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Identification of a novel hydroxylated metabolite of 2,2',3,5',6-pentachlorobiphenyl formed in whole poplar plants

Cunxian Ma¹, Guangshu Zhai², Huimin Wu³, Izabela Kania-Korwel³, Hans-Joachim Lehmler^{1,3}, and Jerald L Schnoor^{1,2,3,*}

¹Interdisciplinary Graduate Program in Human Toxicology, University of Iowa, Iowa City, Iowa 52242, United States

²Department of Civil and Environmental Engineering and IIHR Hydrosience and Engineering, University of Iowa, Iowa City, Iowa 52242, United States

³Department of Occupational and Environmental Health, the University of Iowa, Iowa City, Iowa 52242, United States

Abstract

Polychlorinated biphenyls (PCBs) are a group of persistent organic pollutants consisting of 209 congeners. Oxidation of several PCB congeners to hydroxylated PCBs (OH-PCBs) in whole poplar plants has been reported before. Moreover, 2,2',3,5',6-pentachlorobiphenyl (PCB95), as a chiral congener, has been previously shown to be atropselectively taken up and transformed in whole poplar plants. The objective of this study was to determine if PCB95 is atropselectively metabolized to OH-PCBs in whole poplar plants. Two hydroxylated PCB95s were detected by high performance liquid chromatography-mass spectrometry in the roots of whole poplar plants exposed to racemic PCB95 for 30 days. The major metabolite was confirmed to be 4'-hydroxy-2,2',3,5',6-pentachlorobiphenyl (4'-OH-PCB95) by gas chromatography-mass spectrometry (GC-MS) using an authentic reference standard. Enantioselective analysis showed that 4'-OH-PCB95 was formed atropselectively, with the atropisomer eluting second on the Nucleodex β -PM column (E2-4'-OH-PCB95) being slightly more abundant in the roots of whole poplar plants. Therefore, PCB95 can at least be metabolized into 4'-OH-PCB95 and another unknown hydroxylated PCB95 (as a minor metabolite) in whole poplar plants. Both atropisomers of 4'-OH-PCB95 are formed, but E2-4'-OH-PCB95 has greater atropisomeric enrichment in the roots of whole poplar plants. A comparison with mammalian biotransformation studies indicates a distinctively different metabolite profile of OH-PCB95 metabolites in whole poplar plants. Our observations suggest that biotransformation of chiral PCBs to OH-PCBs by plants may represent an important source of enantiomerically enriched OH-PCBs in the environment.

*Corresponding author: Tel: +1 319 335 5649, jerald-schnoor@uiowa.edu.

Competing interests

The authors declare that they have no competing interests.

Keywords

Polychlorinated biphenyls; Hydroxylated polychlorinated biphenyls; Whole poplars; HPLC-MS; GC-MS; Atropselective

Introduction

Polychlorinated biphenyls (PCBs) are a class of man-made persistent environmental contaminants (Robertson and Hansen 2001), which were recently classified as human carcinogens (Group 1) by the International Agency for Research on Cancer (IARC) (Lauby-Secretan et al. 2013). There are 209 congeners of PCBs with different numbers and substitution patterns of chlorine atoms. Even though they were banned from production decades ago, PCBs are still ubiquitous in the ecosystem (Nouira et al. 2013; Melymuk et al. 2014), including humans (Jotaki et al. 2011). These widely distributed PCBs can be transformed by different species (Sundstrom and Jansson 1975; Buckman et al. 2006; Zhai et al. 2010a). PCBs are also reported to be oxidized to hydroxylated PCBs (OH-PCBs) by cytochromes P450 (CYPs) (McGraw and Waller 2006; Zhai et al. 2013a). These OH-PCBs can be directly excreted in urine (Sato et al. 2012), or further metabolized into dihydroxylated PCBs (Lu et al. 2013), glucuronide conjugates (Sacco et al. 2008) and/or sulfate conjugates (Dhakal et al. 2012; Zhai et al. 2013b).

Of the 209 PCB congeners, nineteen congeners are chiral and form stable rotational isomers that are nonsuperimposable mirror images of each other (Kaiser 1974; Püttmann et al. 1986). Since the two atropisomers of a chiral compound cannot be distinguished by any physical and chemical properties other than their optical rotation, enantioselective biotransformation is of vital importance and interest. The characterization of chiral properties in the environment can provide insight for studying the transportation, distribution, biological transformation and decomposition of PCBs (Lehmle et al. 2010). 2,2',3,5',6-Pentachlorobiphenyl (PCB95), a chiral PCB congener, has been associated with neurodevelopmental disorders (Wayman et al. 2012). *Meta* and *para* hydroxylated derivatives of PCB95 were recently shown to interact differently with the ryanodine receptor (RyR1) and affect calcium signaling pathway in skeletal muscle in a regioisomer-specific manner (Niknam et al. 2013).

The oxidation of PCB95 has been studied in many different life forms. The structures of PCB95 metabolites in the feces of rats and mice or excreta of quails were analyzed by GC-MS (Sundstrom and Jansson 1975). It was suggested that the metabolite profiles are tax-dependent, where *para* hydroxylation in the lower chlorinated ring system is the major metabolite in birds, while *meta* hydroxylation in the higher chlorinated ring is the major metabolite in rats and mice. In addition, cytochrome P450 2B enzymes (CYP2B) are known to stereoselectively metabolize chiral PCBs into OH-PCBs (Warner et al. 2009; Lu and Wong 2011; Kania-Korwel et al. 2011). *In vitro* studies reported that 5-OH-PCB95 was formed atropselectively as the major metabolite in incubations of PCB95 with recombinant rat CYP2B1, rat liver microsomes and liver tissue slices from phenobarbital (CYP2B inducer) pretreated mice (Lu et al. 2013; Kania-Korwel et al. 2011; Wu et al. 2013).

Additional OH-PCB95, including 5-OH-PCB95, 4-OH-PCB95, 4, 5-diOH-PCB95 and an NIH shift derivative 3-OH-PCB103, were identified in female mice (Kania-Korwel et al. 2012). It was found that either 5-OH-PCB95 or 4-OH-PCB95 was the major metabolite depending on the tissue analyzed and dose applied. An unknown hydroxylated metabolite was detected in previous studies, which was assumed to be 3'-OH-PCB95 with oxidation occurring in the lower chlorinated ring system (Kania-Korwel et al. 2011; 2012).

Oxidation of PCBs by cytochrome P450 enzymes can occur via two potential mechanisms: (1) direct insertion of an oxygen atom into an aromatic C-H bond; or (2) formation of an epoxide intermediate which is unstable and rearranges to form OH-PCBs (Robertson and Hansen 2001). For the second mechanism, a phenomenon called NIH shift might take place, which would result in migration of a chlorine atom to the adjacent position on the aromatic ring (Guroff et al. 1967). Based on this information, a maximum of 10 mono-hydroxylated PCB95 could be generated during the biotransformation of PCB95. Theoretically, all these OH-PCB95s would have two atropisomers.

As a model plant in phytoremediation, whole poplar plants (*Populus deltoides* × *nigra*, DN34) have been shown to facilitate the degradation of PCBs (Meggo and Schnoor 2013). In addition, PCBs can be transformed into hydroxylated metabolites and subsequently, sulfate conjugates in whole poplar plants (Liu et al. 2009; Zhai et al. 2010a; 2010b; 2011a; 2013b). Previous work showed that PCB95 can be enantioselectively taken up and translocated in whole poplar plants (Zhai et al. 2011b). A mass difference of 12 ± 2 ng between the two atropisomers of PCB95 (around 1% of total added mass) was observed in plant tissues. Also, the change of atropisomeric fraction during chronic exposure to racemic PCB 95, especially in the middle xylem, was consistent with an enantiospecific biotransformation of PCB95.

However, no studies of the oxidation of PCB95 or the atropisomeric enrichment of OH-PCB95s have been carried out in whole plants, primary producers at the base of the food chain. In this work, two unknown hydroxylated PCB95s were detected by HPLC-MS in the roots of whole poplar plants exposed to PCB95 for 30 days. The more abundant one was identified to be 4'-OH-PCB95 by GC-MS. A subsequent enantioselective analysis of 4'-OH-PCB95 showed greater atropisomeric enrichment of E2-4'-OH-PCB95 in the roots of whole poplar plants.

Experimental

Reagents and Materials

PCBs used in this work, including 2,4,6-trichlorobiphenyl (PCB30), 2,2',3,5',6-pentachlorobiphenyl (PCB95) and 2,2',3,4,4',5,6,6'-octachlorobiphenyl (PCB204), were purchased from AccuStandard (New Haven, CT, USA) with a purity of 99% or better. Authentic 2,2',3,5',6-pentachlorobiphenyl-4-ol (4-OH-PCB95) and 2,2',3,5',6-pentachlorobiphenyl-5-ol (5-OH-PCB95) were synthesized as previously described (Joshi et al. 2011a). The synthesis and characterization of the authentic standard 4'-methoxy-2,2',3,5',6-pentachlorobiphenyl (4'-MeO-PCB95) are described below. The standards for HPLC-MS

were prepared in acetonitrile. The standards for GC-MS were prepared in hexane. All standards were stored in amber glass vials at 4 °C.

Methyl-*tert* butyl ether (MTBE) (HPLC grade), dichloromethane (DCM) (HPLC grade), acetone (pesticide grade), acetonitrile (HPLC grade) and hexane (pesticide grade) were purchased from Fisher Scientific (Pittsburgh, PA, USA). 2-Propanol (>99.5%) and chloroform-*d* (99.8% atom D) were obtained from Sigma-Aldrich (St. Louis, MO, USA). The deionized water (18.3MΩ) was from an ultrapure water system (Barnstead International, Dubuque, IA, USA). Florisil (60–100mesh, Acros Organics) was activated at 450 °C for 12 h (deactivated 1% (w/w) water). All other chemicals and reagents used in this project were of analytical grade or better.

All chemicals for the synthesis of the standard 4'-methoxy-2,2',3,5',6-pentachlorobiphenyl were purchased from commercial sources and used without further purification. Column chromatography was carried out on silica gel (100–200 mesh; Sorbent Technologies, Atlanta, GA, USA). Melting points were determined on a Mel-Temp melting point apparatus and are uncorrected. NMR spectra were measured on a Bruker Avance DRX-400 spectrometer in the University of Iowa Central NMR Research Facility (Iowa City, IA, USA). Chemical shifts are reported in parts per million relative to deuterated chloroform (CDCl₃) (¹H, δ 7.26; ¹³C, δ 77.70) and use tetramethylsilane (TMS) as internal standard.

Synthesis and characterization of 4'-MeO-PCB95

Scheme 1 illustrates the two-step synthesis of 4'-methoxy-2,2',3,5',6-pentachlorobiphenyl.

Scheme 1 Two-step synthesis of 4'-methoxy-2,2',3,5',6-pentachlorobiphenyl, the methylated standard of 4'-OH-PCB95. Reagents and conditions: a) Ag₂SO₄, I₂, DCM, rt, 48 h; b) 2,3,6-trichloroiodobenzene, Cu-Bronze, N₂, 230 °C, 7 days.

Synthesis of 4-iodo-2,5-dichloroanisole

Ag₂SO₄ (916mg) was added to the solution of 2,5-dichloroanisole (433mg) in DCM (30mL), followed by addition of I₂ (727mg) (Joshi et al. 2011b). The reaction mixture was stirred at ambient temperature for 48h, then filtered through celite, decolorized with saturated Na₂S₂O₅, washed by water (2×10mL), dried over Na₂SO₄ and then concentrated. The residue was purified by column chromatography (ethyl acetate/hexane, 1:20, *v/v*) to afford white crystals (720mg). Yield: 97%; Mp 94–95 °C. ¹H NMR (400MHz, CDCl₃) δ/ppm 7.77 (s, 1 H), 7.01 (s, 1 H), 3.88 (s, 3 H); ¹³C NMR (100MHz, CDCl₃) δ/ppm 155.7, 139.7, 137.5, 121.9, 112.9, 85.9, 56.4; Mass spectrum *m/z* (relative abundance %): 302 (100), 287 (75), 259 (60), 160 (20), 97 (50).

Synthesis of 4'-MeO-PCB95

A mixture of 4-iodo-2,5-dichloroanisole (303mg), 2,3,6-trichloroiodobenzene (614mg) and Cu-Bronze (2g) in a sealed ampoule flushed with N₂ was heated in a sand bath at 230 °C for 7 days (Kania-Korwel et al. 2007). The reaction mixture was extracted with boiling DCM (3×10mL). The combined extracts were dried (Na₂SO₄), filtered and the solvent was evaporated under reduced pressure to give a brown viscous oil, purified through silica gel

chromatography to afford a mixture of the desired product and a dechlorinated byproduct. An aliquot of the crude mixture was further purified on a Shimadzu HPLC system on an Eclipse XDB-C18 column (5 μ m, 9.4mm $\times$ 250mm; Agilent, Santa Clara, CA, USA) using CH₃CN/H₂O (65/35) as the eluent, to afford 9.7mg of 4'-methoxy-2,2',3,5',6-pentachlorobiphenyl as a white powder after lyophilization. Yield: 11%. ¹H NMR (400 MHz, CDCl₃) δ /ppm 7.46 (d, J = 8.8Hz, 1 H), 7.36 (d, J = 8.8Hz, 1 H), 7.20 (s, 1 H), 7.07 (s, 1 H), 3.96 (s, 3 H); ¹³C NMR (100MHz, CDCl₃) δ /ppm 156.7, 138.7, 135.2, 134.9, 133.5, 133.0, 132.5, 131.7, 130.0, 129.3, 122.4, 114.2, 57.5; mass spectrum *m/z* (relative abundance %): 356 (M⁺, 100), 313 (50), 241 (40), 120 (15).

Exposure of PCB95

Cuttings of the Imperial Carolina hybrid poplar tree (*Populus deltoides* $\times$ *nigra*, DN34) were used as the model. The exposure procedure was the same as described previously (Zhai et al. 2010a). The final concentration of PCB95 was 0.05mg/L.

Extraction and cleanup of OH-PCB95s

The extraction and cleanup procedures were slightly modified from prior literature (Zhai et al. 2010a). In brief, the roots were ground in liquid nitrogen to very fine powder by a mortar and pestle. The root powder was transferred to different tubes with around 3g in each. Two mL of 37% HCl and 5mL of 2-propanol were added to each sample. After thorough mixing, 10mL/g of hexane/MTBE (1:1, v/v) was added to the sample and shaken overnight at a speed of 250rpm. The extracts containing OH-PCB95s were centrifuged at 5000rpm (Centrifuge: Beckman, Model J2-21M, with rotor PTI F14B-6 $\times$ 250) for 5min to spin down the pellet tissues. The supernatants were then transferred to pre-dried distilling flasks. The extracts were evaporated using a rotary evaporator with water bath temperature at 60 $^{\circ}$ C. After drying, the extracts were dissolved in 1 mL of hexane and transferred to a clean amber glass vial. The rotary evaporator was washed again with 1 mL of hexane and then the hexane was transferred and combined. Then, to each extract 500 μ L of 0.5M NaOH solution (prepared in 50% ethanol) was added and shaken vigorously to mix them. The hexane phase was removed after separation. Another 2mL of hexane was added into the extract and then removed after separation. The ionic OH-PCB95s in the solution were converted to neutral OH-PCB95s by adding 125 μ L of 2M HCl, and then the OH-PCB95s were re-extracted using 2mL of hexane.

The extracts containing OH-PCB95s were cleaned through a florisil column. The column was prepared using a 15mL pipette with 5.0g of florisil at the bottom and 2.0g of anhydrous sodium sulfate on the top. Three mL of dichloromethane/hexane/methanol (50:20:30) was used to activate the column and another 12mL was applied to elute OH-PCB95s. The eluate was evaporated to dryness under a flow of nitrogen and dissolved in 2mL of hexane. The OH-PCB95s dissolved in the hexane were carefully transferred to another clean glass vial. After evaporation under a gentle flow of nitrogen, 1mL of acetonitrile was applied to dissolve the OH-PCB95s for HPLC-MS analysis.

HPLC-MS analysis

HPLC-MS (Agilent 1100 Series LC/MSD) was used to perform the analysis of the samples. The OH-PCB95s were separated on an Agilent Zorbax 80A Extended C18 column (2.1 × 150mm, 5μm) with a mobile phase flow rate of 0.200mL/min at room temperature. The ratio of acetonitrile and water in the mobile phase was 65:35. The injection volume was 20μL. The electrospray in negative ionization mode of MS (LC-ESI (-)-MS) was utilized and the ion mass detected in the SIM mode was 341. Other analysis parameters of the LC-MS were: fragmentor, 115V; capillary voltage, 3500V; gain, 7.00; drying gas flow, 10L/min; nebulizer pressure, 35psi; and drying gas temperature, 250 °C.

Enantioselective analysis of OH-PCB95 was carried out by HPLC-MS (Agilent 1100 Series LCMSD) with chiral column Nucleodex β-PM (4mm × 200mm, 5μm, Macherey-Nagel, Germany). The injection volume was 20μL. MS electrospray in negative ionization mode (LC-ESI (-)-MS) was applied. The mobile phase was water (A) and acetonitrile (B) using the following gradient conditions (Zhai et al. 2013c): 0 min (0.2 mL/min, A:B=55:45) → 25 min (0.2 mL/min, A:B=55:45) → 26 min (0.2 mL/min, A:B=50:50) → 55 min (0.2 mL/min, A:B=50:50) → 56 min (0.2 mL/min, A:B=45:55) → 60 min (0.2 mL/min, A:B=45:55) → 61 min (0.35 mL/min, A:B=45:55) → 89 min (0.35 mL/min, A:B=45:55) → 90 min (0.35 mL/min, A:B=55:45) → Total 100 min. Other parameters were as follows: SIM ion mass, $m/z = 341$; fragmentor, 100V; capillary voltage, 4000V; gain, 7.00; drying gas flow, 10L/min; nebulizer pressure, 35psi; and drying gas temperature, 250 °C.

GC-MS analysis

The root extracts (OH-PCB95) were derivatized by diazomethane as described previously (Kania-Korwel et al. 2008) and spiked with internal standards (PCB30 and PCB204). Analysis of the methoxylated derivatives of OH-PCB95s were performed on an Agilent 7890A gas chromatograph equipped with a 5975 C mass selective detector (MSD) configured in electron impact (EI) mode. The sample separation was performed on a SLB-5ms (30 m×250 μm×0.25μm) from Sigma-Aldrich, St Louis, MO, USA. In the total ion scan mode, a mass range of m/z 50 to 550 was recorded. Helium with a flow rate of 0.75mL/min was used as carrier gas. The following temperature program was utilized to separate the analytes: 100°C for 1min, then 10 °C/min to 280 °C, held for 10min. The injector temperature, ion source temperature and quadrupole temperature were 250 °C, 230 °C and 150 °C, respectively.

Results and discussion

Detection of hydroxylated PCB95 metabolites by HPLC-MS

Tentative analysis of the samples extracted from the roots of whole poplar plants after 30-day exposure was performed by HPLC-MS. Representative chromatograms of the root extract and a mixture of two authentic standards (i.e., 5-OH-PCB95 and 4-OH-PCB95) are shown in Figure 1.

The data showed that at least two OH-PCB95 metabolites (X-OH-PCB95 and Y-OH-PCB95), other than 5-OH-PCB95 or 4-OH-PCB95, were formed in whole poplar plants, one

of which was highly abundant. This is the first direct evidence for the transformation of PCB95 into hydroxylated metabolites by whole poplars. Since Y-OH-PCB95 was apparently a minor metabolite, its low amount represents a limitation for any structural analysis. Therefore, the investigation in this work was focused on elucidation of the structure of X-OH-PCB95.

Potential structures of the unknown OH-PCB95

As mentioned earlier, oxidation of PCBs could occur through two possible mechanisms: direct insertion of an oxygen atom into an aromatic C-H bond or formation of an epoxide intermediate. Five OH-PCB metabolites could be generated via the direct insertion mechanism as shown in Figure 2. When oxidation occurs via an epoxide intermediate, then ten potential metabolites could be produced as shown in Figure 3. In fact, five of the ten potential structures (4-OH-PCB95, 5-OH-PCB95, 3'-OH-PCB95, 4'-OH-PCB95 and 6'-OH-PCB95) are the same as those formed by direct insertion mechanism. Therefore, a maximum of 10 mono-hydroxylated PCB95 could be formed from PCB95.

Identification of the unknown OH-PCB95 metabolite by GC-MS

The root extract containing X-OH-PCB95 was derivatized by diazomethane, concentrated and injected manually into GC-MS to further characterize the unknown PCB95 metabolite. Authentic standards of 5-MeO-PCB95, 4'-MeO-PCB95 and 4-MeO-PCB95 were analyzed in parallel. To allow the calculation of relative retention time, all GC-MS analyses were performed in the presence of PCB 30 and PCB 204 as internal standards (IS).

Retention times and relative retention times (RRTs) for the unknown OH-PCB95 metabolite (as the corresponding X'-MeO-PCB95 derivative) and the authentic standards of 5-MeO-PCB95, 4'-MeO-PCB95 and 4-MeO-PCB95 are shown in Table 1. The RRTs were calculated as the ratio of the retention time between a peak of interest and an internal standard. As shown in Table 1, the RRTs for 4'-MeO-PCB95 (1.442 and 0.925) and X-MeO-PCB95 (1.443 and 0.926) are consistent in terms of both internal standards of PCB30 and PCB204. In contrast, the RRTs of the other two standards are clearly different from X-MeO-PCB95. This observation indicates that X-OH-PCB95 is the hydroxylated PCB95 metabolite with the hydroxyl substituent in the *para* position of lower chlorinated ring, i.e., 4'-OH-PCB95.

Published retention time orders for methoxylated PCB95 derivatives (and other structurally related PCBs, such as PCB91, 132, 136, 149) provide additional, albeit indirect, evidence that the unknown X-OH-PCB95 metabolite is indeed 4'-OH-PCB95. Specifically, *meta* methoxylated PCB95 derivatives have a shorter gas chromatographic retention time compared to the respective *para* methoxylated metabolites (Kania-Korwel et al. 2011; 2012; Wu et al. 2013). For example, 5-MeO-PCB95 has a shorter retention time than 4-MeO-PCB95 (Table 1). Moreover, NIH shift metabolites always have significantly shorter retention times than non-NIH shift metabolites (Kania-Korwel et al. 2011; 2012; Wu et al. 2013). While no authentic 3'-MeO-PCB95 standard is currently available, the formation of the corresponding 3'-OH-PCB95 metabolite by rat liver microsomes has been proposed (Kania-Korwel et al. 2011; 2012; Lu et al. 2013). This putative 3'-OH-PCB95 metabolite (as

the corresponding 3'-MeO-PCB95 derivative) has a shorter retention time than 5-MeO-PCB95 under analysis conditions comparable to the present study. Taken together, these findings suggest an elution order of 3'-MeO-PCB95 < 5-MeO-PCB95 < 4'-MeO-PCB95 (X-MeO-PCB95) < 4-MeO-PCB95. The same elution order has also been reported in an earlier *in vivo* study (Sundstrom and Jansson 1975).

The fragmentation pattern of the unknown OH-PCB95 metabolite is identical to that of the authentic compound (Figure 4A and 4B). Specifically, prominent fragments displaying isotope patterns consistent with the fragmentation of a pentachlorinated OH-PCB (i.e., m/z 355.9, 340.9, 312.9, 286.9, 276.9, and 240.9) are observed in the mass spectra of both the unknown methylated OH-PCB95 metabolite and the authentic 4'-MeO-PCB95 standard. These fragments correspond to [M], [M-CH₃], [M-CH₃CO], [M-CH₃CO-C₂H₂], [M-CH₃CO-HCl] and [M-CH₃CO-Cl₂] (Jansson and Sundström. 197; Bergman et al. 1995; Li et al. 2009) and display relative intensities that are comparable to the intensities observed for 4'-MeO-PCB95. Overall, this fragmentation pattern is consistent with PCB derivatives that have a methoxy group in *meta* or *para* position (Figure 4). In contrast, MeO-PCB derivatives of OH-PCB95 metabolites with a hydroxyl group in *ortho* position are expected to display a prominent dibenzofuran ion with [M-CH₃Cl] (m/z 305.9) (Jansson and Sundström. 197; Bergman et al. 1995; Li et al. 2009), which is not observed in the mass spectrum of the unknown metabolites.

Analysis of the atropisomeric enrichment of 4'-OH-PCB95 by HPLC-MS

Atropisomeric enrichment of 4'-OH-PCB95 in the roots sample was examined on chiral column Nucleodex β -PM (4mm $\times$ 200mm, 5 μ m) by HPLC-MS. A representative chromatogram is shown in Figure 5. Although the two atropisomers of 4'-OH-PCB95 were not very well separated under the analytical conditions employed, it was determined quantitatively that E2-4'-OH-PCB95 had a slightly greater enrichment in the roots of whole poplar plants.

Many factors could contribute to an enantiomeric enrichment of E2-4'-OH-PCB95. One likely explanation is that E2-4'-OH-PCB95 was preferentially formed by cytochrome P450 enzymes in the roots of whole poplars. Another potential explanation is that E1-4'-OH-PCB95 was preferentially selected as the substrate for subsequent transformation, perhaps to sulfated metabolites. For example, previous work has shown that hydroxylated PCBs could be further metabolized into PCB-sulfates in whole poplar plants (Zhai et al. 2013b). In addition, a recent study has shown that racemic mono-hydroxylated PCBs can also act as substrates and be enantioselectively transformed into dihydroxylated PCBs by mammalian cytochrome P450 enzymes (Lu et al. 2013). Other factors like an active selective transport process could also result in atropisomeric enrichment of either chiral PCBs or their OH-PCB metabolites.

Comparison of metabolite profiles of OH-PCB95 in different species

An earlier *in vivo* study suggested that *para* hydroxylation in the lower chlorinated ring system was the major metabolism pathway in birds, while *meta* hydroxylation in the higher chlorinated ring was the major metabolism pathway in rats and mice (Sundstrom and

Jansson 1975). Similarly, metabolism studies using rat liver microsomes and disposition studies in mice reported the formation of either 4-OH-PCB95 or 5-OH-PCB95 as the major metabolite (Kania-Korwel et al. 2011; 2012; Wu et al. 2013). In the present study, 4'-OH-PCB95 (*para* hydroxylation) was identified as the major metabolite of PCB95 oxidation in poplar. Therefore, the metabolite profile of PCB95 in whole poplar plants is in contrast to that in rats and mice but consistent with that in birds. These observations suggest that birds (e.g. quails) and plants (e.g., whole poplars) may have similar functions and specificities of cytochrome monooxygenases. As a result, *para* hydroxylation of PCB95 in the lower chlorinated ring is preferred in those organisms. A distinct hydroxylated metabolite profile of PCB77 between animals and plants has been described in previous work (Zhai et al. 2010b).

Conclusions

PCB95 can be metabolized into 4'-OH-PCB95 and another unknown hydroxylated PCB95 in whole poplar plants with 4'-OH-PCB95 being the major metabolite. A comparison with mammal metabolism studies indicates that poplar plants have a significantly different OH-PCB95 metabolite profile than mammals. Moreover, 4'-OH-PCB95 displayed some enantiomeric enrichment in the roots of whole poplar plants, with the second eluting atropisomer being more abundant. Taken together our observations suggest that biotransformation of chiral PCBs to OH-PCBs by plants may represent an important source of enantiomerically enriched OH-PCBs in the environment.

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Figure 1.

Chromatograms showing the two unknown OH-PCB95 metabolites in the roots of whole poplar plants after 30-day exposure (the solid line). The two unknowns are given the names X-OH-PCB95 and -OH-PCB95, respectively. The dashed line represents the chromatogram of two standards (5-OH-PCB95 and 4-OH-PCB95). Chromatograms were recorded in the selective ion monitoring (SIM) mode $m/z=341$) by HPLC-MS as described under Experimental.

Figure 2.
Formation of OH-PCB95s by direct insertion mechanism

Figure 3.
Formation of OH-PCB95s by an epoxide intermediate mechanism (including NIH shift)

Figure 4.

Electron ionization (EI) mass spectra of 4'-MeO-PCB95 (A), X-MeO-PCB95 (B), 5-MeO-PCB95 (C) and 4-MeO-PCB95 (D). The analysis was performed as described under Experimental. The spectra were recorded in total ion current (TIC) mode. The ion with m/z 149 in (A) represents a background signal.

Figure 5. Analysis of relative abundance of the two atropisomers of 4'-OH-PCB95 on chiral column by HPLC-MS. The chromatogram was recorded in the SIM mode ($m/z=341$).

Scheme 1.

Two-step synthesis of 4'-methoxy-2,2',3,5',6-pentachlorobiphenyl, the methylated standard of 4'-OH-PCB95. Reagents and conditions: a) Ag_2SO_4 , I_2 , DCM, rt, 48 h; b) 2,3,6-trichloriodobenzene, Cu-Bronze, N_2 , 230 °C, 7 days.

Table 1

Comparison of relative retention time (RRT) of 5-MeO-PCB95, 4'-MeO-PCB95, X-MeO-PCB95 and 4-MeO-PCB95

Samples	Retention time (min) in an individual injection		Relative Retention Time (RRT)	
	Analytes	IS-PCB30	IS-PCB204	[*] /IS-PCB30
5-MeO-PCB95	19.27	13.44	20.93	1.434
4'-MeO-PCB95	19.35	13.42	20.91	1.442
X-MeO-PCB95	19.37	13.42	20.92	1.443
4-MeO-PCB95	19.45	13.44	20.93	1.448

Note: IS: Internal Standard.

^{*} The retention time of the corresponding analyte from the same individual injection as internal standards.