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Sulfate Metabolites of 4-Monochlorobiphenyl in Whole Poplar Plants

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Abstract

4-Monochlorobiphenyl (PCB3) has been proven to be transformed into hydroxylated metabolites of PCB3 (OH-PCB3s) in whole poplar plants in our previous work. However, hydroxylated metabolites of PCBs, including OH-PCB3s, as the substrates of sulfotransferases have not been studied in many organisms including plants *in vivo*. Poplar (*Populus deltoides* × *nigra*, DN34) was used to investigate the further metabolism from OH-PCB3s to PCB3 sulfates because it is a model plant and one that is frequently utilized in phytoremediation. Results showed poplar plants could metabolize PCB3 into PCB3 sulfates during 25 day exposures. Three sulfate metabolites, including 2'-PCB3 sulfate, 3'-PCB3 sulfate and 4'-PCB3 sulfate, were identified in poplar roots and their concentrations increased in the roots from day 10 to day 25. The major products were 2'-PCB3 sulfate and 4'-PCB3 sulfate. However, the concentrations of PCB3 sulfates were much lower than those of OH-PCB3s in the roots, suggesting the sequential transformation of these hydroxylated PCB3 metabolites into PCB3 sulfates in whole poplars. In addition, 2'-PCB3 sulfate or 4'-PCB3 sulfate was also found in the bottom wood samples indicating some translocation or metabolism in woody tissue. Results suggested that OH-PCB3s were the substrates of sulfotransferases which catalyzed the formation of PCB3 sulfates in the metabolic pathway of PCB3.

Introduction

Extensive use of polychlorinated biphenyls (PCBs) in past decades has led to their ubiquity in the environment (1). As a group of persistent organic pollutants with lipophilic properties, PCBs exhibit various toxicities to biota and human beings (2–3). In addition, PCBs can be bioaccumulated and biomagnified in higher trophic levels of the food chain to further cause serious damage in humans and wildlife (4). More importantly, without any known anthropogenic source, the hydroxylated metabolites of PCBs (OH-PCBs) have been detected in many species and habitats (5–9). PCBs have been found to be metabolized to hydroxylated PCBs in reactions catalyzed by cytochrome P450 (CYP) isoforms such as CYP2B and CYP1A (1, 10–11). Furthermore, the concentrations of hydroxylated PCB metabolites found in the blood, liver and other tissues of humans, have reached the same concentration range as those of PCBs (7, 12–13).

Hydroxy-PCBs were expected to be further converted to either the glucuronic acid or the sulfate conjugates via glucuronidation and sulfonation of OH-PCBs by phase II enzymes. Glucuronidation is catalyzed by a family of microsomal enzymes, the UDP-

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glucuronosyltransferases (UGTs) (14–16). Sulfation plays a critical role in conjugation reactions for drugs, environmental chemicals and endogenous compounds by the transfer of a sulfonate group (SO_3^{-1}) from the universal sulfate donor 3'-phosphoadenosine 5'-phosphosulfate (PAPS) to an appropriate acceptor molecule catalyzed by sulfotransferase (SULT) enzymes (17–18). Unfortunately, very little is currently known about the biological properties and metabolic disposition of PCB sulfates.

Up to now, evidence suggested that OH-PCBs had double functions as inhibitors and substrates on SULTs. First, OH-PCBs potentially inhibited the activity of phenol SULTs, which might interfere with sulfation of organic compounds (19–23). Schuur et al. (20) found that selected OH-PCBs exhibited a dose-dependent inhibition of 3,3'-diiodothyronine sulfotransferase activity using male rat liver cytosol. Van den Hurk et al. (21) found that OH-PCBs were potent inhibitors on two conjugating enzymes, a phenol-type sulfotransferase and glucuronosyltransferase. Wang et al. (23) further reported OH-PCBs might inhibit the sulfation rate of those xenobiotics by inhibiting the activity of SULT1A1 in human liver cytosol. Second, there was also evidence that some OH-PCBs were substrates for SULTs. Sacco and James (24) have demonstrated that several OH-PCBs were sulfated by polar bear liver cytosol. They found that OH-PCBs, such as 4'-OH-PCB79, 4'-OH-PCB165 and 4'-OH-PCB159, could be produced as sulfate conjugates in the incubation of liver cytosol or microsomes derived from three adult male polar bears. Liu et al. (25) recently reported that two OH-PCBs, including 4-OH-PCB34 and 4'-OH-PCB68, were substrates for SULT2A1.

Plants contain similar functional enzymes such that sulfation in plants plays an important role in the metabolism of both endogenous chemicals, such as steroids (26) and xenobiotics (27–28). Taking sulfation of xenobiotics in plants as examples, Laurent et al. (27) found that 2,4-dichlorophenoxyacetic acid (2,4-D) herbicide was metabolized to 2,4-dichlorophenol (2,4-DCP) by cotton (*Gossypium hirsutum*). Then, 2,4-DCP was further transformed to produce the glucoside conjugate of 2,4-DCP (2,4-DCP- β -O-glucoside), which could be subsequently sulfated to form 2,4-DCP-(6-O-sulfate) glucoside. Capps et al. (28) also found that the insecticide profenofos yielded 4-bromo-2-chlorophenol followed by conjugation with sugars, which was further sulfated to form glucosylsulfate conjugate of 4-bromo-2-chlorophenol in cotton. However, up to now, there were no reports of PCB sulfates *in vivo* in living organisms. Therefore, it was necessary to use PCB3 as a probe to study the sulfate metabolites of PCBs in living organisms, including plants.

In this paper, the sulfate metabolites of PCB3 were studied to elucidate the further transformation of OH-PCB3s in whole poplar plants. Possible pathways of PCB3 metabolism in whole poplar plants were explored using a newly developed, highly sensitive and selective HPLC-MS method, which can directly determine PCB3 sulfates. In this research, we provided evidence for a metabolic pathway of PCB3 in whole poplar plants based on novel determination of PCB3 sulfates. To our best knowledge, it was the first time that PCB sulfates have been reported *in vivo* in plants or other living organisms.

Experimental Section

Chemicals

Three PCB3 sulfates (2'-PCB3 sulfate, 3'-PCB3 sulfate and 4'-PCB3 sulfate; 98% purity or better) were synthesized using established procedures (29) as the standards.

Stock solutions of 2'-PCB3 sulfate, 3'-PCB3 sulfate and 4'-PCB3 sulfate were prepared in water: acetonitrile (65:35) at 1.0 mg mL^{-1} . Working solutions of 2'-PCB3 sulfate, 3'-PCB3 sulfate and 4'-PCB3 sulfate were prepared by gradual dilution of the stock solution with

water: acetonitrile (65:35). All standard solutions of 2'-PCB3 sulfate, 3'-PCB3 sulfate and 4'-PCB3 sulfate were stored in amber glass vials at 4 °C.

Ion pair reagent dibutylamine acetate (0.5M) was purchased from Sigma-Aldrich. Acetonitrile (HPLC grade) and methanol (HPLC grade) were obtained from Fisher Scientific. The deionized water (18.3MΩ) was from an ultrapure water system (Barnstead International, Dubuque, IA, USA). All other chemicals and reagents used in this experiment were of analytical reagent grade or better.

Hydroponic Exposure

Cuttings of hybrid poplar tree (*Populus deltoides* × *nigra*, DN34) were utilized in exposure experiments after 25 days of growth in Hoagland solution. The preparation of cuttings, their growth, and exposure method were the same as those in previous work (30). In brief, healthy vigorously-growing poplar plants were selected for the PCB3 exposure experiments. The exposure reactors consisted of 500 mL glass conical flasks with a PTFE-faced septum sampling port. All the exposure reactors and de-ionized water for Hoagland nutrient solution were autoclaved prior to the use to decrease influence of microorganisms on the hydroxylation and sulfation of PCB3. Then, 400 mL of Hoagland solution and PCB3 were added into the reactors. The final exposure concentration of PCB3 in each reactor was 1.0 mg L⁻¹ except for the blank poplar control without PCB3. Various "controls" were used to deduce whether it was, indeed, poplar plants that can metabolize PCB3 into sulfate metabolites of PCB3. Blank plant controls (three whole poplar plants without PCB3) were used as contamination controls; Dead plant controls (three wilted, dead whole poplar plants with PCB3) were used as inactive plant controls with active microorganisms; Whole poplar plants (three whole, growing, intact poplar plants with PCB3) were used to test the metabolism of PCB3 in poplar plants. All exposure reactors were wrapped with aluminum foil to protect PCB3 from photolysis and were kept at 23±1°C. The photoperiod was set 16 h per day and autoclaved de-ionized water saturated with oxygen was injected into the reactors to compensate for the transpiration losses. Approximately 60 mL d⁻¹ of water was transpired from each reactor during the experiment.

In order to investigate the translocation and distribution of PCB3 sulfates in different parts of poplar plants and at different times, the exposure time points were set as 10 days, 15 days and 25 days; and poplar plants were divided into root, bottom bark, bottom wood, middle bark, middle wood, top bark, top wood and leaf as described previously (30). The root and leaf samples were ground in liquid nitrogen with a ceramic mortar and pestle. Other parts of the poplar plants were cut into very small pieces to efficiently extract PCB3 sulfates.

Pretreatment

The pretreatment procedure in this work for PCB3 sulfates was as follows. Poplar tissue samples were ultrasonically extracted overnight at 4 °C with 5 mL of methanol/g of sample. The 2 mL of extract was transferred after centrifugation at 3000 rpm for 5 min. The extract was evaporated to dryness under a nitrogen flow and dissolved in 1 mL of water: acetonitrile (65:35) for HPLC-MS analysis after filtration using a 0.4 μm membrane.

Instrumental Analysis

Qualitative and quantitative analysis of 2'-PCB3 sulfate, 3'-PCB3 sulfate and 4'-PCB3 sulfate was performed on HPLC-MS (Agilent 1100 Series LC/MSD) with an autosampler. The separation methods of these three PCB3 sulfates were optimized and compared on the Agilent Zorbax Bonus RP columns (2.1×150 mm, 5 μm), Agilent Zorbax 80A Extended C18 column (2.1×150 mm, 5 μm) and Agilent Zorbax SB-C8 column (3.0×150 mm, 3.5 μm) with a mobile phase flow rate of 0.20 mL min⁻¹ at room temperature. The ratio of

acetonitrile and water (ion pair 5 mM, pH=7.5) in isocratic mobile phase was 35:65. The injection volume was 20 μ L. The electrospray in negative ionization mode of MS (LC-ESI (-)-MS) was utilized and the ion mass detected in SIM was 283. Other analysis parameters of the LC-MS were: fragmentor, 80 V; capillary voltage, 3500 V; gain, 7.00; drying gas flow, 13 L min⁻¹; nebulizer pressure, 35 psi; drying gas temperature, 300 °C.

Results and discussion

Method development of PCB3 sulfates

A sensitive and rapid detection method facilitated the study of sulfate metabolites of PCB3. Sulfate metabolites of PCB3 have the similar ionic properties and high solubility in water so that they could not be separated directly by reversed phase liquid chromatography. Thus, ion pair chromatographic method might be a good option because it has been used to separate sulfate compounds, such as aromatic sulfates (31, 32). However, to date, there were no reports in the literature on the detection of PCB sulfates by ion pair chromatography. Therefore, an ion pair chromatographic method was developed to detect three sulfate metabolites of PCB3 (2'-PCB3 sulfate, 3'-PCB3 sulfate and 4'-PCB3 sulfate) by HPLC-MS.

Some important parameters in this method were optimized to separate these three PCB3 sulfates. First, the effect of concentration of ion pair reagent on the separation of PCB3 sulfates was studied on a Bonus RP column. As shown in Figure 1(A), at 1.0 mM of ion pair reagent, 2'-PCB3 sulfate was baseline separated with 3'-PCB3 sulfate and 4'-PCB3 sulfate. However, 3'-PCB3 sulfate and 4'-PCB3 sulfate could not be separated on the Bonus RP column. When the concentration of ion pair reagent increased from 1.0 mM to 5.0 mM, these three PCB3 sulfates were well separated with satisfactory peak shapes. Therefore, 5.0 mM of ion pair reagent was selected in the following experiment. Second, different chromatographic columns, including C8, C18 and Bonus RP, were used to investigate the separation performance of these three PCB3 sulfates. As shown in Figure 1(B), the chromatographic columns had a great influence on the separation of the three PCB3 sulfates. A C8 column could not separate the three PCB3 sulfates completely and displayed poor peak shapes. Both C18 and Bonus RP columns could completely separate the three PCB3 sulfates with good peak shapes. Considering also that the three PCB3 sulfates showed longer retention times on the Bonus RP, which might decrease the interference with real samples, the Bonus RP column was selected for the experiment.

With the parameters above, these three PCB3 sulfates were completely separated with the elution sequence of 2'-PCB3 sulfate, 3'-PCB3 sulfate and 4'-PCB3 sulfate. This ion pair HPLC-MS method provided good analytical performance for these three sulfate metabolites of PCB3. Linear calibration curves, based on peak areas to concentrations, were obtained in the range of 1.0–20 ng mL⁻¹ for the three PCB3 sulfates with correlation coefficients of 0.9995, 0.9998 and 0.9999 for 2'-PCB3 sulfate, 3'-PCB3 sulfate and 4'-PCB3 sulfate, respectively. The relative standard deviations measured at 10 ng mL⁻¹ level were in the range of 1.0–1.5% (n=5). The calculated detection limits (S/N=3) of this HPLC-MS method for 2'-PCB3 sulfate, 3'-PCB3 sulfate and 4'-PCB3 sulfate were 0.16, 0.27, 0.24 ng mL⁻¹, respectively. Furthermore, the recoveries of these three PCB3 sulfates were calculated using the extraction procedure described above. However, there was a big variation among the different tissues. The recoveries of these three PCB3 sulfates obtained in the root samples with spikes of 100 ng each of 2'-PCB3 sulfate, 3'-PCB3 sulfate and 4'-PCB3 sulfate were 90.7±6.63%, 93.2±5.93% and 88.7±5.23%, respectively. On the other hand, the mass recoveries of spikes of these three PCB3 sulfates obtained in the wood samples (100 ng) of 2'-PCB3 sulfate, 3'-PCB3 sulfate and 4'-PCB3 sulfate were 81.5±1.74%, 79.6±2.71% and 64.1±0.63%, respectively. Lastly, the mass recoveries of these three PCB3 sulfates in the

leaf and bark samples were less than 10% because the matrixes in the bark and leaf samples contain pigments which have a great influence on the extraction and detection of these three PCB3 sulfates. The samples with standard additions were conducted to clarify the influence of the matrix, and results showed significant electrospray-induced ion suppression for bark and leaf samples, but little suppression was determined for root and wood samples. Therefore, PCB3 sulfates in the poplar root and wood samples were the main targets of this experiment analyzed by the HPLC-MS method.

PCB3 sulfates in poplar plants

Hydroxylated metabolites of PCB3 were found in PCB3 exposed poplar plants in our previous work (30, 33). Other researchers also proved that hydroxylated metabolites of PCBs could be further metabolized into PCB sulfates by sulfotransferases (SULT) *in vitro* (23, 24). However, *in vivo* studies of sulfate metabolites of PCB3 were still lacking in whole living organisms, including plants.

Sulfate metabolites of PCB3 were studied in the root and wood samples of PCB3 exposed whole poplars. No PCB3 sulfates were detected in the roots of blank control samples (poplars without PCB3 exposure) which indicated that poplar plants were not contaminated during the experimental process. As seen from Figure 2 (A), 2'-PCB3 sulfate, 3'-PCB3 sulfate and 4'-PCB3 sulfate were detected in the roots of poplar plants exposed to PCB3 on day 25. However, 3'-PCB3 sulfate was not found in the roots on day 10 and day 15. Furthermore, the concentrations of 2'-PCB3 sulfate and 4'-PCB3 sulfate increased from $3.10 \pm 1.43 \text{ ng g}^{-1}$ and $2.07 \pm 0.25 \text{ ng g}^{-1}$ on day 10, to $7.99 \pm 5.45 \text{ ng g}^{-1}$ and $5.12 \pm 1.36 \text{ ng g}^{-1}$ on day 15, to $12.69 \pm 7.49 \text{ ng g}^{-1}$ and $18.85 \pm 12.50 \text{ ng g}^{-1}$ on day 25. Moreover, 3'-PCB3 sulfate was only detected in the roots on day 25 with a low concentration of $2.22 \pm 0.51 \text{ ng g}^{-1}$. All these results provided evidence that sulfate metabolites of PCB3 were produced in whole poplar roots during the 25 day exposure from precursor OH-PCBs – the first report of sulfate metabolites of PCB congeners *in vivo*. The concentrations of these three sulfate metabolites of PCB3 were compared with the related hydroxylated metabolites of PCB3 in the roots on day 10 (30) because sulfate metabolites of PCB3 emanated from the corresponding hydroxylated metabolites. It could be seen from Figure 2(B) that the concentrations of sulfate metabolites of PCB3 had the same proportionality as those of the corresponding hydroxylated metabolites of PCB3 in the roots on day 10 (as seen in Figure 2(A)). As expected, the concentrations of sulfate metabolites of PCB3 were much lower than those of hydroxylated metabolites of PCB3 in the roots on day 10 with the ratios being 1:17.6 and 1:16.3 for 2'-PCB3 sulfate to 2'-OH-PCB3 and 4'-PCB3 sulfate to 4'-OH-PCB3, respectively. Of course, the ratio of sulfate metabolites to the starting compound, OH-PCBs, would be influenced by the formation of other metabolites formed in parallel from OH-PCBs, such as methoxyderivatives.

In addition, sulfate metabolites of PCB3 in the wood samples were also studied to further elucidate the transformation in whole poplar plants. The sulfate metabolites of PCB3 were not detected in the wood samples of blank control poplars without PCB3 exposure which excluded the possibility of background contamination. Nor were they found in the wood samples of dead poplar controls with PCB3 exposure, which indicated that living plant tissues were required for production of the PCB3 sulfates. However, the production of PCB3 sulfates was different in the wood from that in the roots of whole poplar during the same exposure times. It could be seen from Figure 2(C) that the metabolite 2'-PCB3 sulfate was only detected in day 10 bottom wood samples and 4'-PCB3 sulfate was only detected in day 15 and day 25 bottom wood samples. Furthermore, 3'-PCB3 sulfate was never detected in the bottom wood samples at any of the time points, and all three PCB3 sulfates were not detected in the top wood and middle wood samples at any of the time points. Clearly, PCB sulfates were not produced in the top and middle wood of poplar, nor in the shoots or leaves,

nor were they translocated from below. However, unequivocal results from the roots and bottom wood clearly demonstrated that whole poplars could metabolize PCB3 to PCB3 sulfates via OH-PCB3s.

Interestingly, two other mono-PCB3 sulfates were detected in the roots on day 25 besides 2'-PCB3 sulfate, 3'-PCB3 sulfate and 4'-PCB3 sulfate with the same mass weights as 2'-PCB3 sulfate, 3'-PCB3 sulfate and 4'-PCB3 sulfate using SIM mode of LC-MS (Figure 3). The structures of these two unknown PCB3 sulfates could not be unambiguously determined because there are other possible PCB3 sulfate congeners. However, the chromatogram provided preliminary evidence for the metabolism of PCB3 to more PCB3 sulfates *in vivo* in whole plants.

Metabolic pathway of PCB3 sulfates in poplar plants

The sulfonation of endogenous and foreign chemicals, including drugs, toxic chemicals and hormones, was a pervasive biological phenomenon with the transfer of a sulfonate group (SO_3^{-1}) from the universal sulfonate donor 3'-phosphoadenosine 5'-phosphosulfate (PAPS) to an appropriate acceptor molecule by sulfotransferases (17, 18). As a lower chlorinated component of commercial PCB products (34), 4-monochlorobiphenyl (PCB3) was an airborne environmental pollutant (35, 36). Furthermore, PCB3 was easily converted by oxidative enzymes to monohydroxy-PCB3s and further to dihydroxy metabolites (37, 38), which were potentially transferred to PCB sulfates. James (39) summarized the potential pathways for the biotransformation of PCBs, including the metabolic chain from PCBs, via OH-PCBs, to PCB sulfates, by different enzymes.

In this work, three sulfate metabolites of PCB3 were identified in whole poplar plants: 2'-PCB3 sulfate, 3'-PCB3 sulfate and 4'-PCB3 sulfate, with the chlorine and sulfate in a different phenyl ring. Two other potential sulfate metabolites of PCB3 were detected but not yet confirmed. Considering our previous OH-PCB3 data (30), the metabolic pathway from PCB3 to PCB3 sulfates could be easily explained via the intermediate OH-PCB3s. The proposed metabolic pathway from PCB3 to PCB3 sulfates was shown in Figure 4. The parent compound, 4-monochlorobiphenyl, was likely first catalyzed by cytochrome P450 enzymes to yield 2',3'-epoxide and 3',4'-epoxide. These epoxide intermediates then were isomerized to 2'OH-PCB3, 3'OH-PCB3, 4'OH-PCB3. The three OH-PCB3s were likely further metabolized into the corresponding 2'-PCB3 sulfate, 3'-PCB3 sulfate and 4'-PCB3 sulfate by sulfotransferases, which suggested that OH-PCBs were the substrates of sulfotransferases to produce PCB sulfates in whole poplars.

Except for PCBs, other hydroxylated polyhalogenated aromatic hydrocarbons (PHAHs) with similar structures, such as polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans, and polybrominated diphenylethers as the substrate, also have shown sulfation *in vitro* by human enzymes SULT1E1 and SULT1A1 (40). Sulfation of persistent organic pollutants was a common process in living organisms. Plants can take-up and translocate volatile PCBs from air, water and soil. Plants have many of the same functional enzymes as animals and humans, which suggested that the sulfation process might also occur in higher animals. In this research, we have provided evidence for metabolism of PCB3 in whole poplar, via OH-PCB3 intermediates, to form a new class of PCB3 sulfate metabolites.

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Literature Cited

1. Safe SH. Polychlorinated biphenyls (PCBs): environmental impact, biochemical and toxic responses, and implications for risk assessment. *Crit Rev Toxicol.* 1994; 24:87–149. [PubMed: 8037844]
2. Knerr S, Schrenk D. Carcinogenicity of “non-dioxinlike” polychlorinated biphenyls. *Crit Rev Toxicol.* 2006; 36:663–694. [PubMed: 17050081]
3. Kimbrough RD. Polychlorinated biphenyls (PCBs) and human health: an update. *Crit Rev Toxicol.* 1995; 25:133–163. [PubMed: 7612174]
4. Soechitram SD, Athanasiadou M, Hovander L, Bergman A, Jacob Sa PJ. Fetal exposure to PCBs and their hydroxylated metabolites in a dutch cohort. *Environ Health Perspect.* 2004; 112:1208–1212. [PubMed: 15289169]
5. Buckman AH, Wong CS, Chow EA, Brown SB, Solomon KR, Fisk AT. Biotransformation of polychlorinated biphenyls (PCBs) and bioformation of hydroxylated PCBs in fish. *Aquat Toxicol.* 2006; 78:176–185. [PubMed: 16621064]
6. Kunisue T, Tanabe S. Hydroxylated polychlorinated biphenyls (OH-PCBs) in the blood of mammals and birds from Japan: lower chlorinated OH-PCBs and profiles. *Chemosphere.* 2009; 74:950–961. [PubMed: 19054541]
7. Park J, Linderholm L, Charles MJ, Athanasiadou M, Petrik J, Kocan A, Drobna B, Trnovec T, Bergman A, Hertz-Picciotto I. Polychlorinated biphenyls and their hydroxylated metabolites (OH-PCBs) in pregnant women from eastern Slovakia. *Environ Health Perspect.* 2007; 115:20–27. [PubMed: 17366814]
8. Sandanger TM, Dumas P, Berger U, Burkow IC. Analysis of HO-PCBs and PCP in blood plasma from individuals with high PCB exposure living on the chukotka peninsula in the Russian arctic. *J Environ Monit.* 2004; 6:758–765. [PubMed: 15346180]
9. Sandala GM, Sonne-Hansen C, Dietz R, Muir DCG, Valters K, Bennett ER, Born EW, Letcher RJ. Hydroxylated and methyl sulfone PCB metabolites in adipose and whole blood of polar bear (*Ursus maritimus*) from East Greenland. *Sci Total Environ.* 2004; 331:125–141. [PubMed: 15325145]
10. Kaminsky LS, Kennedy MW, Adams SM, Guengerich FP. Metabolism of dichlorobiphenyls by highly purified isozymes of rat liver cytochrome P-450. *Biochemistry.* 1981; 20:7379–7384. [PubMed: 6798990]
11. Matsusue K, Ariyoshi N, Oguri K, Koga N, Yoshimura H. Involvement of cytochrome b5 in the metabolism of tetrachlorobiphenyls catalyzed by CYP2B1 and CYP1A1. *Chemosphere.* 1996; 32:517–523. [PubMed: 8907228]
12. Bergman A, Klasson-Wehler E, Kuroki H. Selective retention of hydroxylated PCB metabolites in blood. *Environ Health Perspect.* 1994; 102:464–469. [PubMed: 8593850]
13. Hovander L, Linderholm L, Athanasiadou M, Athanassiadis I, Bignert A, Faengstroem B, Kocan A, Petrik J, Trnovec T, Bergman A. Levels of PCBs their metabolites in the serum of residents of a highly contaminated area in eastern Slovakia. *Environ Sci Technol.* 2006; 40:3696–3703. [PubMed: 16830529]
14. Tampal N, Lehmler H-J, Espandiari P, Malmberg T, Robertson LW. Glucuronidation of hydroxylated polychlorinated biphenyls (PCBs). *Chem Res Toxicol.* 2002; 15:1259–1266. [PubMed: 12387623]
15. Daidoji T, Gozu K, Iwano H, Inoue H, Yokota H. UDP-glucuronosyltransferase isoforms catalyzing glucuronidation of hydroxy-polychlorinated biphenyls in rat. *Drug Metab Dispos.* 2005; 33:1466–1476. [PubMed: 16006569]
16. Sacco JC, Lehmler HJ, Robertson LW, Li W, James MO. Glucuronidation of polychlorinated biphenyls and UDP-glucuronic acid concentrations in channel catfish liver and intestine. *Drug Metab Dispos.* 2008; 36:623–630. [PubMed: 18180271]
17. Strott CA. Sulfonation and molecular action. *Endocrine Rev.* 2002; 23:703–732. [PubMed: 12372849]

18. Kauffman FC. Sulfonation in pharmacology and toxicology. *Drug Metab Rev.* 2004; 36:823–843. [PubMed: 15554249]
19. Kester MHA, Bulduk S, Tibboel D, Meinel W, Glatt H, Falany CN, Coughtrie MWH, Bergman A, Safe SH, Kuiper GGJM, Schuur AG, Brouwer A, Visser TJ. Potent inhibition of estrogen sulfotransferase by hydroxylated PCB metabolites: a novel pathway explaining the estrogenic activity of PCBs. *Endocrinology.* 2000; 141:1897–1900. [PubMed: 10803601]
20. Schuur AG, Brouwer A, Bergman A, Coughtrie MWH, Visser TJ. Inhibition of thyroid hormone sulfation by hydroxylated metabolites of polychlorinated biphenyls. *Chem-Biol Interact.* 1998; 109:293–297. [PubMed: 9566753]
21. van den Hurk P, Kubiczak GA, Lehmler HJ, James MO. Hydroxylated polychlorinated biphenyls as inhibitors of the sulfation and glucuronidation of 3-hydroxy-benzo[a] pyrene. *Environ Health Perspect.* 2002; 110:343–348. [PubMed: 11940451]
22. Wang L, James MO. Sulfonation of 17 β -estradiol and inhibition of sulfotransferase activity by polychlorobiphenyls and celecoxib in channel catfish, *Ictalurus punctatus*. *Aquat Toxicol.* 2007; 81:286–292. [PubMed: 17239972]
23. Wang L, Lehmler HJ, Robertson LW, James MO. Polychlorobiphenyls are selective inhibitors of human phenol sulfotransferase 1A1 with 4-nitrophenol as a substrate. *Chem Biol Interact.* 2006; 159:235–246. [PubMed: 16413005]
24. Sacco JC, James MO. Sulfonation of environmental chemicals and their metabolites in the polar bear (*Ursus maritimus*). *Drug Metab Dispos.* 2005; 33:1341–1348. [PubMed: 15951448]
25. Liu Y, Apak TI, Lehmler HJ, Robertson LW, Duffel MW. Hydroxylated polychlorinated biphenyls are substrates and inhibitors of human hydroxysteroid sulfotransferase SULT2A1. *Chem Res Toxicol.* 2006; 19:1420–1425. [PubMed: 17112228]
26. Pauli GF, Matthiesen U, Fronczek FR. Sulfates as novel steroid metabolites in higher plants. *Phytochemistry.* 1999; 52:1075–1084.
27. Laurent F, Debrauwer L, Rathahao E, Scalla R. 2,4-Dichlorophenoxyacetic acid metabolism in transgenic tolerant cotton (*Gossypium hirsutum*). *J Agric Food Chem.* 2000; 48:5307–5311. [PubMed: 11087477]
28. Capps TM, Barringer VM, Eberle WJ, Brown DR, Sanson DR. Identification of a unique glycosyl sulfate conjugate metabolite of profenofos in cotton. *J Agric Food Chem.* 1996; 44:2408–2411.
29. Li X, Parkin S, Duffel MW, Robertson LW, Lehmler HJ. An efficient approach to sulfate metabolites of polychlorinated biphenyls. *Environ Intern.* 2010; 36:843–848.
30. Zhai G, Lehmler HJ, Schnoor JL. Hydroxylated metabolites of 4-monochlorobiphenyl and its metabolic pathway in whole poplar plants. *Environ Sci Technol.* 2010; 44:3901–3907. [PubMed: 20402517]
31. Zerbinati O, Ostacoli G. Determination of aromatic sulphonates in surface waters by high-performance liquid chromatography with coupled fluorescence and UV detection. *J Chromatogr A.* 1994; 671:217–223.
32. Altenbach B, Giger W. Determination of benzene- and naphthalenesulfonates in wastewater by solid-phase extraction with graphitized carbon black and ion-pair liquid chromatography with UV detection. *Anal Chem.* 1995; 67:2325–2333.
33. Zhai G, Lehmler HJ, Schnoor JL. New hydroxylated metabolites of 4-monochlorobiphenyl in whole poplar plants. *Chemistry Central Journal.* 2011; 5:87. [PubMed: 22185578]
34. Schulz DE, Petrick G, Duinker JC. Complete characterization of polychlorinated biphenyl congeners in commercial Aroclor and Clophen mixtures by multidimensional gas chromatography-electron capture detection. *Environ Sci Technol.* 1989; 23:852–859.
35. Harrad S, Hazrati S, Ibarra C. Concentrations of polychlorinated biphenyls in indoor air and polybrominated diphenyl ethers in indoor air and dust in Birmingham, United Kingdom: implications for human exposure. *Environ Sci Technol.* 2006; 40:4633–4638. [PubMed: 16913117]
36. Kohler M, Zennegg M, Waeber R. Coplanar polychlorinated biphenyls (PCB) in indoor air. *Environ Sci Technol.* 2002; 36:4735–4740. [PubMed: 12487293]
37. McLean MR, Bauer U, Amaro AR, Robertson LW. Identification of catechol and hydroquinone metabolites of 4-monochlorobiphenyl. *Chem Res Toxicol.* 1996; 9:158–164. [PubMed: 8924585]

38. Song Y, Wagner BA, Lehmler HJ, Buettner GR. Semiquinone radicals from oxygenated polychlorinated biphenyls: electron paramagnetic resonance studies. *Chem Res Toxicol.* 2008; 21:1359–1367. [PubMed: 18549251]
39. James, MO. Polychlorinated biphenyls: metabolism and metabolites. In: Robertson, LW.; Hansen, LG., editors. *PCBs-recent advances in environmental toxicology and health effects*. University of Kentucky Press; Lexington: 2001. p. 35-46.
40. Kester MHA, Bulduk S, van Toor H, Tibboel D, Meinel W, Glatt H, Falany CN, Coughtrie MWH, Schuur AG, Brouwer A, Visser TJ. Potent inhibition of estrogen sulfotransferase by hydroxylated metabolites of polyhalogenated aromatic hydrocarbons reveals alternative mechanism for estrogenic activity of endocrine disrupters. *J Clin Endocrinol Metab.* 2002; 87:1142–1150. [PubMed: 11889178]

A

B

Figure 1. Comparison of PCB3 sulfates at 10 ng mL^{-1} on Bonus RP column using different concentrations of ion pair reagent (IP) (A) and PCB3 sulfates at 10 ng mL^{-1} using different columns (C8, C18 and Bonus RP) (B). Peak sequence is 2'-PCB3 sulfate, 3'-PCB3 sulfate and 4'-PCB3 sulfate.

A

C

B

Figure 2. Comparison of concentration of PCB3 sulfates in poplar roots at different times (n=3) (A), concentration of PCB3 sulfates and OH-PCB3 in poplar roots at day 10 (n=3) (B) and concentration of PCB3 sulfates in poplar wood samples at different times (n=3) (C).

Figure 3. SIM mode of LC-MS and comparison of a poplar root sample on day 25 (A) and the PCB3 sulfates standard at a concentration of 10 ng mL^{-1} each (B). Two unknown PCB3 sulfates in poplar roots (A) were shown with arrows.