SULFONATION OF ENVIRONMENTAL CHEMICALS AND THEIR METABOLITES IN THE POLAR BEAR (*Ursus maritimus*)

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ABSTRACT:

Although its habitat comprises mostly remote regions of the Arctic, the polar bear is subject to bioaccumulation of persistent environmental pollutants. Along with their phase I metabolites, they are potential substrates for detoxification via sulfonation and glucuronidation. The capability of polar bear liver to sulfonate a structurally diverse group of environmental chemicals, that is, 3-hydroxybenzo[a]pyrene (3-OH-B[a]P), triclosan, 4'-hydroxy-3,3',4,5'-tetrachlorobiphenyl (4'-OH-PCB79), 4'-hydroxy-2,3,3',4,5,5'-hexachlorobiphenyl (4'-OH-PCB159), 4'-hydroxy-2,3,3',5,5',6'-hexachlorobiphenyl (4'-OH-PCB165), the methoxylchlor metabolite 2-(4-methoxyphenyl)-2-(4-hydroxyphenyl)-1,1,1-trichloroethane (OHMXC), tris(4-chlorophenyl)-methanol (TCPM), and pentachlorophenol (PCP) was investigated. The glucuronidation of 3-OH-B[a]P was also studied. Enzyme activity was assayed by incubation of liver cytosol or microsomes derived from three adult male polar bears with 3'-phosphoadenosine-5'-phosphosulfate or uridine 5'-diphosphoglucuronic acid and substrate, followed by fluorometric or radiochemical thin-layer chromatographic analysis. The efficiency of sulfonation decreased in the order 3-OH-B[a]P ≫ triclosan > 4'-OH-PCB79 > OHMXC > 4'-OH-PCB165 > TCPM > 4'-OH-PCB159 > PCP, all of which produced detectable sulfate conjugates. The 3-OH-B[a]P substrate was readily sulfonated and glucuronidated (apparent $K_m$ 0.41, 1.4 $\mu$M, and apparent $V_{max}$ 0.50, 3.00 nmol/min/mg, respectively). UDP-glucuronic acid kinetics suggested the presence of multiple enzymes glucuronidating 3-OH-B[a]P. Substrate inhibition was observed for the sulfonation of 3-OH-B[a]P and 4'-OH-PCB79 ($K_i$ 1.0 and 217 $\mu$M, respectively). Triclosan was the most rapidly sulfated (apparent $V_{max}$ 1008 pmol/min/mg) of the substrates tested. Since sulfonation of an acyclic tertiary alcoholic group, as in TCPM, has not previously been reported, we also examined TCPM conjugation in humans and catfish, both of which formed TCPM-sulfate. The hexachlorinated polychlorinated biphenyls, TCPM, and PCP were poor substrates for sulfonation, suggesting that this may be one reason why these substances and structurally similar xenobiotics persist in polar bears.

Sulfonation is an important pathway in the biotransformation of a wide range of endogenous compounds and xenobiotics, including phenols, alcohols, amines, and steroids. These substrates may be sulfonated either directly, as in phase II metabolism, or following oxidation by cytochrome P450 enzymes. Sulfotransferases (SULTs) catalyze the transfer of a sulfonic group from 3'-phosphoadenosine-5'-phosphosulfate (PAPS) to these substrates to form the sulfate or sulfamate conjugates. Five SULT gene families have been identified in mammals (SULTs 1–5). Whereas SULT1A1 and SULT1E1 are responsible for most of the phenol and estrogen SULT hepatic activity, respectively, SULT2A1 (hydroxysteroid SULT) shows greater affinity for alcohols and benzylic alcohols (Glatt, 2002).

The lipophilicity and inherent chemical stability of persistent organic pollutants renders them excellent candidates for absorption through biological membranes as well as accumulation in both organisms and their environment. Many persistent organic pollutants have been shown to biomagnify in food webs to potentially toxic levels in top predators such as the polar bear (*Ursus maritimus*), whose diet mainly consists of ringed seal (*Phoca hispida*) blubber (Kucklick et al., 2002).

Since the sulfonation of xenobiotics has never been studied in the polar bear, the objective of this study was to investigate the efficiency of this route of detoxification on a select group of known environmental pollutants: 4'-hydroxy-3,3',4,5'-tetrachlorobiphenyl (4'-OH-P-PCB79), 4'-hydroxy-2,3,3',4,5,5'-hexachlorobiphenyl (4'-OH-PCB159), 4'-hydroxy-2,3,3',5,5',6'-hexachlorobiphenyl (4'-OH-PCB165), pentachlorophenol (PCP), tris(4-chlorophenyl)-methanol (TCPM), 2-(4-methoxyphenyl)-2-(4-hydroxyphenyl)-1,1,1-trichloroethane (OHMXC), 3-hydroxybenzo[a]pyrene (3-OH-B[a]P), and triclosan (2,4',5'-trichloro-2'-hydroxyphenyl ether) (Fig. 1).

Polychlorinated biphenyls (OH-PCBs), major biotransformation products of PCBs (James, 2001), have been shown to be present in relatively high concentrations in polar bears (Sandau and Norstrom, 1998; Sandau et al., 2000). The abundance of these hydroxylated metabolites may be due to cytochrome P450 induction (Letcher et al., 1998; Sandau et al., 2000). The abundance of these hydroxylated metabolites may be due to cytochrome P450 induction (Letcher et al., 1998; Sandau et al., 2000).

**ABBREVIATIONS:** SULT, sulfotransferase; BSA, bovine serum albumin; PAPS, 3'-phosphoadenosine-5'-phosphosulfate; OH-PCB, polychlorinated biphenyl; 4'-OH-PCB79, 4'-hydroxy-3,3',4,5'-tetrachlorobiphenyl; 4'-OH-PCB159, 4'-hydroxy-2,3,3',4,5,5'-hexachlorobiphenyl; 4'-OH-PCB165, 4'-hydroxy-2,3,3',5,5',6'-hexachlorobiphenyl; PCP, pentachlorophenol; TCPM, tris(4-chlorophenyl)-methanol; OHMXC, 2-(4-methoxyphenyl)-2-(4-hydroxyphenyl)-1,1,1-trichloroethane; 3-OH-B[a]P, 3-hydroxybenzo[a]pyrene; UDPGA, uridine 5'-diphosphoglucuronic acid; UGT, UDP-glucuronosyltransferase; TLC, thin-layer chromatography.
inefficient phase II detoxication, and inhibition of their own biotransformation. The 4'-OH-PCB79 (an oxidation product of PCB congener 77) is a potent inhibitor of the sulfonation of several substrates, including 3-OH-B[a]P in channel catfish intestine and human liver (van den Hurk et al., 2002; Wang et al., 2005), 3,5-diiodothyronine (T₃) in rat liver (Schuur et al., 1998), and estradiol by recombinant human SULT1E1 (Kester et al., 2000). Both 4'-OH-PCB159 and 4'-OH-PCB165 have been shown to inhibit the sulfonation of 3-OH-B[a]P by human SULT (Wang et al., 2005). Another compound detected in polar bears is PCP (Sandau and Norstrom, 1998), a commonly used wood preservative that has been implicated in thyroid hormone disruption in Arctic Inuit populations (Sandau et al., 2002). TCPM is a globally distributed organochlorine compound of uncertain origin, which was reported in human adipose tissue (Minh et al., 2000). Polar bear liver contains 4000 to 6800 ppb lipid weight TCPM, the highest levels recorded for this compound in all species studied (Jarman et al., 1992). TCPM is a potent androgen receptor antagonist in vitro (Schrader and Cooke, 2002). OHMXC, formed by demethylation of the organochlorine pesticide methoxychlor, is an estrogen receptor α agonist, an estrogen receptor β antagonist, and an androgen receptor antagonist (Gaido et al., 2000). The ubiquitous environmental pollutant benzo[a]pyrene is mainly metabolized to 3-OH-B[a]P, a procarcinogen that can be eliminated via sulfonation (Tong and James, 2000). Together with its 7,8-dihydrodiol-9,10-oxide and 7,8-oxide metabolites, 3-OH-B[a]P can form adducts with macromolecules and initiate carcinogenesis (Ribeiro et al., 1986). Triclosan is an antimicrobial agent that has been detected in human plasma and breast milk (Adolfsson-Erici et al., 2002). In vitro studies have shown that triclosan inhibits various biotransformation enzymes, including SULT and UDP-glucuronosyltransferases (UGTs) (Wang et al., 2004).
The fact that 3-OH-B[a]P, triclosan, OHMXC, 4′-OH-PCB79, 4′-OH-PCB159, and 4′-OH-PCB165 have not been reported as environmental contaminants in polar bears to date may be due to nonsignificant levels in the Arctic environment or efficient metabolism via, for example, sulfonation. On the other hand, the presence of PCP and, particularly, high amounts of TCPM in these Arctic carnivores may indicate poor sulfonation of these substrates. The polychlorobiphenyls 4′-OH-PCB159 and 4′-OH-PCB165 are of interest since, though they have not been detected in polar bears, they are structurally similar to 4′-OH-PCB172, one of the major OH-PCBs found in polar bear plasma (Sandau et al., 2000). It is thus possible that these compounds are sulfonated with similar efficiencies. The other major phase II biotransformation pathway for the above-mentioned compounds is glucuronidation. Polar bear liver efficiently glucuronidated 3-OH-B[a]P and several OH-PCBs (Sacco and James, 2004).

This study determined that polar bear hepatic SULT activity was highest for 3-OH-B[a]P and triclosan, followed by 4′-OH-PCB79. The other compounds studied, 4′-OH-PCB159, 4′-OH-PCB165, TCPM, and OHMXC, were poor substrates. This is the first time, to our knowledge, that the sulfation of TCPM was investigated for any species.

Materials and Methods

Unlabeled PAPS was purchased from the Dayton Research Institute (Dayton, OH). Uridine 5′-diphosphoglucuronic acid (UDPGA) was obtained from Sigma (St. Louis, MO). Radiolabeled [35S]PAPS (1.82 or 3.56 Ci/mmol) was obtained from PerkinElmer Life and Analytical Sciences (Boston, MA). The benzo[a]pyrene metabolites 3-OH-B[a]P, B[a]P-3-O-sulfate, and B[a]P-3-O-glucuronide were supplied by the Midwest Research Institute (Kansas City, MO), through contact with the Chemical Carcinogen Reference Standard Repository of the National Cancer Institute. Dr. L.W. Robertson, University of Iowa (Iowa City, IA), kindly donated the 4′-OHC-PCB165, triclosan, PCP, TCPM, and OHMXC. Cytoisolic protein concentrations and incubation time were optimized for every test substrate to ensure that the reaction was linear during the incubation period. Substrate consumption did not exceed 5%. The incubation mixture consisted of 0.1 M Tris-Cl buffer (pH 7.0), 0.4% BSA in water, 20 μM PAPS (10% labeled with [35S]), 0.1 mg of polar bear hepatic cytosolic protein, or 0.005 mg in the case of 4′-OH-PCB79 and triclosan, and substrate in a total reaction volume of 0.1 ml or 0.5 ml in the case of TCPM. The OH-PCBs, triclosan, and OHMXC were added to tubes from methanol solutions, and the methanol was removed under N2 prior to addition of other components. The TCPM was dissolved in DMSO, the solvent being present at a concentration not exceeding 1% in the final assay volume. Control determinations utilizing 1% DMSO had no inhibitory effect on sulfonation. Aqueous solutions of sodium pentachlorophenate were utilized in the case of PCP. Tubes containing all components except the cosubstrate were placed in a water bath at 37°C, and PAPS was added to initiate the reaction. Incubation times were 5 min (TCPM), 20 min (4′-OH-PCB79, triclosan), 30 min (PCP), and 40 min (OHMXC, 4′-OH-PCB159, 4′-OH-PCB165). The incubation was terminated by the addition of an equal volume of a 1:1 mixture of 2.5% acetic acid and PIC-A and water. The sulfated product was extracted with 3.0 ml of ethyl acetate as described previously (Wang et al., 2004) and the phases were separated by centrifugation. Duplicate portions of the ethyl acetate phase were counted for quantitation of sulfated conjugates.

Radiochemical TLC Method. Since the ethyl acetate phase contains sulfated conjugates formed from both the substrate of interest and substrates already present in polar bear liver, TLC was used to quantify substrate sulfation in cases where SULT activity was similar in samples and substrate blanks. After evaporating 2 ml of ethyl acetate extract from the SULT assay under N2, the solutes were reconstituted in 40-μl methanol. For 4′-OH-PCB159, 4′-OH-PCB165, TCPM, and OHMXC, the substrate conjugates were separated on RP-18SIV, reverse-phase TLC plates with a fluorescent indicator (Merck, Darmstadt, Germany) using methanol/water (80:20). For TCPM, Whatman KC8F reverse-phase 200-μm TLC plates with a fluorescent indicator in conjunction with a developing solvent system consisting of methanol/water/0.28 M PIC-A (40:60:1.9 by volume) were used. Electronic autoradiography (PerkinElmer Instant Imager) was used to identify and quantify the radioactive bands separated on the TLC plate. The counts representing the substrate sulfate conjugate products were expressed as a fraction of the total radioactivity determined by scintillation counting, thus enabling the radioactivity due to the substrate conjugate to be accurately determined.

The identity of the conjugate of TCPM as a sulfate ester was verified by studying its sensitivity to sulfatase. Polar bear cytosol (0.5 mg) was incubated for 75 min with or without 200 μM TCPM. The incubation was terminated and the product extracted into ethyl acetate as above. The ethyl acetate was evaporated to dryness and dissolved in 0.25 ml of Tris buffer, pH 7.5, containing 0 or 0.08 unit of sulfatase. After an overnight incubation at 35°C, the reaction was stopped by the addition of methanol and the tubes were centrifuged. The supernatants were evaporated to dryness, reconstituted in methanol, and analyzed by TLC as described above.

UDP-Glucuronosyltransferase Assay. The fluorometric assay for 3-OH-B[a]P was performed according to a method described previously (James et al., 1997). Preliminary studies established the conditions for linearity of reaction with respect to time, protein, and detergent concentrations, at the same time ensuring that substrate consumption did not exceed 10%. The apparent Km for UDPGA was determined by performing experiments at a fixed concentration on the basis that at alkaline pH, the benzo[a]pyrene-3-O-sulfate has different wavelength optima for fluorescence excitation and emission (294/415 nm) from the benzo[a]pyrene-3-O-phenoxyanion (390/545 nm) (James et al., 1997). Saturation concentrations of PAPS were determined by performing the assay at 1 μM 3-OH-B[a]P. The reaction mixture for the detection of the sulfation of 3-OH-B[a]P by polar bear liver cytosol consisted of 0.1 M Tris-HCl buffer (pH 7.6), 0.4% BSA, PAPS (0.02 mM), 25 μg of polar bear hepatic cytosolic protein, and 3-OH-B[a]P (0.05–25 μM) in a total reaction volume of 1.0 ml. SULT activity (pmol/min/mg) was calculated from a standard curve prepared with B[a]P-3-O-sulfate standards. Substrate consumption did not exceed 10%.
of 3-OH-B[a]P (10 μM). Saturating UDPGA concentrations were used to determine 3-OH-B[a]P glucuronidation kinetics.

**Kinetic Analysis.** Duplicate values for the rate of conjugate formation at each substrate concentration were used to calculate kinetic parameters using Prism v 4.0 (GraphPad Software Inc., San Diego, CA). Equations used to fit the data were the Michaelis-Menten hyperbola for one-site binding (eq. 1), the Hill plot (eq. 2), substrate inhibition for one-site binding (eq. 3) (Houston and Kenworthy, 2000), and partial substrate inhibition due to binding to an allosteric site (eq. 4) (Zhang et al., 1998).

\[
\begin{align*}
V &= V_{\text{max}}[S](K_{m} + [S]) \\
V &= V_{\text{max}}[S](S_{0}b + [S]p) \\
V &= V_{\text{max}}[S](K_{m} + [S] + ([S]^{2}/K_{i})) \\
V &= V_{\text{max}}(1 + (V_{\text{max}}[S]V_{\text{max}}[K])/(1 + K_{i}[S] + [S]K))
\end{align*}
\]

Values for \(K_{m}\) and \(V_{\text{max}}\) derived from eq. 1 were used as initial values in the fitting of data to eqs. 3 and 4. Eadie-Hofstee plots were used to analyze the biphasic kinetics observed.

**Results**

**Sulfonation and Glucuronidation of 3-OH-B[a]P.** Optimum conditions for sulfonation were a 10-min incubation time and 25 μg of cytosolic protein. A concentration of 0.02 mM PAPS provided saturating concentrations of the cosubstrate and enabled kinetic parameters at 1.0 μM 3-OH-B[a]P to be calculated by the application of eq. 1 (Table 1, Sulfonation). The data for the sulfonation of 3-OH-B[a]P was fit to a two-substrate model (eq. 3), whereby the binding of a second substrate to the enzyme is responsible for the steep decline in enzyme activity at concentrations exceeding 1 μM (Fig. 2a). Initial estimates of \(V_{\text{max}}\) and \(K_{m}\) were provided by the initial data obtained at low [S] (noninhibitory), whereas \(V_{\text{max}}\) was constrained to 65 ± 20 pmol/min/mg, which is slightly below the plateau in Fig. 2a. The kinetic scheme (Fig. 2b) illustrates the proposed partial substrate inhibition process, which assumes that substrate binding is at equilibrium, which is probable due to the low turnover rate of SULT. The best fit of the data was provided by a \(K_{i}\) of 1.0 ± 0.1 μM. Binding of the second substrate molecule results in a 10-fold reduction in the rate of sulfonation formation.

Optimum conditions for the glucuronidation of 3-OH-B[a]P by polar bear microsomes were found to be 5 μg of microsomal protein and a 20-min incubation. A concentration of 4 mM UDPGA was determined to be suitable for providing saturating concentrations of the cosubstrate. The binding of UDPGA to UGT at 10 μM 3-OH-B[a]P was shown to be biphasic, with a 5-fold reduction in affinity at higher UDPGA concentrations (Table 1, Glucuronidation). The kinetic parameters for the cosubstrate were calculated by deconvoluting the curvilinear data in the Eadie-Hofstee plot (Fig. 3). In the presence of 4 mM UDPGA, the formation of B[a]P-3-O-glucuronide followed Michaelis-Menten kinetics (Table 1, Glucuronidation).

**Sulfonation of Other Substrates.** Triclosan sulfate was formed rapidly, with the overall kinetics conforming to a hyperbolic curve (eq. 1) (Table 2). Substrate inhibition was observed for 4′-OH-PCB79 (Fig. 4), with the data fitting eq. 3. The value of \(K_{i}\) that gave the best fit was 217 ± 25 μM (Table 2). Sulfate conjugation of 4′-OH-PCB159 and 4′-OH-PCB165, which proceeded via Michaelis-Menten kinetics, was, respectively, 11 and 5 times less efficient than the sulfonation of 4′-OH-PCB79 (Table 2). At a concentration of 10 μM, 4′-OH-PCB165 was observed to inhibit sulfonation of substrates already present in polar bear liver cytosol by 60%. Due to variable rates of sulfonation of these unknown substrates, autoradiographic counts corresponding to the OHMXC-O-sulfate band were used to correct the activities calculated from the scintillation counter data (Fig. 5). This enabled the transformed data to be fit into a Michaelis-Menten model (Table 2). The autoradiograms obtained showed that increasing concentrations of OHMXC resulted in decreased counts for the unknown sulfate conjugates (Fig. 5). Sulfonation of the unknown substrates in polar bear cytosol was reduced by half at OHMXC concentrations <20 μM.

The total TCPM sulfate conjugate production formed after 5 min under initial rate conditions did not exceed 30 pmol. TLC, followed by autoradiography, was thus used to distinguish the TCPM-sulfate band (\(R_{f} 0.54\)) from other sulfate conjugates (\(R_{f} 0.05\) and 0.72) originating from compounds in the polar bear liver cytosol (Fig. 6). The data obtained followed hyperbolic kinetics (Table 2). Even though the TLC from the kinetic experiments showed a TCPM concentration-dependent increase of the band corresponding to the purported TCPM-sulfate, and this band was absent in the substrate blank, the fact remained that we were apparently looking at the only instance of the TCPM conjugate at \(R_{f} 0.54\) and 0.72 (Table 2).

**Table 1**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>(V_{\text{max}}) (pmol/min/mg)</th>
<th>(K_{m}) (μM)</th>
<th>(V_{\text{max}}/K_{m}) (μmol/min/mg)</th>
<th>(K_{i}) (μM)</th>
<th>(V_{\text{max}}/K_{i}) (μmol/min/mg)</th>
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<tbody>
<tr>
<td><strong>Sulfonation</strong></td>
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<tr>
<td>3-OH-B[a]P</td>
<td>500 ± 8</td>
<td>0.41 ± 0.03</td>
<td>1220 ± 70</td>
<td>65.0 ± 20.0</td>
<td>1.01 ± 0.10</td>
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<tr>
<td>PAPS</td>
<td>162 ± 35</td>
<td>0.22 ± 0.07</td>
<td></td>
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<td></td>
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<tr>
<td><strong>Glucuronidation</strong></td>
<td></td>
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<tr>
<td>3-OH-B[a]P</td>
<td>3.00 ± 1.18</td>
<td>1.4 ± 0.2</td>
<td>1900 ± 544</td>
<td></td>
<td></td>
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<tr>
<td>UDPGA</td>
<td>1.53 ± 0.56</td>
<td>1.47 ± 0.48</td>
<td>42.9 ± 2.5</td>
<td>200 ± 68</td>
<td></td>
</tr>
</tbody>
</table>

\(\text{a}\) Constrained variables to obtain best fit.

\(\text{b}\) Values for high-affinity component.

\(\text{c}\) Values for low-affinity component.
same position (Fig. 6). The TCPM sulfate conjugate from polar bear could be hydrolyzed by sulfatase (Fig. 7), providing further evidence of the sulfonation of this alcohol.

Inhibition of sulfonation of substrates already present in the polar bear liver was noted upon adding $1/_{H9262}M$ PCP (Fig. 8). The data for PCP sulfonation fitted the nonlinear Hill plot (eq. 2) (Table 2).

**Discussion**

The sulfonation of hydroxylated metabolites of benzo[a]pyrene has been reported in various species, including fish (James et al., 2001) and humans (Wang et al., 2004). Benzo[a]pyrene-3-glucuronide has been shown to be produced by fish (James et al., 1997), rats (Lilienblum et al., 1987), and humans (Wang et al., 2004). There are, however, few studies investigating the kinetics of these conjugation reactions. Glucuronidation of 3-OH-B[a]P was more efficient in polar bear liver than in human liver or catfish intestine. On the other hand, the efficiency of sulfonation was similar to that shown in human liver but around 3 times less than that in catfish intestine (James et al., 2001; Wang et al., 2004). From the limited comparative data available, it can be surmised that, in general, polar bear liver is an important site of 3-OH-B[a]P detoxication, particularly with respect to glucuronidation.

Substrate inhibition for the sulfonation of 3-OH-B[a]P has been observed at relatively low concentrations of the xenobiotic in other species such as catfish and human (Tong and James, 2000; Wang et al., 2005). Data from the polar bear sulfonation assay fitted a two-substrate model developed for the sulfonation of 17β-estradiol by SULT1E1 (Zhang et al., 1998). This model was also used to explain the sulfonation profile observed for the biotransformation of 1-hydroxypyrene, a compound structurally similar to 3-OH-B[a]P, by SULTs 1A1 and 1A3 (Ma et al., 2003). In the original model, SULT1E1 was saturated with PAPS, and each of the estradiol substrate molecules bound independently to the enzyme. The estradiol binding sites were proposed to consist of a catalytic site, and an allosteric site that regulates turnover of the substrate (Zhang et al., 1998). The substrate inhibition observed with polar bear liver cytosol
at higher 3-OH-B[a]P concentrations (>0.75 μM) can thus be explained by the binding of a second substrate molecule to an allosteric site, which leads to a 2-fold decrease in affinity and an 8-fold decrease in \( V_{\text{max}} \).

SULTs are generally high-affinity, low-capacity biotransformation enzymes that operate effectively at low substrate concentrations. Thus, typical \( K_m \) values for the sulfonation of xenobiotic substrates are usually significantly lower than \( K_m \) values for the same substrates undergoing biotransformation by low-affinity, high-capacity glucuronosyltransferases (UGTs). In polar bear liver, both pathways showed similar apparent affinities for 3-OH-B[a]P, with \( K_m \) values of 0.4 and 1.4 μM for sulfonation and glucuronidation, respectively, suggesting that these two pathways of phase II metabolism compete at similar 3-OH-B[a]P concentrations. However, the apparent maximal rate of sulfonation was about 7.5 times lower than the rate of glucuronidation.

It was previously reported that the maximum rate of glucuronidation of 3-OH-B[a]P by polar bear liver was 1.26 nmol/min/mg, or around half the \( V_{\text{max}} \) value obtained in this study (Sacco and James, 2004). However, the preceding study utilized 0.2 mM UDPGA, which, as seen from Table 1, Glucuronidation, is equivalent to the \( K_m \) (for UDPGA) of the low-affinity enzyme and, thus, does not represent saturating concentrations of the cosubstrate. The affinity of the enzyme for 3-OH-B[a]P did not change significantly with a 20-fold increase in UDPGA concentrations, suggesting that substrate binding is independent of the binding of cosubstrate. The binding of UDPGA was biphasic, indicating that multiple hepatic UGTs may be responsible for the biotransformation. Biphasic UDPGA kinetics have also been demonstrated in human liver and kidney for 1-naphthol, morphine, and 4-methylumbelliferone (Miners et al., 1988a,b; Tsoutsikos et al., 2004). Although \( V_{\text{max}} \) was similar for both components, there was a 5-fold decrease in enzyme affinity for UDPGA as the cosubstrate concentration was increased. The involvement of at least two enzymes can be physiologically advantageous since it enables the maintenance of a high turnover rate even as UDPGA is consumed. Although physiologically UDPGA concentrations in polar bear liver are unknown, mammalian hepatic UDPGA has been determined to be around 200 to 400 μM (Zhivkov et al., 1975; Cappiello et al., 1991), implying that the observed nonlinear kinetics in the polar bear may operate in vivo.

The rate of triclosan sulfonation was the highest of all the substrates studied; apparent \( V_{\text{max}} \) was twice as high as for 3-OH-B[a]P. However, the overall efficiency of sulfonation of the hydroxylated polyaromatic hydrocarbon was still 13 times higher than for triclosan sulfonation. The presence of three chlorine substituents (though none flanking the phenol group) does not hinder the sulfonation of triclosan when compared with the “chlorine-free” 3-OH-B[a]P. Triclosan sulfonation in polar bear liver was similar to that in human liver with respect to enzyme affinity; however the maximum rate was 10-fold higher in polar bears than in humans (Wang et al., 2004). This may be one reason why triclosan has not been detected in polar bear plasma or liver to date.

Our data fitted a model that indicates that the substrate inhibition observed for 4′-OH-PCB79 may be due to a second substrate molecule interacting with the enzyme-substrate complex at the active site rather than an allosteric site, resulting in a dead-end complex. Unlike 3-OH-B[a]P, sulfonation can only proceed via the single substrate-SULT complex. Models of SULT1A1 and 1A3, with two molecules of p-nitrophenol or dopamine at the active site, respectively, have been proposed as a mechanism of substrate inhibition (Gamage et al.,...
The inclusion of two additional chlorine substituents on the nonphenol ring (with respect to 4'-OH-PCB79) resulted in both 4'-OH-PCB159 and 4'-OH-PCB165 being very poor substrates. Inefficient sulfonation may be one reason why the related compound 4'-OH-PCB172 accumulates in polar bears. Some degree of substrate inhibition may also be expected to contribute to this accumulation, as was observed with 4'-OH-PCB165.

Sulfonation was not an efficient pathway of OHMXC detoxification. The rate of OHMXC-sulfonation formation was around 7 times lower than for 4'-OH-PCB79. Since resonance delocalization of negative charge on the phenolic oxygen by the flanking chlorines in chlorophenols may decrease $V_{\text{max}}$ by increasing the energy of the transition state of the reaction (Duffel and Jakoby, 1981), it is possible that in the case of OHMXC (with no chlorines flanking the phenolic group), product release, rather than sulfonate transfer, may have been the rate-limiting step.

TCPM was a poor substrate for sulfonation, and this may be one reason why it has been measured in such high amounts in polar bear liver. To our knowledge, sulfonation of acyclic tertiary alcohols has not been reported in the literature. Despite the considerable steric hindrance of three phenyl groups, the alcohol group could be sulfonated. Although the alcohol in TCPM is not of the benzylic type, the presence of three proximal phenyl groups may give this group some benzylic character, rendering sulfonation of the alcohol possible. Both SULT1E1 and SULT2A1 have been shown to sulfonate benzylic alcohol groups attached to large molecules (Glatt, 2000). Sulfation of the benzylic hydroxyl group leads to an unstable sulfate conjugate that readily degrades to the reactive carbocation or spontaneously hydrolyzes back to the alcohol. Attempts to recover TCPM-O-sulfonate from TLC plates resulted in recovery of TCPM from the conjugate band, perhaps because of the conjugate’s instability (data not shown).

A study of the sulfonation of PCP was complicated by the fact that it is a known SULT inhibitor, often with $K_I$ values in the submicromolar range. In our experiments, this was seen as a 74% decrease in formation of the unidentified sulfonate conjugates (band shown at the solvent front in Fig. 8) upon addition of 1 $\mu$M PCP. Although PCP was a strong inhibitor of SULT1E1 (Kester et al., 2000) and has been postulated to be a dead-end inhibitor for phenol sulfotransferases (Duffel and Jakoby, 1981), it was possible that polar bear SULT 1A isoforms were not completely inhibited by PCP, or that other SULT isoform(s) were responsible for the limited sulfonation activity observed. Thus, we have shown that, in vitro, at least, one mammalian species is capable of limited PCP sulfonation. Even though the tertiary alcohol of TCPM was a poor candidate for sulfonation, it was metabolized at twice the efficiency of PCP, which has a phenolic group that is usually more susceptible to sulfonation. This demonstrates the extent of the decreased nucleophilicity on the phenolic oxygen due to the resonance delocalization afforded by the five chlorine substituents.

In summary, this study has demonstrated that, in polar bear liver, 3-OH-B[a]P was a good substrate for sulfonation and glucuronidation. Other, chlorinated substrates were biotransformed with less efficiency, implying that reduced rates of sulfonation may contribute to the persistence of compounds such as hexachlorinated OH-PCBs, TCPM, and PCP in polar bear tissues.

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References


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