

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

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Received: 16 February 2015 / Accepted: 7 December 2015 / Published online: 17 December 2015
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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.

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

Responsible editor: Roland Kallenbom

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conjugates (Sacco et al. 2008), and/or sulfate conjugates (Dhakal et al. 2012; Zhai et al. 2013b).

Of the 209 PCB congeners, 19 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 (Lehmler 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 gas chromatography-mass spectrometry (GC-MS) (Sundstrom and Jansson 1975). It was suggested that the metabolite profiles are taxa-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, was 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 ten 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, b, 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 high-performance liquid chromatography-mass spectrometry (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 (18.3 MΩ) was from an ultrapure water system (Barnstead International, Dubuque, IA, USA). Florisil (60–100 mesh, 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.

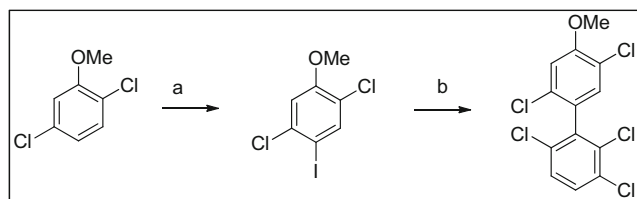
All chemicals for the synthesis of the standard 4'-methoxy-2,2',3,5',6-pentachlorobiphenyl were purchased from commercial sources and used without further purification. Column chromatography was carried out on silica gel (100–200 mesh; Sorbent Technologies, Atlanta, GA, USA). Melting points were determined 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.

Synthesis of 4-iodo-2,5-dichloroanisole

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



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-trichloriodobenzene, Cu-Bronze, N₂, 230 °C, 7 days

Synthesis of 4'-MeO-PCB95

A mixture of 4-iodo-2,5-dichloroanisole (303 mg), 2,3,6-trichloriodobenzene (614 mg) and Cu-Bronze (2 g) 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 × 10 mL). 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 by-product. An aliquot of the crude mixture was further purified on a Shimadzu HPLC system on an Eclipse XDB-C18 column (5 μm, 9.4 × 250 mm; Agilent, Santa Clara, CA, USA) using CH₃CN/H₂O (65/35) as the eluent, to afford 9.7 mg 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 (100 MHz, 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; and 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* × *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 0.05 mg/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 3 g in each. Two milliliter of 37 % HCl and 5 mL of 2-propanol were added to each sample. After thorough mixing, 10 mL/g of hexane/MTBE (1:1, v/v) was added to the sample and shaken overnight at a speed of 250 rpm. The extracts containing OH-PCB95s were centrifuged at 5000 rpm (Centrifuge: Beckman, Model J2-21 M, with rotor PTI F14B-6 × 250) for 5 min 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 °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 μL of 0.5-M NaOH solution (prepared in 50 % ethanol) was added and

shaken vigorously to mix them. The hexane phase was removed after separation. Another 2 mL 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 μL of 2 M HCl, and then, the OH-PCB95s were re-extracted using 2 mL of hexane.

The extracts containing OH-PCB95s were cleaned through a florisil column. The column was prepared using a 15-mL pipette with 5.0 g of florisil at the bottom and 2.0 g of anhydrous sodium sulfate on the top. Three milliliter of dichloromethane/hexane/methanol (50:20:30) was used to activate the column, and another 12 mL was applied to elute OH-PCB95s. The eluate was evaporated to dryness under a flow of nitrogen and dissolved in 2 mL 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, 1 mL 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 \times 150 mm, 5 μm) with a mobile phase flow rate of 0.200 mL/min at room temperature. The ratio of acetonitrile and water in the mobile phase was 65:35. The injection volume was 20 μL . The electrospray in negative ionization mode of MS (LC-ESI (-)-MS) was utilized and the ion mass detected in the selective ion monitoring (SIM) mode was 341. Other analysis parameters of the LC-MS were: fragmentor, 115 V; capillary voltage, 3500 V; gain, 7.00; drying gas flow, 10 L/min; nebulizer pressure, 35 psi; and drying gas temperature, 250 $^{\circ}\text{C}$.

Enantioselective analysis of OH-PCB95 was carried out by HPLC-MS (Agilent 1100 Series LCMSD) with chiral column Nucleodex β -PM (4 \times 200 mm, 5 μm , Macherey-Nagel, Germany). The injection volume was 20 μL . 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) \rightarrow 25 min (0.2 mL/min, A:B=55:45) \rightarrow 26 min (0.2 mL/min, A:B=50:50) \rightarrow 55 min (0.2 mL/min, A:B=50:50) \rightarrow 56 min (0.2 mL/min, A:B=45:55) \rightarrow 60 min (0.2 mL/min, A:B=45:55) \rightarrow 61 min (0.35 mL/min, A:B=45:55) \rightarrow 89 min (0.35 mL/min, A:B=45:55) \rightarrow 90 min (0.35 mL/min, A:B=55:45) \rightarrow total 100 min. Other parameters were as follows: SIM ion mass, m/z =341; fragmentor, 100 V; capillary voltage, 4000 V; gain, 7.00; drying gas flow, 10 L/min; nebulizer pressure, 35 psi; and drying gas temperature, 250 $^{\circ}\text{C}$.

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 was 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-5 ms (30 m \times 250 μm \times 0.25 μm) 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 0.75 mL/min was used as carrier gas. The following temperature program was utilized to separate the analytes: 100 $^{\circ}\text{C}$ for 1 min, then 10 $^{\circ}\text{C}/\text{min}$ to 280 $^{\circ}\text{C}$, held for 10 min. The injector temperature, ion source temperature, and quadrupole temperature were 250, 230, and 150 $^{\circ}\text{C}$, respectively.

Results and discussion

Detection of hydroxylated PCB95 metabolites by HPLC-MS

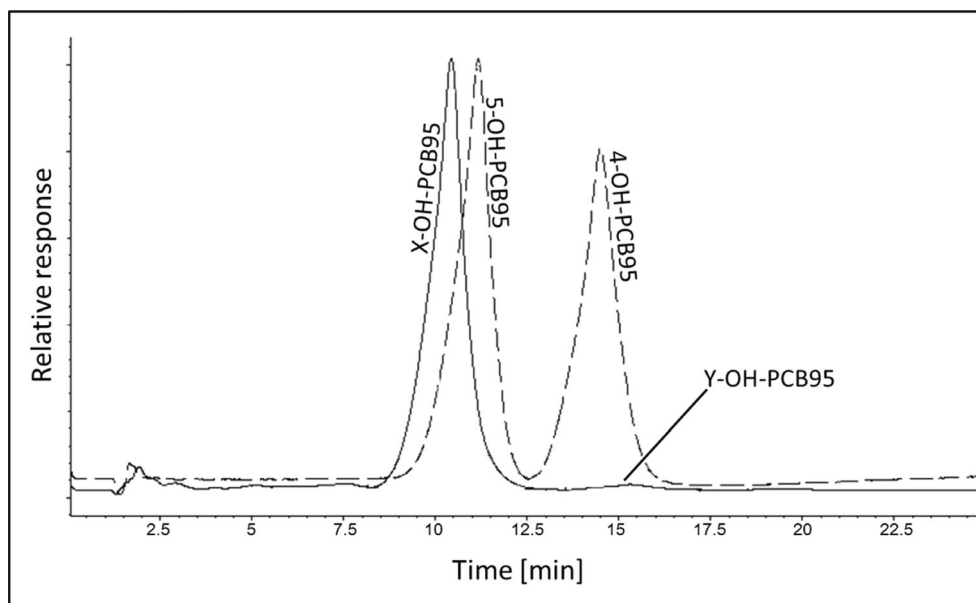
Tentative analysis of the samples extracted from the roots of whole poplar plants after 30-day exposure was performed by HPLC-MS. Representative chromatograms of the root extract and a mixture of two authentic standards (i.e., 5-OH-PCB95 and 4-OH-PCB95) are shown in Fig. 1.

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 Fig. 2. When oxidation occurs via an epoxide intermediate, then ten potential metabolites could be produced as shown in Fig. 3. In fact, five of the ten potential structures (4-OH-PCB95, 5-OH-PCB95,

Fig. 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 Y-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” section



3'-OH-PCB95, 4'-OH-PCB95, and 6'-OH-PCB95) are the same as those formed by direct insertion

mechanism. Therefore, a maximum of ten mono-hydroxylated PCB95 could be formed from PCB95.

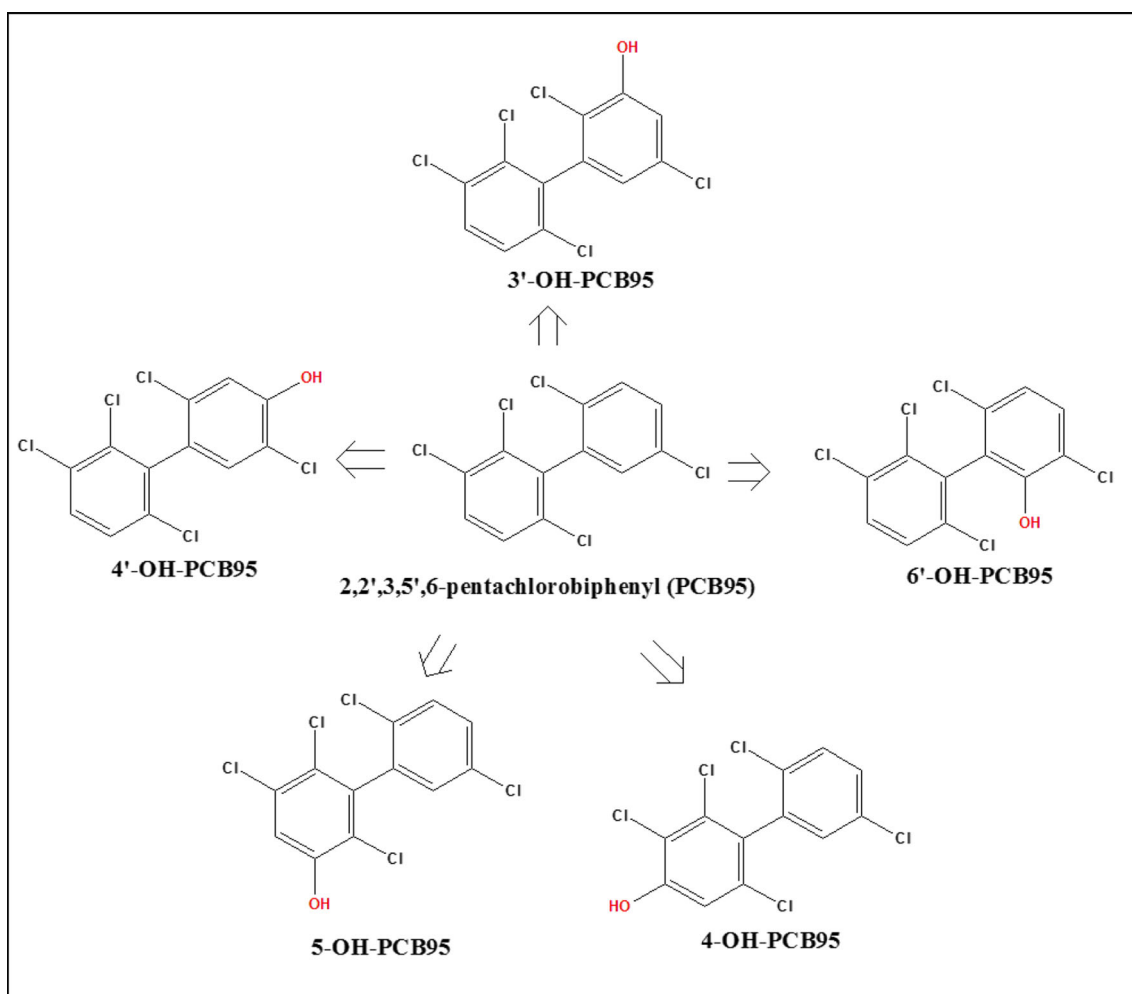


Fig. 2 Formation of OH-PCB95s by direct insertion mechanism

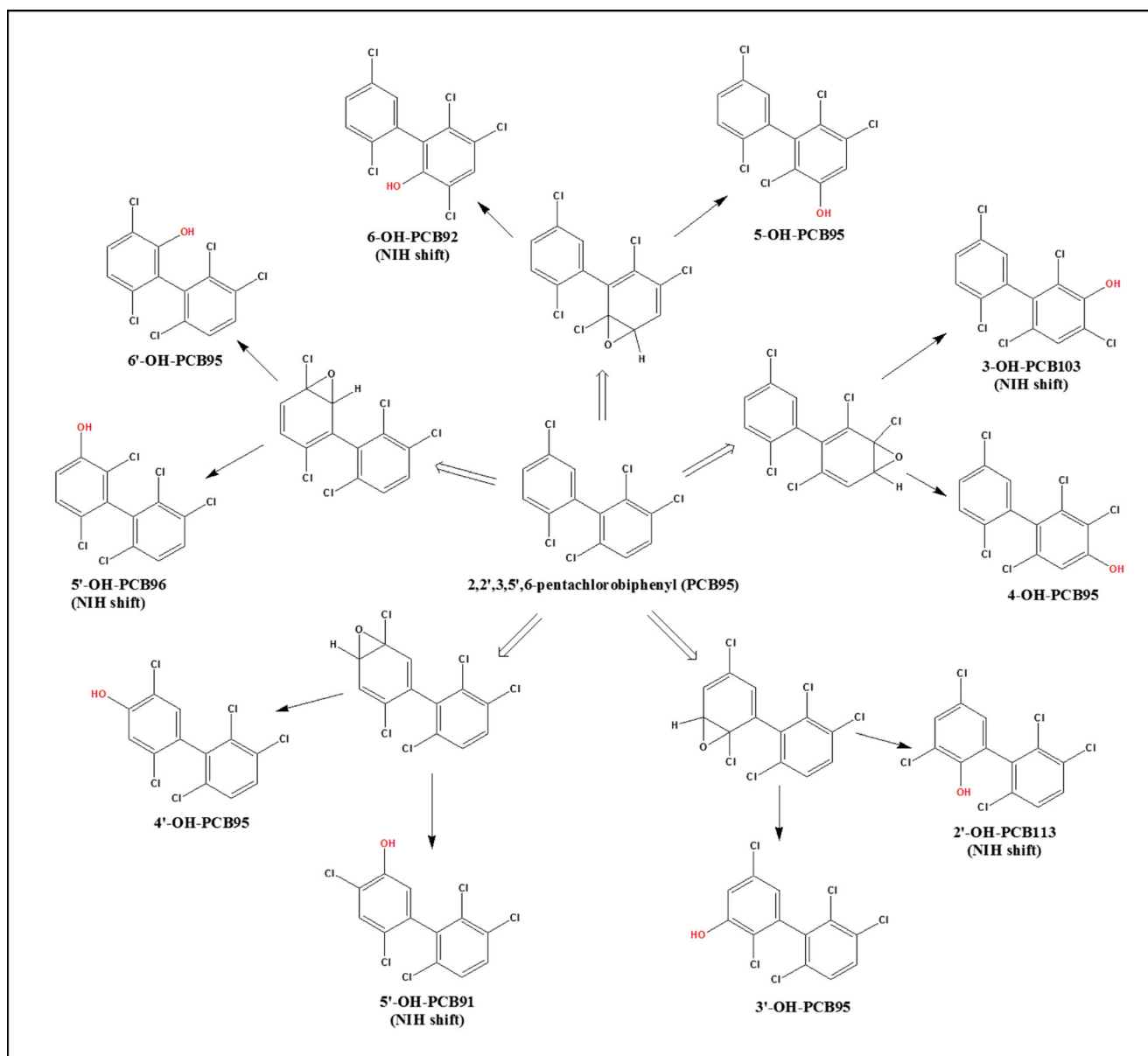


Fig. 3 Formation of OH-PCB95s by an epoxide intermediate mechanism (including NIH shift)

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,

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 ^a	IS-PCB204 ^a
5-MeO-PCB95	19.27	13.44	20.93	1.434	0.921
4'-MeO-PCB95	19.35	13.42	20.91	1.442	0.925
X-MeO-PCB95	19.37	13.42	20.92	1.443	0.926
4-MeO-PCB95	19.45	13.44	20.93	1.448	0.929

IS internal standard

^a The retention time of the corresponding analyte from the same individual injection as internal standards

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 (Fig. 4a b). 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 1974; 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 (Fig. 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 (4 × 200 mm, 5 μm) by HPLC-MS. A representative chromatogram is shown in Fig. 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

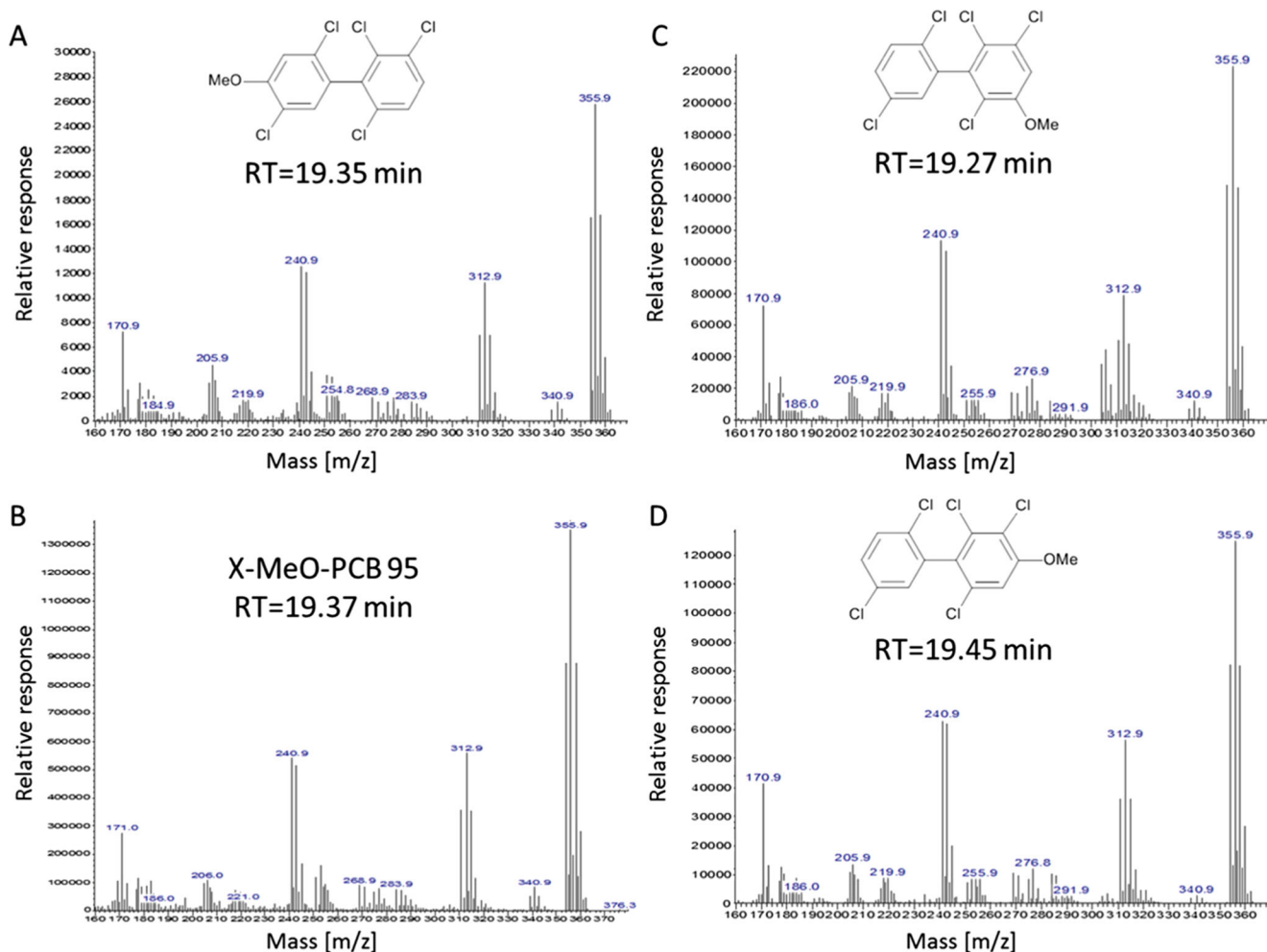


Fig. 4 Electron ionization (EI) mass spectra of 4'-MeO-PCB95 (a), X-MeO-PCB95 (b), 5-MeO-PCB95 (c), and 4-MeO-PCB95 (d). The analysis was performed as described under “Experimental” section. The

spectra were recorded in total ion current (TIC) mode. The ion with m/z 149 in (a) represents a background signal

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).

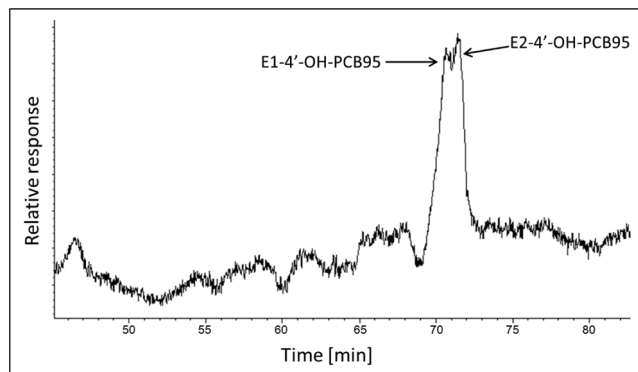


Fig. 5 Analysis of relative abundance of the two atropisomers of 4'-OH-PCB95 on chiral column by HPLC-MS. The chromatogram was recorded in the SIM mode ($m/z=341$)

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.

Acknowledgments We would like to thank Dr. Sudhir N. Joshi (University of Iowa) and Dr. Sandhya M. Vyas (University of Iowa) for the synthesis of several OH-PCB95 standards. This work was supported by the Iowa Superfund Research Program (ISRP), National Institute of Environmental Health Science, Grant Number P42ES013661. We also thank the Center for Global and Regional Environmental Research (CGRER) at the University of Iowa for additional financial support.

Compliance with ethical standards

Competing interests The authors declare that they have no competing interests.

References

- Bergman Å, Wehler EK, Kuroki H, Nilsson A (1995) Synthesis and mass spectrometry of some methoxylated PCB. *Chemosphere* 30: 1921–1938
- Buckman AH, Wong CS, Chow EA, Brown SB, Solomon KR, Fisk AT (2006) Biotransformation of polychlorinated biphenyls (PCBs) and bioformation of hydroxylated PCBs in fish. *Aquat Toxicol* 78:176–185
- Dhakal K, He X, Lehmler HJ, Teesch LM, Duffel MW, Robertson LW (2012) Identification of sulfated metabolites of 4-chlorobiphenyl (PCB3) in the serum and urine of male rats. *Chem Res Toxicol* 25: 2796–2804
- Guroff G, Daly JW, Jerina DM, Renson J, Witkop B, Udenfriend S (1967) Hydroxylation-induced migration: the NIH shift. Recent experiments reveal an unexpected and general result of enzymatic hydroxylation of aromatic compounds. *Science* 157:1524–1530
- Jansson B, Sundström G (1974) Mass spectrometry of the methyl esters of isomeric hydroxychlorobiphenyls—potential metabolites of chlorobiphenyls. *Biomed Mass Spectrom* 1:386–392
- Joshi SN, Vyas SM, Duffel MW, Parkin S, Lehmler HJ (2011a) Synthesis of sterically hindered polychlorinated biphenyl derivatives. *Synthesis* 7:1045–1054
- Joshi SN, Vyas SM, Wu H, Duffel MW, Parkin S, Lehmler HJ (2011b) Regioselective iodination of chlorinated aromatic compounds using silver salts. *Tetrahedron* 67:7461–7469
- Jotaki T, Fukata H, Mori C (2011) Confirmation of polychlorinated biphenyl (PCB) distribution in the blood and verification of simple quantitative method for PCBs based on specific congeners. *Chemosphere* 82:107–113
- Kaiser K (1974) On the optical activity of polychlorinated biphenyls. *Environ Pollut* 7:93–101
- Kania-Korwel I, Shaikh NS, Hornbuckle KC, Robertson LW, Lehmler HJ (2007) Enantioselective disposition of PCB 136 (2,2',3,3',6,6'-hexachlorobiphenyl) in C57BL/6 mice after oral and intraperitoneal administration. *Chirality* 19:56–66
- Kania-Korwel I, Zhao H, Norstrom K, Li X, Hornbuckle KC, Lehmler HJ (2008) Simultaneous extraction and clean-up of polychlorinated biphenyls and their metabolites from small tissue samples using pressurized liquid extraction. *J Chromatogr A* 1214:37–46
- Kania-Korwel I, Duffel MW, Lehmler HJ (2011) Gas chromatographic analysis with chiral cyclodextrin phases reveals the enantioselective formation of hydroxylated polychlorinated biphenyls by rat liver microsomes. *Environ Sci Technol* 45:9590–9596
- Kania-Korwel I, Barnhart CD, Stamou M, Truong KM, El-Komy NONE, Mohammed MH, Lein PJ, Veng-Pedersen P, Lehmler HJ (2012) 2, 2',3,5',6-Pentachlorobiphenyl (PCB 95) and its hydroxylated metabolites are enantiomerically enriched in female mice. *Environ Sci Technol* 46:11393–11401
- Lauby-Secretan B, Loomis D, Grosse Y, El Ghissassi F, Bouvard V, Benbrahim-Tallaa L, Guha N, Baan R, Mattock H, Straif K (2013) Carcinogenicity of polychlorinated biphenyls and polybrominated biphenyls. *Lancet Oncol* 14:287–288
- Lehmler HJ, Harrad SJ, Hühnerfuss H, Kania-Korwel I, Lee CM, Lu Z, Wong CS (2010) Chiral polychlorinated biphenyl transport, metabolism, and distribution: a review. *Environ Sci Technol* 44:2757–2766
- Li X, Robertson LW, Lehmler HJ (2009) Electron ionization mass spectral fragmentation study of sulfation derivatives of polychlorinated biphenyls. *Chem Cent J* 3:5
- Liu J, Hu D, Jiang G, Schnoor JL (2009) *In vivo* biotransformation of 3,3', 4,4'-tetrachlorobiphenyl by whole plants-poplars and switchgrass. *Environ Sci Technol* 43:7503–7509
- Lu Z, Wong CS (2011) Factors affecting phase I stereoselective biotransformation of chiral polychlorinated biphenyls by rat cytochrome P450 2B1 isozyme. *Environ Sci Technol* 45: 8298–8305
- Lu Z, Kania-Korwel I, Lehmler HJ, Wong CS (2013) Stereoselective formation of mono- and dihydroxylated polychlorinated biphenyls by rat cytochrome P450 2B1. *Environ Sci Technol* 47:12184–12192
- McGraw JE Sr, Waller DP (2006) Specific human CYP 450 isoform metabolism of a pentachlorobiphenyl (PCB-IUPAC# 101). *Biochem Biophys Res Commun* 344:129–133
- Meggo RE, Schnoor JL (2013) Cleaning polychlorinated biphenyl (PCB) contaminated garden soil by phytoremediation. *Environ Sci (Ruse)* 1:33–52
- Melymuk L, Robson M, Csiszar SA, Helm PA, Kaltenecker G, Backus S, Bradley L, Gilbert B, Blanchard P, Jantunen L, Diamond ML (2014) From the city to the lake: loadings of PCBs, PBDEs, PAHs and PCMs from Toronto to Lake Ontario. *Environ Sci Technol* 48: 3732–3741
- Niknam Y, Feng W, Cherednichenko G, Dong Y, Joshi SN, Vyas SM, Lehmler HJ, Pessah IN (2013) Structure-activity relationship of selected meta- and para-hydroxylated non-dioxin like polychlorinated biphenyls: from single RyR1 channels to muscle dysfunction. *Toxicol Sci* 136:500–513
- Nouira T, Risso C, Chouba L, Budzinski H, Boussetta H (2013) Polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) in surface sediments from Monastir Bay (Tunisia, Central Mediterranean): occurrence, distribution and seasonal variations. *Chemosphere* 93:487–493
- Püttmann M, Oesch F, Robertson LW, Mannschreck A (1986) Characteristics of polychlorinated biphenyl (PCB) atropisomers. *Chemosphere* 15:2061–2064
- Robertson LW, Hansen LG (2001) Recent advances in environmental toxicology and health effects of PCBs. University Press of Kentucky, Lexington
- Sacco JC, Lehmler HJ, Robertson LW, Li W, James MO (2008) Glucuronidation of polychlorinated biphenyls and UDP-glucuronic acid concentrations in channel catfish liver and intestine. *Drug Metab Dispos* 36:623–630
- Sato N, Yonekubo J, Ezaki T, Suzuki M, Matsumura C, Haga Y, Nakano T (2012) Measurement of accumulation of hydroxylated polychlorinated biphenyl (OH-PCBs) in human urine and blood. *Organohalogen Compd* 74:76–79
- Sundstrom G, Jansson B (1975) The metabolism of 2,2',3,5',6-pentachlorobiphenyl in rats, mice and quails. *Chemosphere* 6:361–370

- Warner NA, Martin JW, Wong CS (2009) Chiral polychlorinated biphenyls are biotransformed enantioselectively by mammalian cytochrome P-450 isozymes to form hydroxylated metabolites. *Environ Sci Technol* 43:114–121
- Wayman GA, Yang D, Bose DD, Lesiak A, Ledoux V, Bruun D, Pessah IN, Lein PJ (2012) PCB-95 promotes dendritic growth via ryanodine receptor-dependent mechanisms. *Environ Health Perspect* 120:997–1002
- Wu X, Duffel MW, Lehmler HJ (2013) Oxidation of polychlorinated biphenyls by liver tissue slices from phenobarbital-pretreated mice is congener-specific and atropselective. *Chem Res Toxicol* 26:1642–1651
- Zhai G, Lehmler HJ, Schnoor JL (2010a) Hydroxylated metabolites of 4-monochlorobiphenyl and its metabolic pathway in whole poplar plants. *Environ Sci Technol* 44:3901–3907
- Zhai G, Lehmler HJ, Schnoor JL (2010b) Identification of hydroxylated metabolites of 3,3',4,4'-tetrachlorobiphenyl and metabolic pathway in whole poplar plants. *Chemosphere* 81:523–528
- Zhai G, Lehmler HJ, Schnoor JL (2011a) New hydroxylated metabolites of 4-monochlorobiphenyl in whole poplar plants. *Chem Cent J* 5:87
- Zhai G, Hu D, Lehmler HJ, Schnoor JL (2011b) Enantioselective biotransformation of chiral PCBs in whole poplar plants. *Environ Sci Technol* 45:2308–2316
- Zhai G, Lehmler HJ, Schnoor JL (2013a) Inhibition of cytochromes P450 and the hydroxylation of 4-monochlorobiphenyl in whole poplar. *Environ Sci Technol* 47:6829–6835
- Zhai G, Lehmler HJ, Schnoor JL (2013b) Sulfate metabolites of 4-monochlorobiphenyl in whole poplar plants. *Environ Sci Technol* 47:557–562
- Zhai G, Wu X, Lehmler HJ, Schnoor JL (2013c) Atropisomeric determination of chiral hydroxylated metabolites of polychlorinated biphenyls using HPLC-MS. *Chem Cent J* 7:183