DMD Fast Forward. Published on June 10, 2005 as doi:10.1124/dmd.105.004648

Sulfonation of environmental chemicals and their metabolites in the polar bear (*Ursus maritimus*)

James C. Sacco and Margaret O. James

Department of Medicinal Chemistry, College of Pharmacy, University of Florida, P.O. Box 100485, Gainesville Florida 32610.

Running title: Sulfonation of environmental chemicals in the polar bear

Corresponding author: -

Margaret O. James

Department of Medicinal Chemistry,

College of Pharmacy

University of Florida, P.O. Box 100485,

Gainesville, FL 32610

United States of America

Phone: (352) 846-1952

Fax: (352) 846-1972

Email: mojames@cop.ufl.edu

Number of text pages:	26
Number of tables:	2
Number of figures:	7
Number of references:	40
Number of words, abstract:	250
Number of words, introduction:	750
Number of words, discussion:	1458

Nonstandard abbreviations:

BSA, bovine serum albumin; SULT, sulfotransferase; PAPS, 3´-phosphoadenosine-5´-phosphosulfate; POP, persistent organic pollutant; OH-PCBs, polychlorinated biphenylols; 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-

DMD Fast Forward. Published on June 10, 2005 as DOI: 10.1124/dmd.105.004648
This article has not been copyedited and formatted. The final version may differ from this version.

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-glucuronosyl transferase; PAH, polyaromatic hydrocarbon

DMD Fast Forward. Published on June 10, 2005 as DOI: 10.1124/dmd.105.004648
This article has not been copyedited and formatted. The final version may differ from this version.

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 methoxychlor 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 PAPS or UDPGA and substrate, followed by fluorometric or radiochemical TLC 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 μM, 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 μM 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 OH-PCBs, 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 either be sulfonated directly, as in phase II metabolism, or following oxidation by cytochrome P450 enzymes. Sulfotransferases (SULT) 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 (SULTs1-5). While 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 (POPs) renders them excellent candidates for absorption through biological membranes as well as accumulation in both organisms and their environment. Many POPs 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-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), triclosan (2,4,4´-trichloro-2´-hydroxydiphenyl ether) (Figure 1).

Polychlorinated biphenylols (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 CYP induction (Letcher et al., 1996), 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 (T_2) 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 4000-6800 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 (ER) α agonist, an ERβ 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 (UGT) (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 non-significant 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 polychlorobiphenylols 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, PCP, TCPM, and OHMXC were poor substrates. This is the first time, to our knowledge, that the sulfation of TCPM was investigated for any species.

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 [^{35}S]PAPS (1.82 or 3.56 Ci/mmol) was obtained from Perkin-Elmer Life Sciences, Inc. (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, U of Iowa, kindly donated the 4´-OH-PCB79, and 4´-OH-PCB159 and 4´-OH-PCB165 were purchased from AccuStandard, Inc. (New Haven, CT). PCP from Fluka Chemical (Milwaukee, WI) was used to prepare the water-soluble sodium salt (Meerman et al., 1983). Triclosan and sulfatase (Type VI from *Aerobacter*, S1629) were purchased from Sigma (St.Louis, MO), while methoxychlor and TCPM were purchased from ICN Biomedical (Aurora, OH) and Lancaster Synthesis, Inc. (Pelham, NH), respectively. The OHMXC was prepared by the demethylation of methoxychlor and purified by recrystallization (Hu and Kupfer 2002). Tetrabutyl ammonium hydrogen sulfate PIC-A low UV reagent was from Water Corporation, Milford, MA. Other reagents were the highest grade available from Fisher Scientific (Atlanta, GA) and Sigma.

Animals. The samples used in this study were a kind donation from Dr. S. Bandiera (U British Columbia) and Dr. R. Letcher (Environment Canada). They were derived from the distal portion of the right lobe of livers of three adult male bears G, K and X. Bears G and K were collected as part of a legally-controlled hunt by Inuit in the Canadian Arctic in April 1993 near Resolute Bay, Northwest Territories, while bear X was collected in

November 1993 near Churchill, Manitoba, just after the fasting period. Liver samples were removed within 10-15 minutes after death, cut into small pieces and frozen at -196ºC in liquid N_2. The samples were subsequently stored at -80ºC.

Cytosol and microsomes preparation. Prior to homogenization, the frozen polar bear liver samples (~2g) were gradually thawed in a few ml of homogenizing buffer. Homogenizing buffer consisted of 1.15% KCl, 0.05 M K_3PO_4 pH 7.4, and 0.2 mM phenylmethylsulfonyl fluoride, added from concentrated ethanol solution just before use. Resuspension buffer consisted of 0.25 M sucrose, 0.01 M Hepes pH 7.4, 5% glycerol, 0.1 mM dithiothreitol, 0.1 mM ethylene diamine tetra-acetic acid and 0.1 mM phenyl methyl sulfonyl fluoride. The liver was placed in a volume of fresh ice-cold buffer equal to 4 times the weight of the liver sample. The cytosol and microsomal fractions were obtained using a procedure described previously (Wang et al., 2004). Microsomal and cytosolic protein contents were measured by the Lowry assay, using bovine serum albumin (BSA) as standard.

Sulfotransferase assays

A. *Fluorometric method*. The activity was measured 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-phenolate anion (390/545 nm) (James et al., 1997). Saturating concentrations of PAPS were determined by performing the assay at 1 μM 3-OH-B[a]P. The reaction mixture for detecting the sulfation of 3-OH-BaP by polar bear liver cytosol consisted of 0.1 M Tris-Cl buffer (pH 7.6), 0.4% BSA, PAPS (0.02 mM), 25 μg polar bear hepatic cytosolic protein, and 3-OH-B[a]P (0.05-25 μM) in a total reaction volume of 1.0mL. 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%.

B. *Radiochemical extraction method*. This method, based on Wang and co-workers (2004), was employed in the study of the sulfonation of 4´-OH-PCB79, 4´-OH-PCB159, 4´-OH-PCB165, triclosan, PCP, TCPM and OHMXC. Cytosolic 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% labelled with ^{35}S), 0.1 mg 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 0.1mL, or 0.5mL in the case of TCPM. The OH-PCBs, triclosan and OHMXC were added to tubes from methanol solutions, and the methanol was removed under N_2 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 pentachlorophenolate were utilized in the case of PCP. Tubes containing all components except the co-substrate were placed in a water bath at 37ºC 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 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 sulfate conjugates.

C. *Radiochemical TLC method*. Since the ethyl acetate phase contains sulfate 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 N_2, the solutes were reconstituted in 40 μL methanol. For 4´-OH-PCB159, 4´-OH-PCB165, PCP and OHMXC, the substrate conjugates were separated on RP-18F_{254s} reverse phase TLC plates with fluorescent indicator (Merck, Darmstadt, Germany) using methanol:water (80:20). For TCPM, Whatman $KC_{18}F$ reverse phase 200 μm TLC plates with 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 employed. Electronic autoradiography (Packard Instant Imager, Meriden, CT) 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 minutes 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 units of sulfatase. Following an overnight incubation at 35˚C, the reaction was stopped by the addition of methanol and the tubes

DMD Fast Forward. Published on June 10, 2005 as DOI: 10.1124/dmd.105.004648
This article has not been copyedited and formatted. The final version may differ from this version.

were centrifuged. The supernatants were evaporated to dryness, reconstituted in methanol and analyzed by TLC as described above.

UDP-glucuronosyl transferase 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 K_m for UDPGA was determined by performing experiments at a fixed concentration of 3-OH-B[a]P (10 μM). Saturating UDPGA concentrations were used in order 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 at an allosteric site (eq. 4) (Zhang et al., 1998).

$$v = V_{max}[S] / (K_m + [S]) \quad (1)$$

$$v = V_{max}[S]^h / (S_{50}{}^h + [S]^h) \quad (2)$$

$$v = V_{max}[S] / (K_m + [S] + ([S]^2/K_i)) \quad (3)$$

$$v = V_{max1}(1 + (V_{max2}[S]/V_{max1}K_i)) / (1 + K_m/[S] + [S]/K_i) \quad (4)$$

Values for K_m and V_{max} derived from equation 1 were used as initial values in the fitting of data to equations 3 and 4. Eadie-Hofstee plots were used in order to analyze the biphasic kinetics observed.

DMD Fast Forward. Published on June 10, 2005 as DOI: 10.1124/dmd.105.004648
This article has not been copyedited and formatted. The final version may differ from this version.

Results

Sulfonation and glucuronidation of 3-OH-B[a]P

Optimum conditions for sulfonation were 10 minutes incubation time and 25 μg cytosolic protein. A concentration of 0.02 mM PAPS provided saturating concentrations of the co-substrate and enabled kinetic parameters at 1.0 μM 3-OH-B[a]P to be calculated by the application of eq. 1 (Table 1a). 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 (Figure 2a). Initial estimates of V_{max1} and K_m were provided by the initial data obtained at low [S] (non-inhibitory), while V_{max2} was constrained to 65 ± 20 pmol/min/mg, which is slightly below the plateau in Figure 2a. The kinetic scheme (Figure 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 tenfold reduction in the rate of sulfonate formation.

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

DMD Fast Forward. Published on June 10, 2005 as DOI: 10.1124/dmd.105.004648
This article has not been copyedited and formatted. The final version may differ from this version.

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 (Figure 4), with the data fitting equation (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 (Figure 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 (Figure 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 minutes under initial rate conditions did not exceed 30 pmol. TLC, followed by autoradiography, were 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 (Figure 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 ever reported of a successful sulfonation of a tertiary alcohol. Thus, additional experiments were performed to verify the identity of this conjugate. The purity of the TCPM was tested in the event that the additional band was due to an impurity in the substrate. However, the substrate used was found to be free of contaminants by HPLC (C_{18} reverse phase column, with detection at 268 and 220 nm, using 90% methanol in water and a flow rate of 1 mL/min). A single peak was recorded at 7.3 minutes. Another experiment involved a 60-minute incubation performed with 100 μM TCPM and 0.1 mg cytosolic protein from polar bear, channel catfish and human liver. For each of the three species, we detected a conjugate at R_f = 0.54. The substrate blanks showed no band at the same position (Figure 6). The TCPM sulfate conjugate from polar bear could be hydrolyzed by sulfatase (Figure 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 μM PCP (Figure 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 three times less than in catfish intestine (Wang et al., 2004, James et al., 2001). 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

DMD Fast Forward. Published on June 10, 2005 as DOI: 10.1124/dmd.105.004648
This article has not been copyedited and formatted. The final version may differ from this version.

(>0.75 μM) can thus be explained by the binding of a second substrate molecule to an allosteric site, which leads to a two-fold decrease in affinity and an eightfold decrease in V_{max}.

SULTs are generally high-affinity, low-capacity biotransformation enzymes that operate effectively at low substrate concentrations. Thus, typical K_ms for the sulfonation of xenobiotic substrates are usually significantly lower than K_ms 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_ms of 0.4 and 1.4 μM for sulfonation and glucuronidation respectively, suggesting 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_{max} value obtained in this study (Sacco and James 2004). However, the preceding study utilized 0.2 mM UDPGA, which, as seen from Table 2a, is equivalent to the K_m (for UDPGA) of the low-affinity enzyme, and thus does not represent saturating concentrations of the co-substrate. 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 co-substrate. 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).

While V_{max} was similar for both components, there was a fivefold decrease in enzyme affinity for UDPGA as the co-substrate 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 physiological UDPGA concentrations in polar bear liver are unknown, mammalian hepatic UDPGA has been determined to be around 200-400 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_{max} was twice as high as for 3-OH-B[a]P. However, the overall efficiency of sulfonation of the hydroxylated PAH 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 to the 'chlorine-free' 3-OH-B[a]P. Triclosan sulfonation in polar bear liver was similar to human liver with respect to enzyme affinity; however the maximum rate was tenfold 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 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., 2003, Barnett et al., 2004), while the crystal structure of human EST containing bound 4,4´-OH-3,3´,5,5´-tetrachlorobiphenyl at the active site has not provided any evidence of an allosteric site (Shevtsov et al., 2003). The slower sulfonation of 4´-OH-PCB79 compared with 3-OH-B[a]P may result from the inductive effect of the chlorines flanking the phenolic group rather than steric hindrance (Duffel and Jakoby, 1981). However, polar bear liver sulfonated 4´-OH-PCB79 more rapidly than the other OH-PCB substrates studied.

The inclusion of two additional chlorine substituents on the non-phenol 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-sulfonate 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_{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 SULT 1E1 and SULT 2A1 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_is 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 Figure 8) upon addition of 1 μM 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.

Acknowledgements

The authors thank Dr. Stelvio Bandiera, Dr. Ross Norstrom, Dr. Robert Letcher and Dr. Malcolm Ramsay for polar bear samples, and Leah Stuchal for preparation of OHMXC.

References

Adolfsson-Erici M, Pettersson M, Parkkonen J and Sturve J (2002) Triclosan, a commonly used bactericide found in human milk and in the aquatic environment in Sweden. *Chemosphere* **46:**1485-1489.

Barnett AC, Tsvetanov S, Gamage N, Martin JL, Duggleby RG and McManus M (2004) Active site mutations and substrate inhibition in human sulfotransferase 1A1 and 1A3. *J Biol Chem* **279:**18799-805.

Cappiello M, Giuliani L and Pacifici GM (1991) Distribution of UDP-glucuronosyl-transferase and its endogenous substrate uridine 5'-diphosphoglucuronic acid in human tissues. *Eur J Clin Pharmacol* **41:**345-350.

Duffel MW and Jakoby WB (1981) On the mechanism of aryl sulfotransferase. *J Biol Chem* **256:**11123-11127.

Gaido KW, Maness SC, McDonnell DP, Dehal SS, Kupfer D and Safe S (2000) Interaction of methoxychlor and related compounds with estrogen receptor α and β, and androgen receptor: Structure-activity studies. *Mol Pharmacol* **58:**852-858.

Gamage NU, Duggleby RG, Barnett AC, Tresillian M, Latham CF, Liyou NE, McManus ME and Martin JL (2003) Structure of a human carcinogen-converting enzyme, SULT1A1. Structural and kinetic implications of substrate inhibition. *J Biol Chem* **278:**7655-7662.

Glatt H (2000) Sulfotransferases in the bioactivation of xenobiotics. *Chem-Biol Interact* **129:**141-170.

Glatt HR (2002) Sulphotransferases, in *Enzyme systems that metabolise drugs and other xenobiotics* (Costas I ed) pp 353-439, John Wiley and Sons, London, England.

Glatt H, Pauly K, Czich A, Falany JL and Falany CN (1995) Activation of benzylic alcohols to mutagens by rat and human SULTs expressed in *E.coli*. *Eur J Pharmacol* **293:**173-181.

Hu Y and Kupfer D (2002) Enantioselective metabolism of the endocrine disruptor pesticide methoxychlor by human cytochromes P450 (P450s): major differences in selective enantiomer formation by various P450 isoforms. *Drug Metab Dispos* **30:**1329-1336.

Houston JB and Kenworthy KE (2000) In vitro-in vivo scaling of CYP kinetic data not consistent with the classical Michaelis-Menten model. *Drug Metab Dispos* **28:**246-254.

James MO (2001) Polychlorinated biphenyls: metabolism and metabolites, in *PCBs. Recent Advances in the Environmental Toxicology and Health Effects* (Robertson LW and Hansen LG eds) pp 35-45, The University Press of Kentucky, Lexington, KY.

James MO, Altman AH, Morris K, Kleinow KM and Tong Z (1997) Dietary modulation of phase 1 and phase 2 activities with benzo[*a*]pyrene and related compounds in the intestine but not in the liver of the channel catfish, *Ictalurus punctatus. Drug Metab Dispos* **25:**346-354.

James MO, Tong Z, Rowland-Faux L, Venugopal CS and Kleinow KM (2001) Intestinal bioavailability and biotransformation of 3-hydroxybenzo[a]pyrene in an isolated perfused preparation from channel catfish, *Ictalurus punctatus*. *Drug Metab Dispos* **29:**721-728.

Jarman WM, Simon M, Norstrom RJ, Burns SA, Bacon CA, Simonelt BRT and Risebrough RW (1992) Global distribution of Tris(4-chlorophenyl)methanol in high trophic level birds and mammals. *Environ Sci Technol* **26:**1770-1774.

Kester MH, Bulduk S, Tibboel D, Meinl W, Glatt H, Falany CN, Coughtrie MW, Bergman A, Safe SH, Kuiper GG, Schuur AG, Brouwer A and Visser TJ (2000) Potent inhibition of estrogen sulfotransferase by hydroxylated PCB metabolites: a novel pathway explaining the estrogenic activity of PCBs. *Endocrinology* **141:**1897-1900.

Kucklick JR, Struntz WDJ, Becker PR, York GW, O'Hara TM and Bohonowych JE (2002) Persistent organochlorine pollutants in ringed seals and polar bears collected from northern Alaska. *Sci Total Environ* **287:**45-59.

Letcher RJ, Norstrom RJ, Lin S, Ramsay MA and Bandiera SM (1996) Immunoquantitation and microsomal monooxygenase activities of hepatic cytochromes P450 1A and P450 2B and chlorinated contaminant levels in polar bear *(Ursus maritimus*). *Toxicol Appl Pharmacol* **137:**127-140.

Lilienblum W, Platt KL, Schirmer G, Oesch F and Bock KW (1987) Regioselectivity of rat liver microsomal UDP-glucuronosyltransferase activities toward phenols of benzo(a)pyrene and dibenz(a,h)anthracene. *Mol Pharmacol* **32:**173-177

Ma B, Shou M and Schrag ML (2003) Solvent effect on cDNA-expressed human sulfotransferase (SULT) activities in vitro. *Drug Metab Dispos* **31:**1300-1305.

Maervoet J, Covaci A, Schepens P, Sandau CD, and Letcher RJ (2004) A reassessment of the nomenclature of polychlorinated biphenyl (PCB) metabolites. *Environ Health Perspect* **112:**291-294.

Meerman JHN, Sterneborg HMJ and Mulder GJ (1983) Use of pentachlorophenol as long-term inhibitor of sulfation of phenols and hydroxamic acids in the rat in vivo. *Biochem Pharmacol* **32:**1587-1593.

Miners JO, Lillywhite KJ and Birkett DJ (1988a) Kinetic and inhibitor studies of 4-methylumbelliferone and 1-naphthol glucuronidation in human liver microsomes. *Biochem Pharmacol* **37:**665-671.

Miners JO, Lillywhite KJ and Birkett DJ (1988b) In vitro evidence for the involvement of at least two forms of human liver UDP-glucuronosyl transferase in morphine 3-glucuronidation. *Biochem Pharmacol* **37:**2839-2845.

Minh TB, Watanabe M, Tanabe S, Yamada T, Hata J and Watanabe S (2000) Occurrence of tris(4-chlorophenyl)methane, tris(4-chlorophenyl)methanol and some other persistent organochlorine in Japanese human adipose tissue. *Environ Health Perspect* **108:**599-603

Ribeiro O, Kirkby CA, Hirom PC and Millburn P (1986) Reactive intermediates from 3-hydroxybenzo[a]pyrene and its glucuronide. *Carcinogenesis* **7:**481-484.

Sacco JC and James MO (2004) Glucuronidation in the polar bear (*Ursus maritimus*). *Mar Environ Res* **58:**475-479.

Sandau CD and Norstrom RJ (1998) Analysis of hydroxylated metabolites of PCBs (OH-PCBs) in polar bear plasma and human whole blood, in *Proceedings of the Second Biennial International Conference on Chemical Measurement and Monitoring of the Environment, 11-14 May 1998*, pp 405-410 Ottawa, Canada.

Sandau CD, Meerts IA, Letcher RJ, Mcalees AJ, Chittim B, Brouwer A and Norstrom RJ (2000) Identification of 4-hydroxyheptachlorostyrene in polar bear plasma and its

binding affinity to transthyretin: A metabolite of octachlorostyrene? *Environ Sci Technol* **34:**3871-3877.

Sandau CD, Ayotte P, Dewailly E, Duffe J and Norstrom RJ (2002) Pentachlorophenol and hydroxylated polychlorinated biphenyl metabolites in umbilical cord blood plasma of neonates from coastal populations in Quebec. *Environ Health Perspect* **110:**411-417.

Schrader TJ and Cooke GM (2002) Interaction between tris(4-chlorophenyl)methanol and the human androgen receptor in vitro. *Toxicol Lett* **136:**19-24.

Schuur AG, Legger FF, van Meeteren ME, Moonen MJH, van Leeuwen-Bol I, Bergman A, Visser TJ and Brouwer A (1998) In vitro inhibition of thyroid hormone sulfation by hydroxylated metabolites of halogenated aromatic hydrocarbons. *Chem Res Toxicol* **11:**1075-1081.

Shevtsov S, Petrotchenko EV, Pedersen LC and Negishi M (2003) Crystallographic analysis of a hydroxylated polychlorinated biphenyl (OH-PCB) bound to the catalytic estrogen binding site of human estrogen sulfotransferase. *Environ Health Perspect* **111:** 884-888.

Tong Z and James MO (2000) Purification and characterization of hepatic and intestinal phenol sulfotransferase with high affinity for benzo[a]pyrene phenols from channel catfish, *Ictalurus punctatus*. *Arch Biochem Biophys* **376:**409-419.

Tsoutsikos P, Miners JO, Stapleton A, Thomas A, Sallustio BC and Knights KM (2004) Evidence that unsaturated fatty acids are potent inhibitors of renal UDP-glucuronosyltransferases (UGT): kinetic studies using human kidney cortical

microsomes and recombinant UGT1A9 and UGT2B7. *Biochem Pharmacol* **67:**191-199.

Van den Hurk P, Kubiczak GA, Lehmler H-J and James MO (2002) Hydroxylated polychlorinated biphenyls as inhibitors of the sulfation and glucuronidation of 3-hydroxybenzo[a]pyrene. *Environ Health Perspect* **110:**343-348.

Wang L-Q, Falany CN and James MO (2004) Triclosan as a substrate and inhibitor of 3`-phosphoadenosine 5`-phosphosulfate-sulfotransferase and UDP-glucuronosyl transferase in human liver fractions. *Drug Metab Dispos* **32:**1162-1169.

Wang L-Q, Lehmler H-J, Robertson LW, Falany CN and James MO (2005) *In vitro* inhibition of human hepatic and cDNA-expressed sulfotransferase activity with 3-hydroxybenzo[a]pyrene by polychlorobiphenylols. *Environ Health Perspect* **113**:680-687.

Zhang H, Varlamova O, Vargas FM, Falany CN, Leyh TS and Varmalova O (1998) Sulfuryl transfer: the catalytic mechanism of human estrogen sulfotransferase, *J Biol Chem* **273:**10888-10892.

Zhivkov V, Tosheva R and Zhivkova Y (1975) Concentration of uridine diphosphate sugars in various tissues of vertebrates. *Comp Biochem Physiol* **51:**421-424.

Footnotes

a) Part of this work was presented at the 7th International ISSX Meeting, August 29-September 2, 2004, held in Vancouver, Canada.

b) Address correspondence to:

M.O. James

Department of Medicinal Chemistry,

College of Pharmacy

University of Florida, P.O. Box 100485,

Gainesville, FL 32610-0485

United States of America

Email: mojames@cop.ufl.edu

Legends for figures

Figure 1. Structures of xenobiotic substrates investigated in this study. (1) 3-OH-B[a]P, (2) triclosan, (3) 4´-OH-PCB79, (4) 4´-OH-PCB159, (5) 4´-OH-PCB165, (6) OHMXC, (7) TCPM, (8) PCP. The OH-PCBs were named as PCB metabolites, according to the convention suggested by Maervoet et al. (2004). Full names of each compound are given in the text.

Figure 2. a) Sulfonation of 3-OH-B[a]P at PAPS = 0.02 mM. Each data point represents the average of duplicate assays for each bear, while the error bars represent the standard deviation. The line represents the best fit to the data of equation (3). b) Kinetic model for partial substrate inhibition of SULT by 3-OH-B[a]P, after Zhang et al. (1998). E refers to SULT.

Figure 3. Eadie-Hofstee plot for the glucuronidation of 10 μM 3-OH-B[a]P, over a UDPGA concentration range of 5-3000 μM. Each data point represents the average of duplicate assays for all bears, while the error bars represent the standard deviation.

Figure 4. Sulfonation of 4´-OH-PCB79, PAPS = 0.02 mM. Each data point represents the average of duplicate assays for each bear, while the error bars represent the standard deviation. The line represents the best fit to equation (4) for 4´-OH-PCB79.

Figure 5. Autoradiogram showing the reverse-phase TLC separation of sulfonation products of OHMXC. Incubations were carried out with the indicated concentrations of OHMXC. The arrow indicates the sulfate conjugate of the OHMXC, while other bands represent unidentified sulfate conjugates formed from endobiotics or other xenobiotics in polar bear liver cytosol. The mobile phase was as described in the methods section.

Figure 6. Autoradiogram showing the reverse-phase TLC separation of sulfonation products from incubations with TCPM using polar bear (P), channel catfish (C), and human (H) liver cytosol in the absence of (0), and presence of 100 μM TCPM (100). The arrow indicates the sulfate conjugate of the substrate, while other bands represent unidentified sulfate conjugates formed from endobiotics or other xenobiotics in liver cytosol. The mobile phase was as described in the methods section.

Figure 7. Autoradiogram showing the reverse-phase TLC separation of sulfonation products of TCPM and the effect of sulfatase treatment. A, incubation in the absence of TCPM (lane 1), and following treatment with sulfatase (lane 2). B, incubation with 200 μM TCPM (lane 3), and following treatment with sulfatase (lane 4). The arrow indicates the sulfate conjugate of the TCPM, while other bands represent unidentified sulfate conjugates formed from endobiotics or other xenobiotics in polar bear liver cytosol.

Figure 8. Autoradiogram showing reverse-phase TLC separation of sulfonation products from the study of PCP kinetics. The arrow indicates the sulfate conjugate of PCP, while other bands represent unidentified sulfate conjugates formed from endobiotics or other xenobiotics in polar bear liver cytosol. The mobile phase was as described in the methods section.

Table 1. Estimated kinetic parameters (Mean ± SD) for (a) sulfonation and (b) glucuronidation of 3-OH-B[a]P by polar bear liver cytosol and microsomes. Values were calculated as described in Materials and Methods.

(a) sulfonation

Substrate	V_{max1} (app) (pmol/min/mg)	K_m (app) (μM)	V_{max1}/K_m (μL/min/mg)	V_{max2} (app) [a] (pmol/min/mg)	K_i (app) (μM)	V_{max2}/K_i (μL/min/mg)
3-OH-B[a]P	500 ± 8	0.41 ± 0.03	1220 ± 70	65.0 ± 20.0	1.01 ± 0.10	66.2 ± 26.8
PAPS	162 ± 35	0.22 ± 0.07	--	--	--	--

(b) glucuronidation

Substrate	V_{max} (app) (nmol/min/mg)	K_m (app) (μM)	V_{max} / K_m (μL/min/mg)
3-OH-B[a]P	3.00 ± 1.18	1.4 ± 0.2	1900 ± 544
UDPGA	1.53 ± 0.56[b], 1.47 ± 0.48[c]	42.9 ± 2.5[b], 200 ± 68[c]	--

[a] constrained variables to obtain best fit

[b] values for high-affinity component

[c] values for low-affinity component

DMD Fast Forward. Published on June 10, 2005 as DOI: 10.1124/dmd.105.004648
This article has not been copyedited and formatted. The final version may differ from this version.

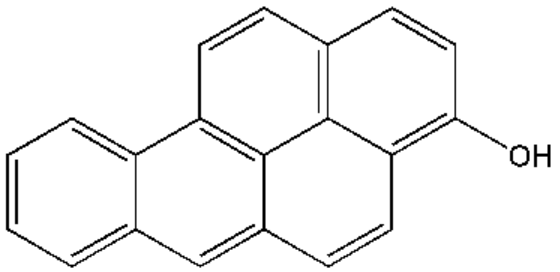
Table 2. Kinetic parameters (Mean ± SD) for the sulfonation of various xenobiotics by polar bear liver cytosol, listed in order of decreasing enzymatic efficiency. All data fit equation (1), except for 4´-OH-PCB79 and PCP, which fit equations (3) and (2) respectively (see Materials and Methods for equations).

Substrate	V_{max} (pmol/min/mg)	K_m (μM)	V_{max} / K_m (μL/min/mg)	K_i (μM)
triclosan	1008 ± 135	11 ± 2	90.8 ± 6.8	-
4´-OH-PCB79	372 ± 38	123 ± 20	3.1 ± 0.3	217 ± 25[a]
OHMXC	51.1 ± 7.8	67 ± 4	0.8 ± 0.1	-
4´-OH-PCB165	8.6 ± 2.0	17 ± 7	0.56 ± 0.17	-
TCPM	62.0 ± 11.2	144 ± 36	0.44 ± 0.06	-
4´-OH-PCB159	14.8 ± 2.3	60 ± 21	0.28 ± 0.12	-
PCP	13.8 ± 1.2	72 ± 14[b]	0.20 ± 0.05	-

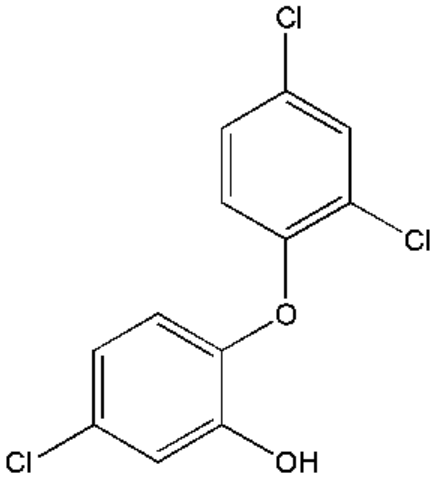
[a] K_i for bears G, K and X were 240, 220 and 190 μM respectively. These values were constrained to obtain the best fit for the data

[b] $S_{50}{}^{h}$; h = 2.0 ± 0.4

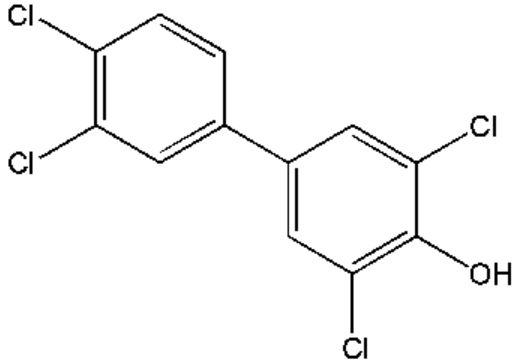
Figure 1



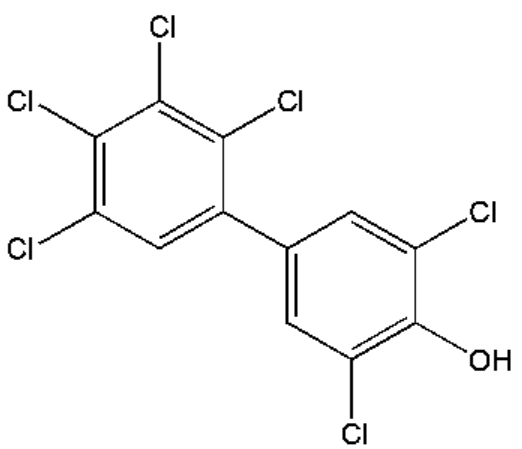
(1)



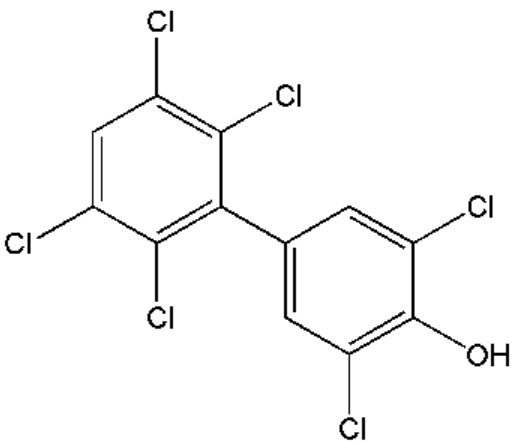
(2)



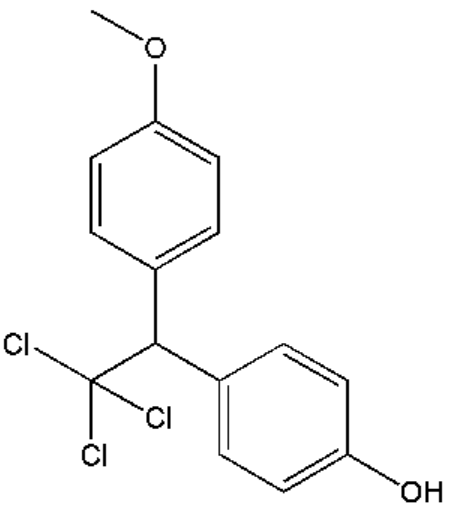
(3)



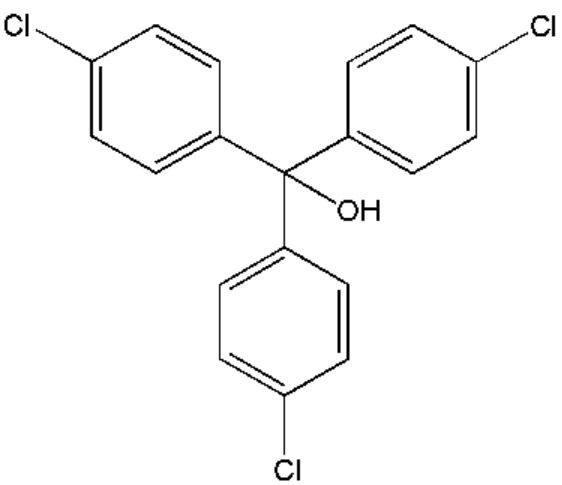
(4)



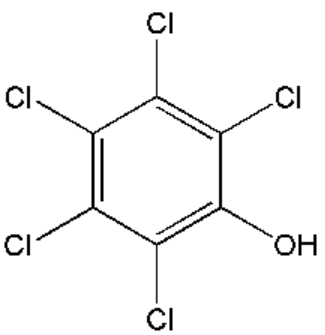
(5)



(6)



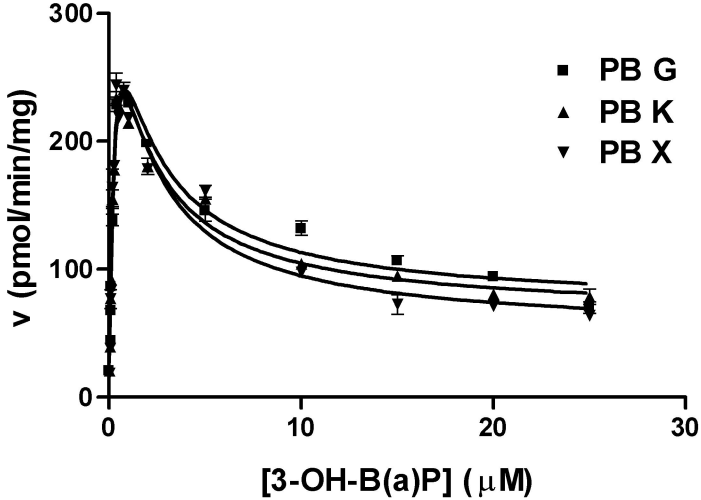
(7)



(8)

Figure 2

(a)


300
200
100
0
v (pmol/min/mg)
0
10
20
30
[3-OH-B(a)P] (μM)
PB G
PB K
PB X

(b)

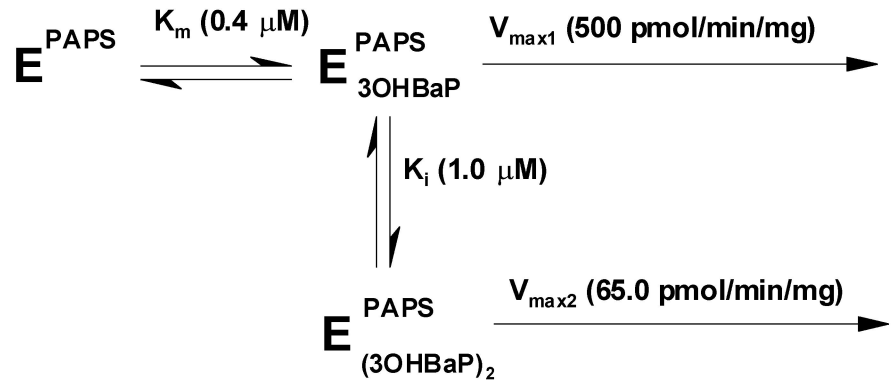

E PAPS
Km (0.4 μM)
E PAPS 3OHBaP
Vmax1 (500 pmol/min/mg)
Ki (1.0 μM)
E PAPS (3OHBaP)2
Vmax2 (65.0 pmol/min/mg)

Figure 3

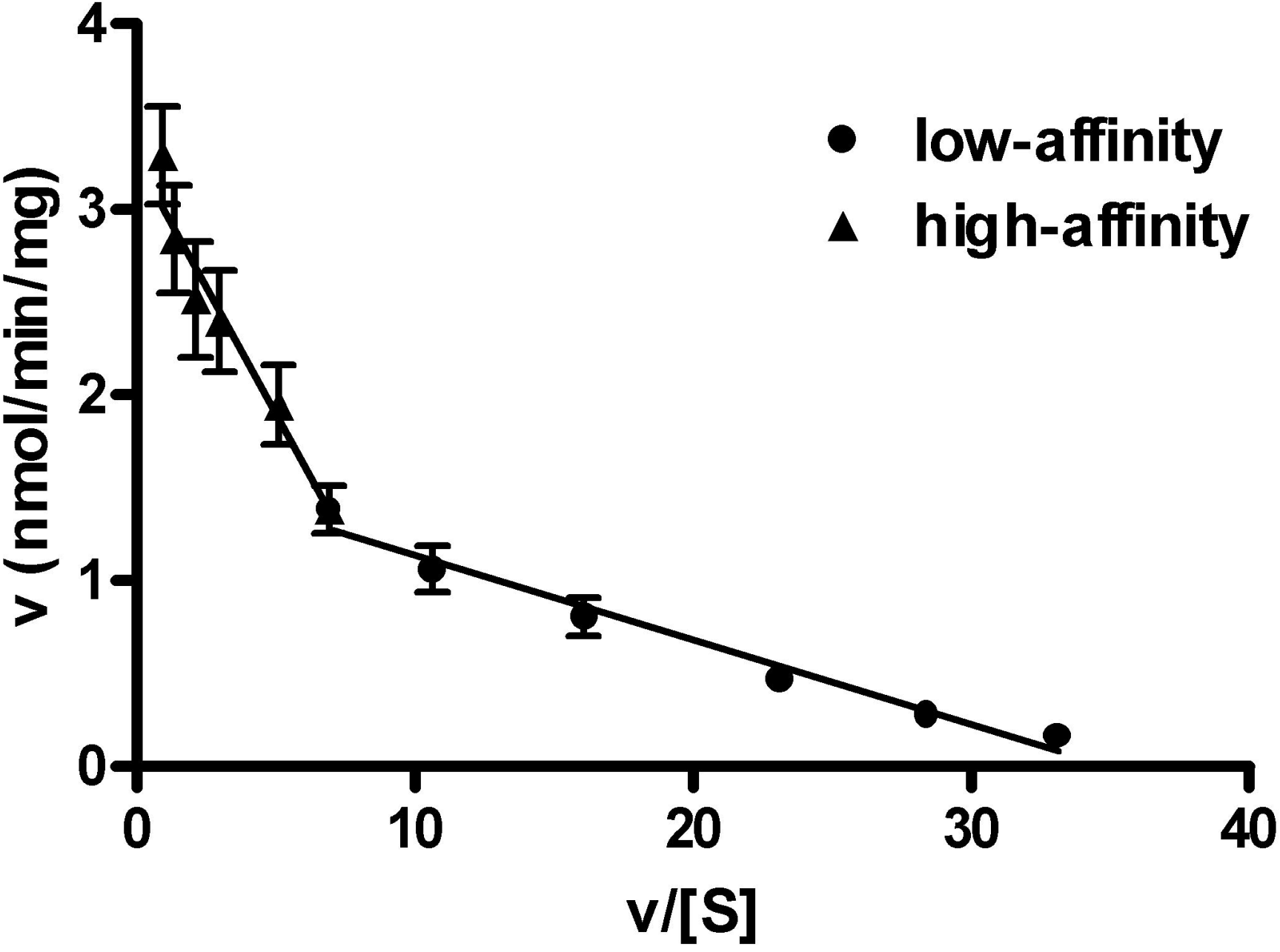


Figure 4

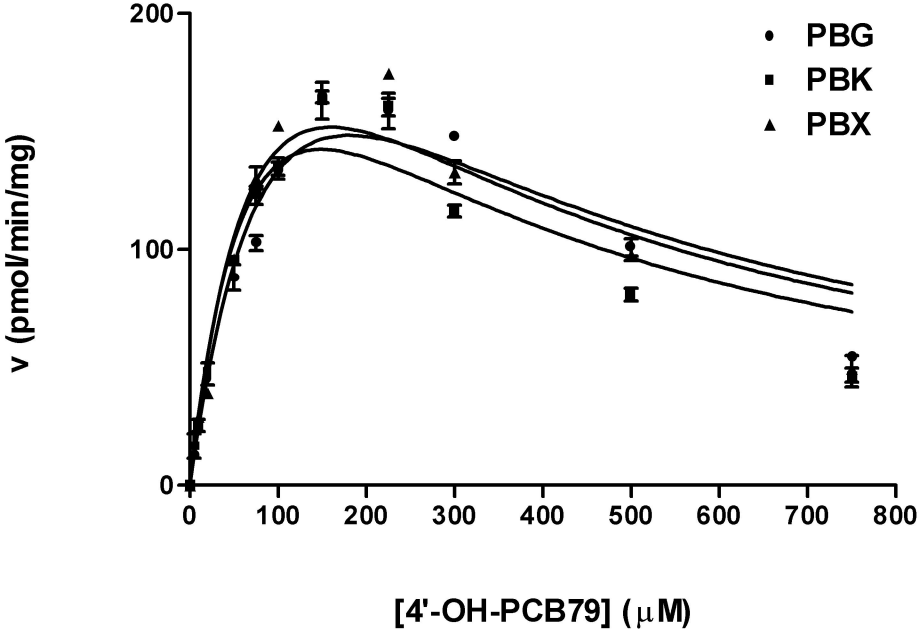

200
100
0
0
100
200
300
400
500
600
700
800
PBG
PBK
PBX
v (pmol/min/mg)
[4'-OH-PCB79] (μM)

Figure 5

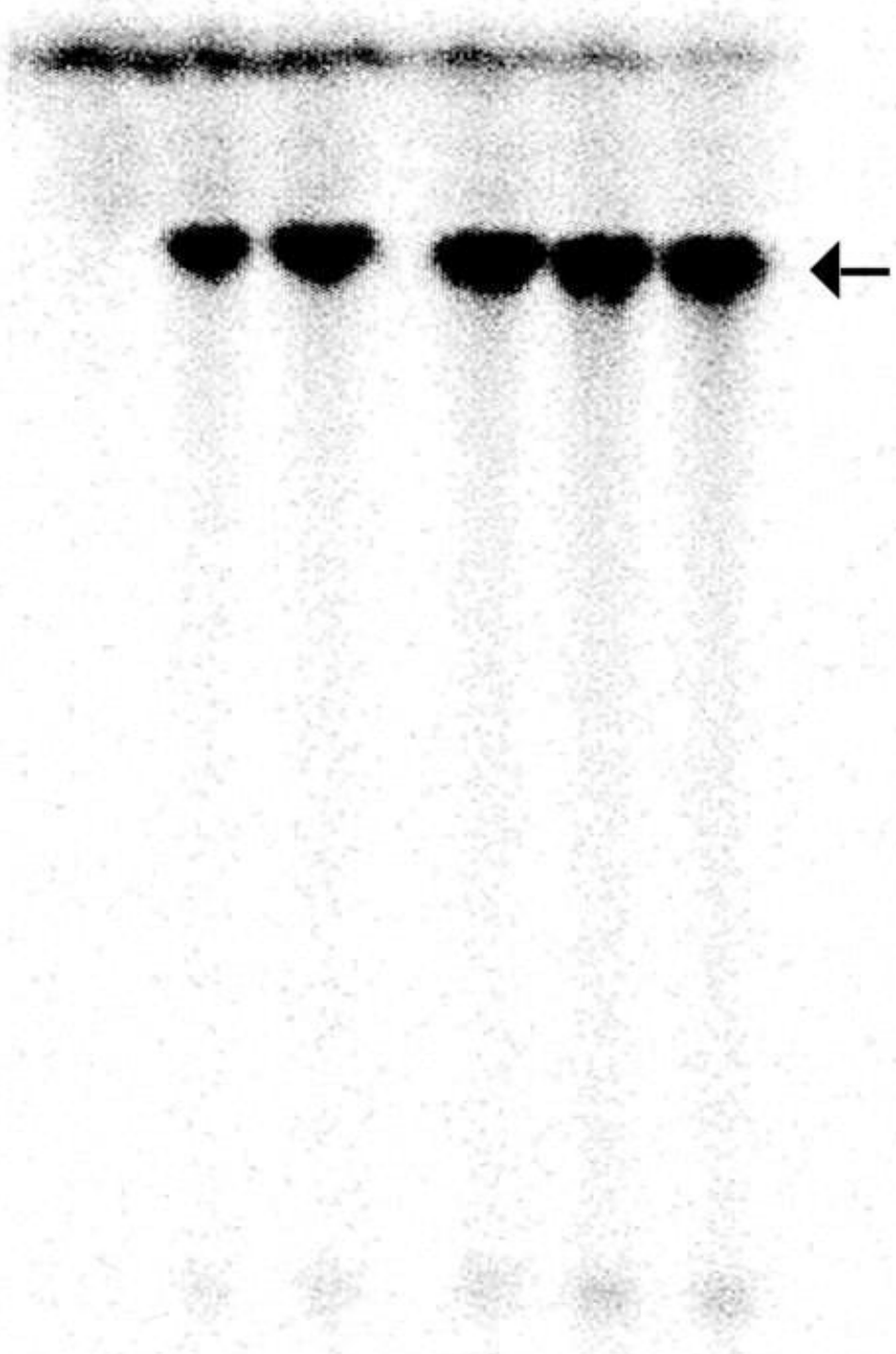


Figure 6

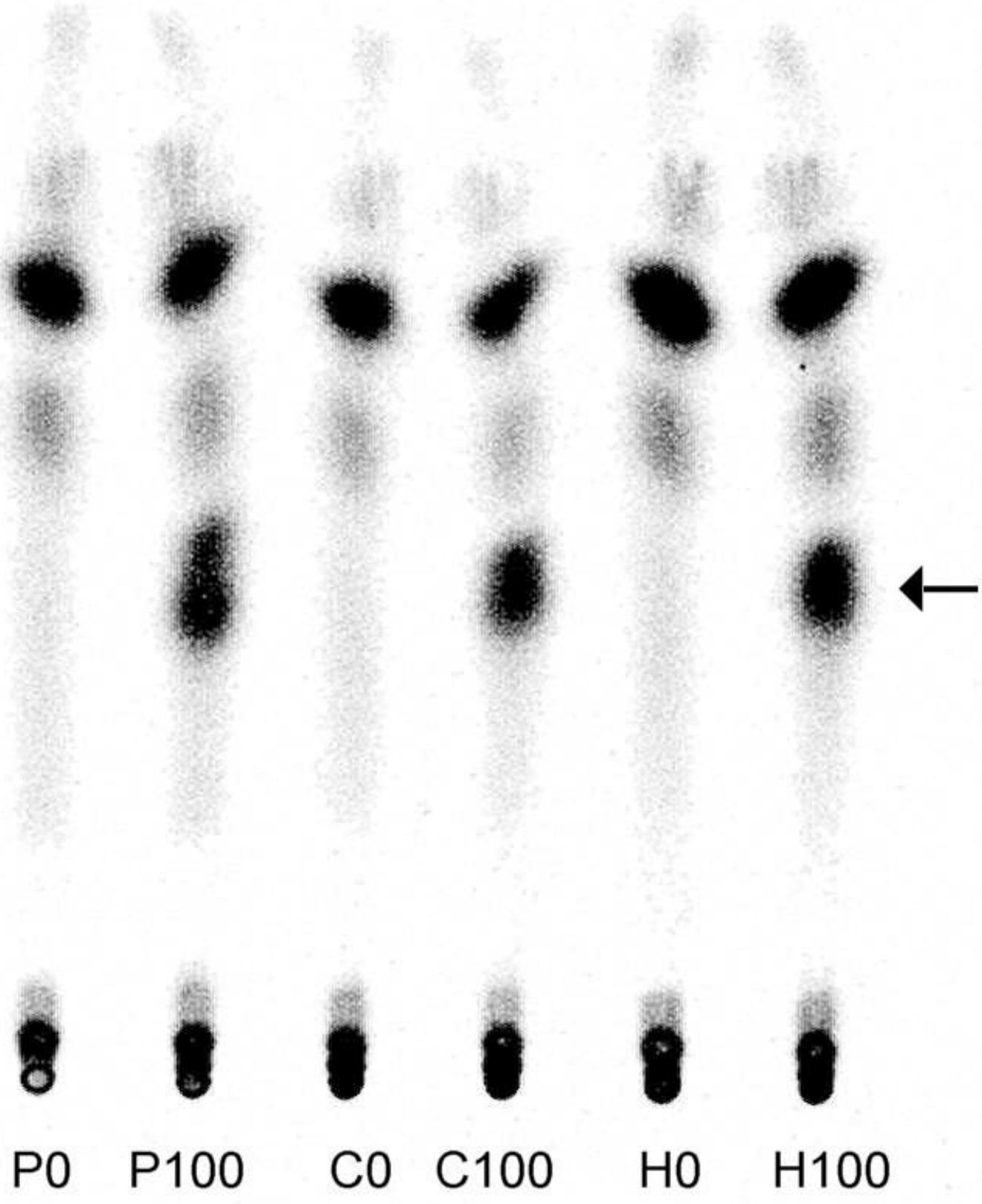


Fig 7

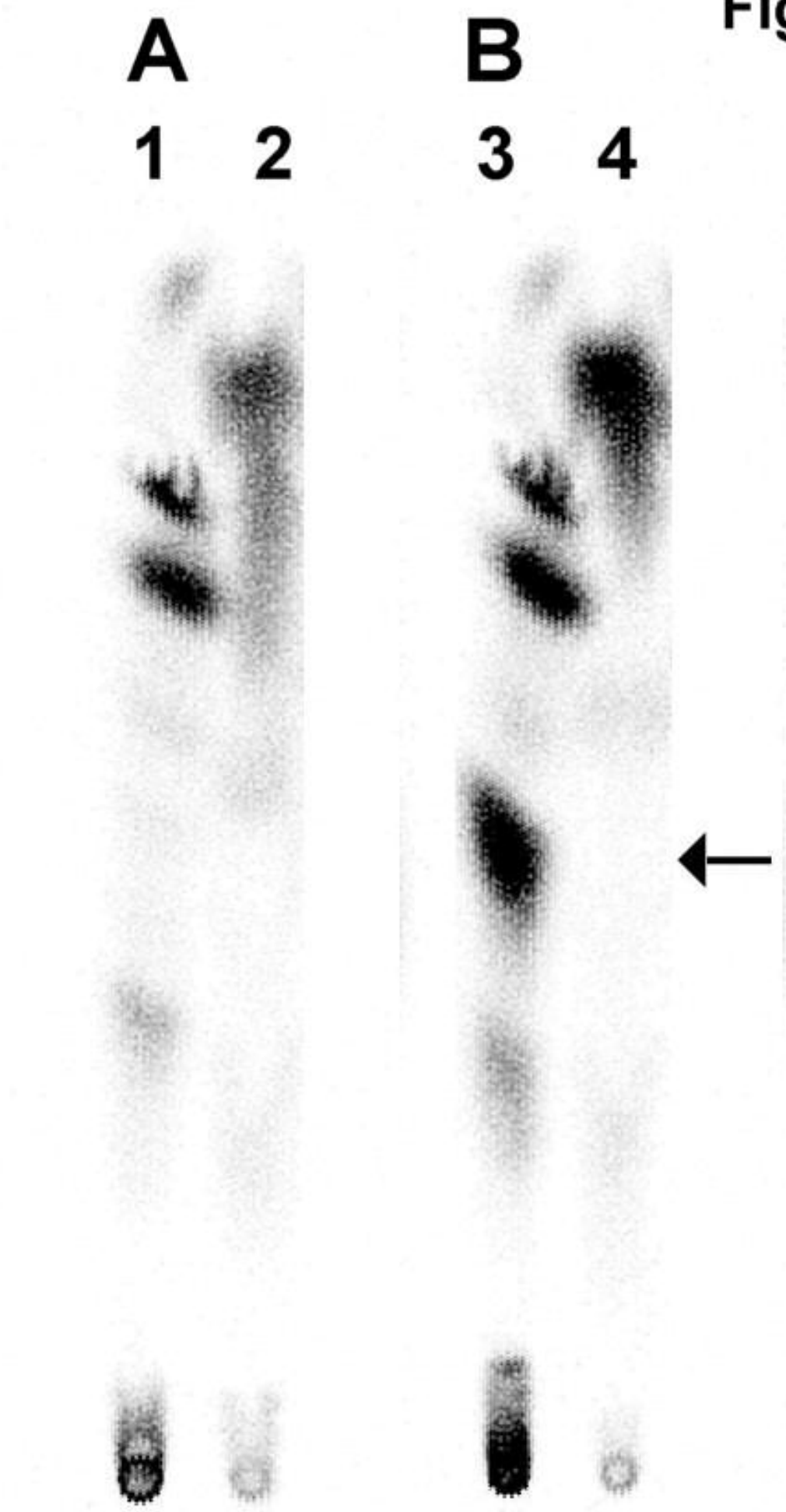


Figure 8