Chlorine substituents are not indicated. *Lower part*, Inhibition of E2 sulfation by recombinant human SULT1E1 by 0.01–1000 nM PCDF-OHs. Reaction conditions: 1 nM [³H]E2, 0.1 μg total cytosolic protein/ml, 50 μM PAPS, and 30-min incubation. Results are the means of two to four experiments; the coefficient of variation was less than 20%.

relatively weak inhibitors of E2 sulfation by SULT1E1, with IC₅₀ values greater than 200 nM (Table 1). Also, the one hydroxylated, polychlorodiphenylether tested, 2-OH-4,2',4'-TrCDE, only inhibited SULT1E1 activity at micromolar con-

centrations (Table 1). BPA did not affect E2 sulfation at concentrations less than 1 μM, whereas its derivatives having halogens in all positions adjacent to the two hydroxyl groups,



PBDE-OH

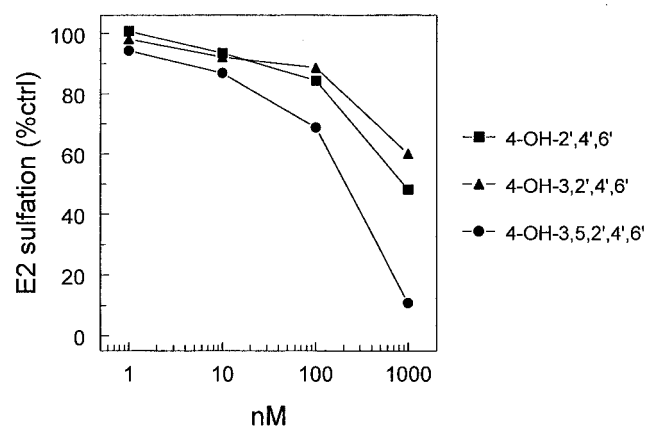
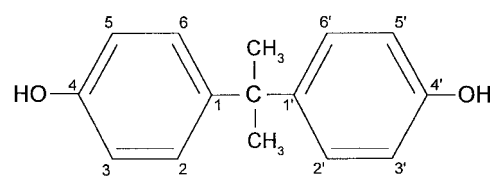


FIG. 3. *Upper part*, Core structure of PBDE-OH. Bromine substituents are not indicated. *Lower part*, Inhibition of E2 sulfation by recombinant human SULT1E1 by 1–1000 nM PBDE-OHs. Reaction conditions: 1 nM [^3H]E2, 0.1 μg total cytosolic protein/ml, 50 μM PAPS, and 30-min incubation. Results are the means of two to four experiments; the coefficient of variation was less than 20%.

3,5,3',5'-TeCBPA and 3,5,3',5'-TeBBPA, were relatively potent inhibitors, with mean IC_{50} values of 40 and 20 nM, respectively (Fig. 4 and Table 1).

The type of inhibition of E2 sulfation by the different PHAH-OHs was studied by Lineweaver-Burk analysis (Fig. 5). Depending on the structure, different types of inhibition were observed. Addition of 2-OH-7,8-DiCDD (Fig. 5A) or 2-OH-7,8-DiCDF (Fig. 5B) changed the slope, but had little effect on the y -axis intercept of these plots, indicative of competitive inhibition. However, 2-OH-1,3,7,8-TeCDD (Fig. 5A) and 3-OH-2,4,7,8,9-PeCDF (Fig. 5B) affected both the slope and the y -axis intercept, and the plots converged at about the same point on the x -axis, indicating primarily noncompetitive inhibition by these potent inhibitors. The Lineweaver-Burk analyses of the effects of BPA and 3,5,3',5'-TeCBPA (Fig. 5C) and of 4-OH-3,5,2',4',6'-PeBDE (Fig. 5D) indicate that these compounds inhibit E2 sulfation primarily in a noncompetitive manner. The K_i values derived from these plots are in good agreement with the corresponding IC_{50} values for the different inhibitors, amounting to 60 nM for 2-OH-7,8-DiCDD, 2 nM for 2-OH-1,3,7,8-TeCDD, 270 nM for 2-OH-7,8-DiCDF, 0.15 nM for 3-OH-2,4,7,8,9-PeCDF, 150 nM for 4-OH-3,5,2',4',6'-PeBDE, 14 μM for BPA, and 35 nM for 3,5,3',5'-TeCBPA.

Binding of PHAH-OHs to the active site of SULT1E1 is likely to result in their sulfation, which was tested directly by incubating 1 μM of the various compounds with 0.3 μM [^{35}S]PAPS and SULT1E1. These experiments also included different PCB-OHs that have previously been shown to in-



BPA

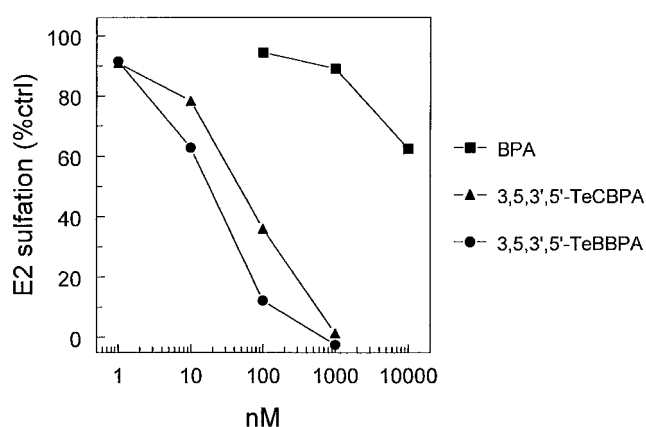


FIG. 4. *Upper part*, Core structure of BPA derivatives. Halogen substituents are not indicated. *Lower part*, Inhibition of E2 sulfation by recombinant human SULT1E1 by 1–10000 nM BPA or halogenated BPA. Reaction conditions: 1 nM [^3H]E2, 0.1 μg total cytosolic protein/ml, 50 μM PAPS, and 30-min incubation. Results are the means of two to four experiments; the coefficient of variation was less than 20%.

hibit E2 sulfation by SULT1E1 (30). Product formation was analyzed by the method of Foldes and Meek (41), which involves precipitation of the remaining [^{35}S]PAPS with BaSO_4 , presumably leaving the radioactive sulfated products in solution. The results of this established procedure were compared with a method developed in our laboratory based on the separation of labeled PAPS and sulfated products by HPLC. Figure 6A shows the HPLC analysis of the sulfation of 2-OH-7,8-DiCDF as a representative example, demonstrating the clear separation between remaining [^{35}S]PAPS and ^{35}S -labeled sulfated product. Figure 6B shows that there was reasonable agreement between the results of the two methods regarding the sulfation of most compounds, although in several instances the BaSO_4 precipitation method significantly underestimated the formation of sulfated products in comparison with HPLC analysis. This was especially the case with sulfated compounds that were strongly retarded on the C_{18} column, suggesting that relatively nonpolar sulfates are partially lost in the BaSO_4 precipitation method.

Table 2 shows the results of the HPLC measurements of the sulfation of the various PHAH-OHs by SULT1E1 in comparison with the sulfation of these compounds by the human phenol sulfotransferase SULT1A1. We found that most PHAH-OHs tested in this study were sulfated by SULT1E1 as well as by SULT1A1 (Table 2).

We also studied the inhibition of E2 sulfation by various PHAH-OHs using human liver cytosol as a source of native human SULT1E1. Figure 7 compares the effects of different PHAH-OHs (0.1 or 1 μM) on E2 sulfation by SULT1E1 and

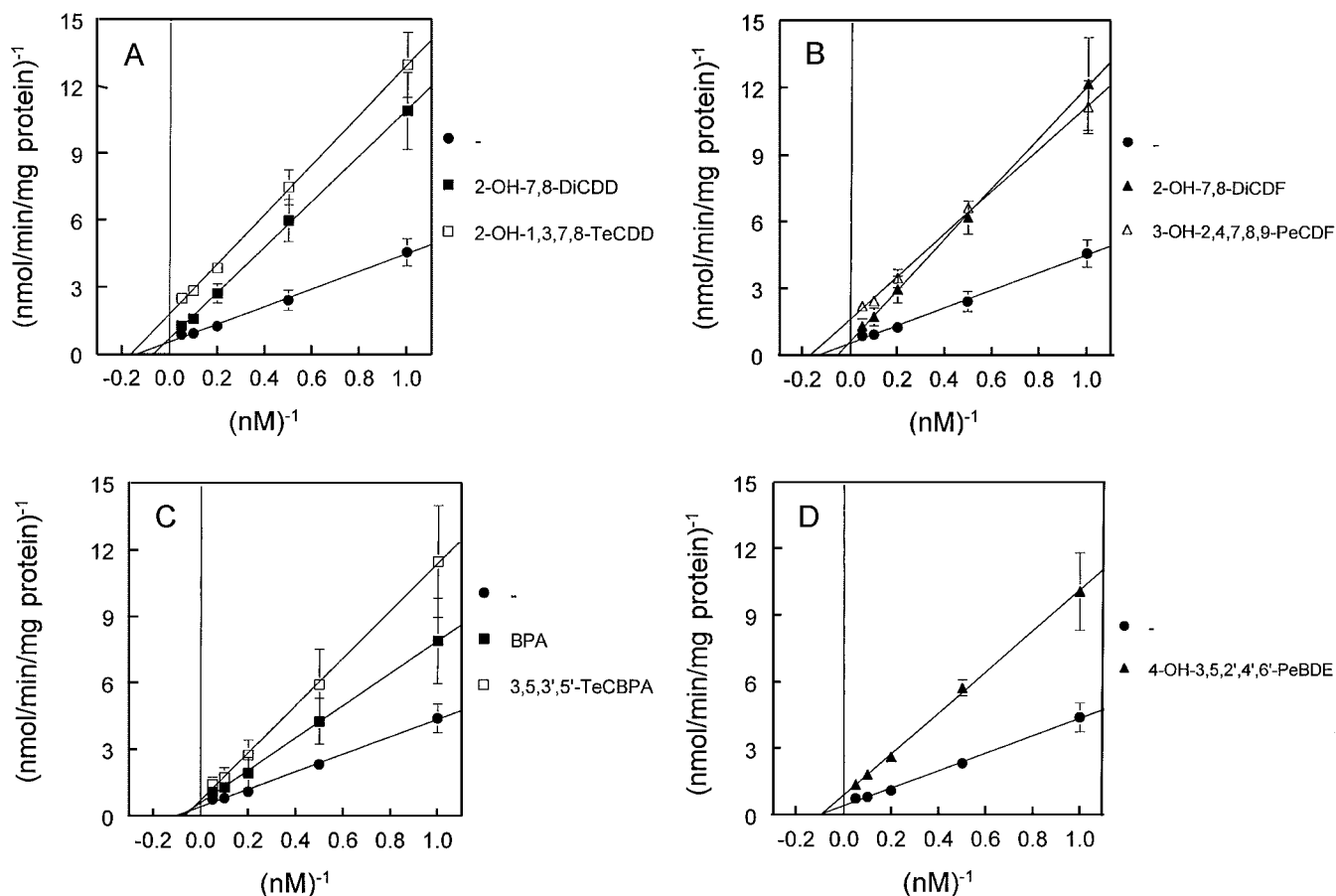


FIG. 5. Lineweaver-Burk analysis of the inhibition of the sulfation of 1–20 nM E2 by 0.1 $\mu\text{g/ml}$ recombinant human SULT1E1 by 250 nM 2-OH-7,8-DiCDD or 4 nM 2-OH-1,3,7,8-TeCDD (A), by 500 nM 2-OH-7,8-DiCDF or 0.2 nM 3-OH-2,4,7,8,9-PeCDF (B), by 12 μM BPA or 60 nM 3,5,3',5'-TeCBPA (C), or by 200 nM 4-OH-3,5,2',4',6'-PeBDE (D). Results are the mean \pm SD of two to four experiments. At points where no error bar is shown the SD is smaller than the symbol.

human liver cytosol, showing a strong correlation between the inhibitions of the recombinant and native enzymes ($r = 0.922$). In general, human liver enzyme was less potently inhibited than recombinant human SULT1E1.

Discussion

Structure-activity relationship of SULT1E1 inhibition by PHAH-OHs

We previously showed that various PCB-OHs potently inhibit E2 sulfation by human SULT1E1 (30). This finding suggested that endocrine-disrupting chemicals may act by increasing the bioavailability of hormones through inhibition of hormone-conjugating enzymes in target tissues (30, 42). In this study we tested the effects of other classes of PHAH-OHs on E2 sulfation by SULT1E1. The results demonstrate that in all classes of PHAH-OHs tested, those with two adjacent halogen substituents around the OH group are the most potent inhibitors of E2 sulfation by SULT1E1, which is in agreement with the structure-activity relationship found for SULT1E1 inhibition by single ring halogenated phenols and hydroxylated PCBs (30). A possible explanation for the increase in potency by adjacent halogen substitutions is the increased dissociation of the OH group. The potency of in-

hibitors with this substitution pattern decreases in the order PCDF-OH > PCDD-OH > BPA > PBDE-OH derivatives. The planar structures of PCDD-OHs and PCDF-OHs *vs.* the nonplanar structures of BPAs and PBDE-OHs may play a role in this, because the patterns for inhibition of SULT1E1 by PCB-OHs suggested preferred binding of coplanar compounds to the enzyme (30). This finding fits with the primarily planar structure of the natural ligand E2. Recently, the crystal structure of the mouse estrogen sulfotransferase has been elucidated (43). Mouse and human SULT1E1 show 77% amino acid sequence identity; both orthologous enzymes have K_m values in the nanomolar range (25, 44). The modeling of hydroxylated PHAHs in E2-binding sites of mouse SULT1E1 or human SULT1E1, when its crystal structure also becomes available, should further our understanding of the structural requirements for inhibition of SULT1E1.

Regarding the varying potencies of the different groups of (hydroxylated) PHAHs, it should be noted that levels of exposure are also different for the various PHAHs. The plasticizer BPA is commonly used in the food-packaging industry and in dentistry. Microgram amounts were found in the liquid from vegetable cans with plastic linings and in the saliva of patients with dental sealings (45). TBBPA, TCBPA,

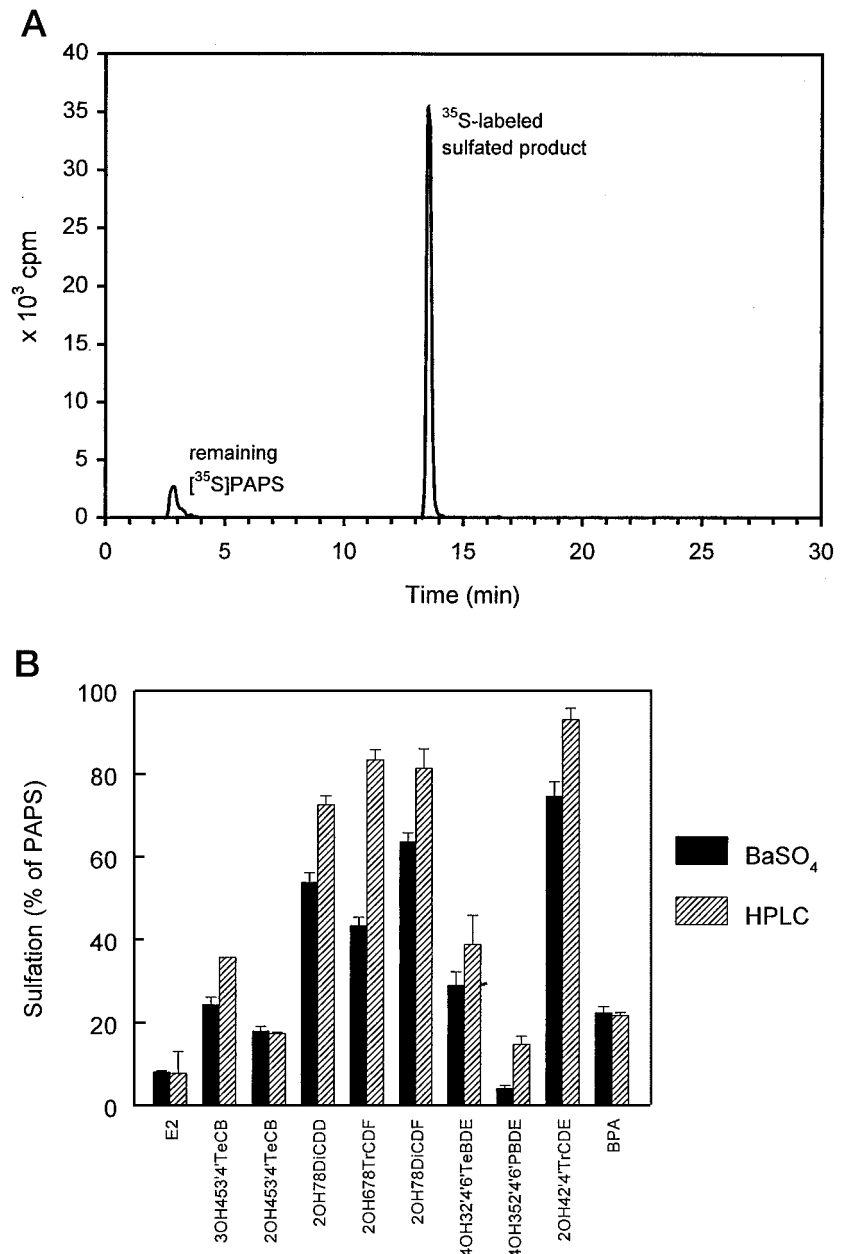


FIG. 6. A, HPLC analysis of the sulfation of 2-OH-7,8-DiCDF by SULT1E1 using [³⁵S]PAPS. B, Sulfation of PHAH-OHs by SULT1E1 measured by the BaSO₄ precipitation method and by HPLC analysis. Reaction conditions: 1 μM substrate, 15 μg total cytosolic protein/ml, 0.3 μM [³⁵S]PAPS, and 30-min incubation. Results are the mean ± SD of triplicate (BaSO₄) or duplicate (HPLC) determinations from a representative experiment.

and polybrominated diphenylethers are high production volume chemicals that are widely used in consumer electronics and many other products as flame retardants; the production volume of TBBPA in 1995 was higher than 50,000 tons/yr (46). The background exposure of humans to PCDDs and PCDFs is much lower. Only trace levels (parts per trillion) of PCDDs and PCDFs have been detected in normal human blood and tissues (47). However, as in our studies IC₅₀ values for some PCDD-OHs and PCDF-OHs were less than 1 nM, and 1 nM equals approximately 20 parts per trillion, the *in vitro* effects of the most potent PCDD-OHs and PCDF-OHs were observed at concentrations that may actually be in the same range that they are present in human tissues.

Depending on the structure, different types of inhibition were found with different PHAH-OHs. SULT1E1 is known

to have two substrate-binding sites, the active site as well as an allosteric site (24, 48). Binding of E2 to the latter site is thought to be largely responsible for the phenomenon of substrate inhibition that is observed at increasing E2 concentrations. The primarily competitive or noncompetitive nature by which the different PHAH-OHs inhibit the sulfation of E2 by SULT1E1 may thus be explained by their preferential affinity for the active or the allosteric site, respectively.

Sulfation of PHAH-OHs by SULT1E1 and SULT1A1

Estrogenic chemicals such as alkylphenols, diethylstilbestrol, and BPA have recently been shown to be substrates for human SULT1A1 (49, 50). In this study we compared the sulfation of PHAH-OHs by SULT1E1 and SULT1A1. Most of

TABLE 2. Sulfation of PHAH-OHs by human SULT1E1 and SULT1A1

Compound	Sulfation (% of PAPS added)	
	SULT1E1	SULT1A1
PCB-OH		
4-OH-3,3',4'-TrCB	9.9 ± 1.0	19.3 ± 1.8
4-OH-2,3,5,3',4'-PeCB	0.6 ± 0.8	0.7 ± 1.0
4-OH-3,2',3',4',5'-PeCB	17.6 ± 2.0	6.6 ± 3.1
3-OH-4,5,3',4'-TeCB	33.4 ± 3.3	10.5 ± 3.2
2-OH-4,5,3',4'-TeCB	19.4 ± 2.8	19.0 ± 4.7
4,4'-(OH) ₂ -3,5,3',5'-TeCB	1.0 ± 1.1	18.3 ± 2.8
PCDD-OH		
2-OH-7,8-DiCDD	70.6 ± 2.7	74.1 ± 10.1
2-OH-3,7,8-TrCDD	22.7 ± 9.5	11.4 ± 1.4
2-OH-1,3,7,8-TeCDD	28.2 ± 3.5	31.7 ± 6.9
PCDF-OH		
4-OH-1,3,6,7-TeCDF	6.9 ± 0.3	10.7 ± 1.4
3-OH-2,6,7,8-TeCDF	7.9 ± 1.1	7.3 ± 4.7
3-OH-2,4,7,8-TeCDF	2.5 ± 0.2	3.1 ± 1.1
3-OH-2,4,7,8,9-PeCDF	1.0 ± 0.0	1.0 ± 0.3
2-OH-7,8-DiCDF	82.4 ± 1.7	31.6 ± 4.4
2-OH-6,7,8-TrCDF	81.1 ± 3.1	55.2 ± 8.7
2-OH-1,3,7,8-TeCDF	7.0 ± 1.4	3.3 ± 2.3
1-OH-2,4,7,8-TeCDF	0.0 ± 0.0	24.8 ± 3.4
PHDE-OH		
4-OH-2',4',6'-TrBDE	2.5 ± 1.4	10.7 ± 0.9
4-OH-3,2',4',6'-TeBDE	43.1 ± 6.1	30.4 ± 6.2
4-OH-3,5,2',4',6'-PeBDE	13.3 ± 1.9	0.7 ± 0.7
2-OH-4,2',4'-TrCDE	89.2 ± 5.1	80.5 ± 7.6
BPA	23.6 ± 2.9	62.0 ± 3.2
3,5,3',5'-TeCBPA	7.7 ± 2.5	26.2 ± 1.1
3,5,3',5'-TeBBPA	5.8 ± 0.8	15.9 ± 0.7

Reaction conditions: 1 μM substrate, 15 (SULT1E1) or 100 (SULT1A1) μg total cytosolic protein/ml, 0.3 μM [^{35}S]PAPS, and 30-min incubation. Data are presented as the mean \pm SD from two to three experiments.

the compounds tested were sulfated by SULT1E1 as well as by SULT1A1. It should be noted, however, that these incubations had to be performed with a limited PAPS concentration and excess substrate, which is very different from the conditions used to test the effects of PHAH-OHs on the sulfation of E2 by SULT1E1. Sulfation of the different PHAH-OHs was tested at a single substrate concentration of 1 μM , although the IC_{50} and K_i values for their inhibition of E2 sulfation ranged from less than 1 nM to more than 10 μM . Therefore, the findings presented in Table 2 are not representative of the rate of sulfation of the different PHAH-OHs by SULT1E1 under the conditions where they were tested as inhibitors of E2 sulfation. Also, in view of the large variation in saturation of the low K_m SULT1E1 and the high K_m SULT1A1 at 1 μM of the various substrates, the data reported in Table 2 are not representative of the substrate preferences of these isoenzymes at lower, more relevant PHAH-OH concentrations. The *in vivo* significance of sulfation of hydroxylated PHAHs in PHAH metabolism remains to be established.

Effects of PHAH-OHs on E2 sulfation by human liver cytosol

The effects of PHAH-OHs on E2 sulfation by SULT1E1 and by human liver cytosol were highly correlated. However, the relationship between the rates of E2 sulfation by the different enzyme preparations was nonlinear. Although SULT1E1 is

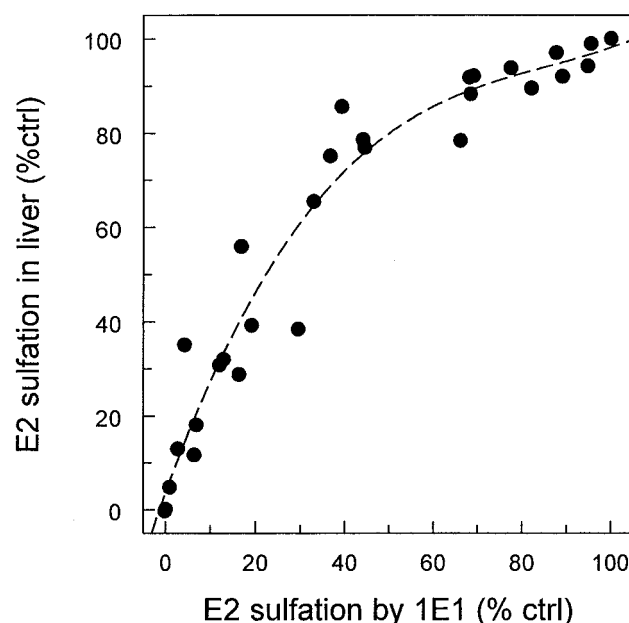


FIG. 7. Sulfation of E2 by human liver cytosol *vs.* SULT1E1 in the presence of different PHAH-OHs. Reaction conditions: 1 nM [^3H]E2, 0.1 or 1 μM PHAH-OH, 0.1 (SULT1E1) or 10 (human liver) μg total cytosolic protein/ml, 50 μM PAPS, and 30-min incubation. The PHAH-OHs tested were the PCDD-OHs and PCDF-OHs listed in Table 1 as well as 12 different PCB-OHs (compounds 5, 8, 10, 14, 16, 17, 23, 24, 26, 28, 31, and 32 from Ref. 30). Results are expressed as a percentage of control E2 sulfation in the absence of inhibitor and are presented as the means of two to four experiments (SULT1E1) or as the means of triplicate determinations from a representative experiment (human liver cytosol).

the better enzyme for E2 sulfation, with a K_m value of approximately 4 nM, SULT1A1 also catalyzes E2 sulfation, with a K_m value of 2–5 μM (51). The potencies of inhibition of SULT1E1 differ by orders of magnitude from those by which the different PHAH-OHs inhibit human SULT1A1 activity, which is characterized by apparent K_i values in the micromolar range (33). The nonlinear relationship between the rates of E2 sulfation by recombinant SULT1E1 and human liver cytosol in the presence of various PHAH-OHs may be explained by the presence of SULT1A1 in human liver. However, at nanomolar concentrations, E2 is predominantly sulfated in human liver by SULT1E1 (29), suggesting that the nonlinear relationship is not due to significant sulfation of E2 in human liver by SULT1A1. A more likely explanation is suggested by our findings that the PHAH-OHs are sulfated by SULT1E1 as well as by SULT1A1. Therefore, compared with recombinant SULT1E1, larger amounts of the PHAH-OHs will be metabolized in human liver cytosol, decreasing their inhibitory effects on native SULT1E1. An additional explanation may be that, compared with recombinant SULT1E1, human liver cytosol contains more proteins to which PHAH-OHs bind, diminishing their availability for native SULT1E1.

Concluding remarks

In this study we have demonstrated that E2 sulfation catalyzed by recombinant human SULT1E1 and that catalyzed by human liver sulfotransferase are both potentially inhib-

ited by different classes of PHAH-OHs, with IC₅₀ values for recombinant SULT1E1 inhibition in the low or even subnanomolar range. The potent inhibitors 2-OH-3,7,8-TrCDD, 2-OH-1,3,7,8-TeCDD, 3-OH-2,4,7,8-TeCDF, and 3-OH-2,4,7,8,9-PeCDF, with IC₅₀ values of 34, 4.1, 1.4, and 0.18 nM, respectively, have been identified in mammalian species (20). The most potent inhibitors have an even higher affinity for human SULT1E1 than its cognate substrate E2, which has a K_m value of 4 nM for the enzyme (25). Therefore, we hypothesize that part of the estrogenic activity of PHAHs is explained by an increase in E2 bioavailability through inhibition of human SULT1E1 by hydroxylated PHAH metabolites. Such a mechanism for the pseudoestrogenic activity of PHAH-OHs is particularly relevant for estrogen-responsive tissues that express SULT1E1, such as the endometrium, mammary gland, and testis (26–28). The effects of PHAH-OHs on the regulation of local estrogen levels in these tissues will depend on a variety of factors, such as the supply or local generation of the various PHAH metabolites, their potency in inhibiting SULT1E1, their rate of inactivation by SULT1E1 and other isoenzymes such as SULT1A1, their urinary or biliary excretion rates, and also on the reversal of the sulfation of E2 and the inhibitors by local estrogen sulfatase expression (52).

PHAHs are known also to affect the thyroid hormone system. In laboratory animals, plasma T₄ is markedly decreased as a result of competitive binding of the hydroxylated metabolites to the plasma carrier transthyretin and induction of hepatic UDP-glucuronyltransferases by the PHAHs themselves (53). PHAH-OHs have also been reported to inhibit the *in vitro* deiodination of thyroid hormone by the type I iodothyronine deiodinase as well as the sulfation of the hormone by human SULT1A1 (33). During human fetal development, sulfation is an important pathway of thyroid hormone inactivation (54). We have recently demonstrated that human SULT1E1 also efficiently catalyzes the sulfation of iodothyronines, among which are the prohormone T₄ and the active hormone T₃ (25). Inhibition of SULT1E1 may thus also have thyroid hormone-disrupting effects during fetal development. Further studies should determine to what extent estrogen and possibly thyroid hormone levels are disrupted by hydroxylated PHAHs through inhibition of estrogen sulfotransferase.

Acknowledgments

We thank Drs. S. H. Safe, A. Bergman, and H. Kuroki for kindly providing hydroxylated PHAH metabolites.

Received July 9, 2001. Accepted November 28, 2001.

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This work was supported by the Sophia Foundation for Medical Research (Project 211), the Commission of the European Communities (BMH1-CT92-0097 and QL93-2000-00930), Deutsche Forschungsgemeinschaft (INK 26), and Tenovus Scotland (Tayside).

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