Title

Effect of Pregnancy on the Disposition of 2,2′,3,5′,6-Pentachlorobiphenyl (PCB 95) Atropisomers and Their Hydroxylated Metabolites in Female Mice

Journal

Chemical Research in Toxicology, 28(9)

ISSN

0893-228X

Authors

Kania-Korwel, Izabela
Barnhart, Christopher D
Lein, Pamela J
et al.

Publication Date

2015-09-21

DOI

10.1021/acs.chemrestox.5b00241

Peer reviewed

Chemical Research in Toxicology

Article

pubs.acs.org/crt

Effect of Pregnancy on the Disposition of 2,2′,3,5′,6-Pentachlorobiphenyl (PCB 95) Atropisomers and Their Hydroxylated Metabolites in Female Mice

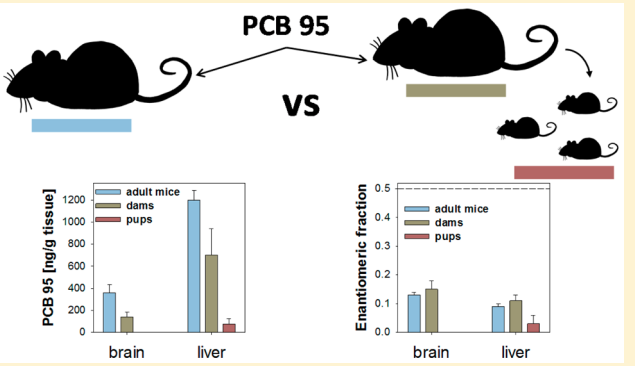
Izabela Kania-Korwel,[†] Christopher D. Barnhart,[‡] Pamela J. Lein,[‡] and Hans-Joachim Lehmler*[,†]

[†]Department of Occupational and Environmental Health, College of Public Health, The University of Iowa, Iowa City, Iowa 52242-5000, United States

[‡]Department of Molecular Biosciences, School of Veterinary Medicine, University of California, Davis, California 95616, United States

Supporting Information

ABSTRACT: Chiral PCBs, such as PCB 95, are developmental neurotoxicants that undergo atropisomeric enrichment in nonpregnant adult mice. Because pregnancy is associated with changes in hepatic cytochrome P450 enzyme activity as well as lipid disposition and metabolism, this study investigates the effect of pregnancy on the maternal disposition of chiral PCBs. Female C57BL/6 mice (8 weeks old) were dosed daily beginning 2 weeks prior to conception and continuing throughout gestation and lactation (56 days total) with racemic PCB 95 (0, 0.1, 1.0, or 6.0 mg/kg body wt/day) in peanut butter. Levels and chiral signatures of PCB 95 and its hydroxylated metabolites (OH-PCBs) were determined in adipose, blood, brain, and liver. Tissue levels of PCB 95 increased 4- to 12-fold with increasing dose, with considerable enrichment of the second eluting atropisomer in all tissues (EF range 0.11 to 0.26). OH-PCBs displayed atropisomeric enrichment in blood and liver but were not detected in adipose and brain. Levels of PCB 95 and its metabolites were 2- to 11-fold lower in pregnant dams relative to those previously reported in nonpregnant age-matched female mice; however, PCB 95 and OH-PCB profiles and chiral signatures were similar between both studies. In contrast, human brain samples contained racemic PCB 95 residues (EF = 0.50). These results demonstrate that changes in cytochrome P450 enzyme activity and lipid disposition during pregnancy reduce the PCB body burden in dams but do not affect metabolite profiles or chiral signatures. The differences in chiral signatures between mice and humans suggest species-specific differences in atropisomeric disposition, the toxicological significance of which remains to be determined.



INTRODUCTION

Polychlorinated biphenyls (PCBs) are persistent organic pollutants that remain a public health concern because of their ongoing, inadvertent production, their environmental persistence, and their presence in human tissues. Humans are exposed to PCBs via the diet[1,2] or dermally in occupational settings.[3] A growing number of studies suggest that inhalation also represents an important route of PCB exposure, especially to lower chlorinated PCBs and PCB congeners with multiple ortho chlorine substituents.[1,4,5] For example, 2,3,6-substituted PCB congeners are present at relatively high concentrations in indoor air in some U.S. schools,[6] and biomonitoring studies detected higher levels of 2,5 and 2,3,6-substituted PCB congeners in school-aged children compared to those in the general U.S. population.[7] Higher levels of PCB 95, a 2,3,6-substituted PCB congener, have also been observed in postmortem brain samples from individuals with a genetic neurodevelopmental disorder compared to those in neurotypical controls.[8] These observations raise human health concerns because experimental studies have implicated these multiple ortho-substituted PCB congeners in developmental neurotoxicity.[9,10]

Several neurotoxic PCB congeners, including PCB 95, are chiral, i.e., they exist as stable rotational isomers that are nonsuperimposable mirror images of each other. Numerous studies have reported a variable degree of atropisomeric enrichment in wildlife, laboratory animals, and humans.[11] *In vitro* studies by several groups including ours suggest that this atropisomeric enrichment is at least partly due to the atropselective metabolism of PCBs to hydroxylated (OH-PCBs) and other metabolites by cytochrome P450 (P450) enzymes.[12−15] In addition, dietary exposure to atropisomerically enriched PCBs can contribute to chiral signatures

Received: June 8, 2015

DOI: 10.1021/acs.chemrestox.5b00241
Chem. Res. Toxicol. XXXX, XXX, XXX−XXX

observed *in vivo*, as has been shown in studies of PCBs' movement through aquatic food webs.[11] Several studies suggest that the atropisomeric enrichment of PCBs and their metabolites have toxicological implications. Specifically, chiral PCBs atropselectively interfere with cellular targets implicated in cellular calcium homeostasis[16,17] and, as we have shown recently, atropselectively affect neuronal connectivity in cultured rat hippocampal neurons.[18]

A number of changes in maternal body composition and physiological processes, such as changes in maternal lipid and lipoprotein plasma concentrations and hepatic P450 enzyme activities, contribute to an altered maternal PCB disposition during pregnancy and lactation in mammals, including humans.[19−24] There is evidence that these physiological changes enantioselectively alter the disposition of chiral drugs, such as bronchodilators[25] or antidepressants.[26] These observations raise the question of whether pregnancy-related physiological changes similarly alter the atropselective maternal disposition of chiral PCBs, thereby exposing the fetus and/or neonate to neurotoxic PCBs with metabolite profiles and chiral signatures that differ from those of PCB-exposed but nonpregnant adult females. Such differences are expected to be toxicologically relevant because PCBs atropselectively affect molecular and cellular targets implicated in PCB developmental neurotoxicity.[16−18]

To address this knowledge gap, the present study investigated the levels and chiral signatures of PCB 95 and its hydroxylated metabolites in pregnant mice exposed daily to racemic PCB 95 beginning 2 weeks prior to mating and continued throughout gestation and lactation. PCB 95 was selected for this study because it is an environmentally relevant PCB congener[11,27] that has been associated with developmental neurotoxicity in experimental studies.[28,29] Considerable enantiomeric enrichment of PCB 95 and its hydroxylated metabolites was observed in dams and their offspring, which is in contrast to racemic signatures of PCB 95 residues present in several postmortem human brain samples.

EXPERIMENTAL PROCEDURES

Caution: *Polychlorinated biphenyls and their metabolites are toxic and should be handled carefully with appropriate personal protective equipment. The derivatization reagent diazomethane is highly toxic and explosive. Its preparation and use should occur under a fume hood and behind a safety shield. All glassware used to handle diazomethane should have fire-polished ends.*

Chemicals. 2,2′,3,5′,6-Pentachlorobiphenyl (PCB 95; 99.7% purity), 2,3,4′,5,6-pentachlorobiphenyl (PCB 117; 99% purity), 2,2′,3,4,4′,5,6,6′-octachlorobiphenyl (PCB 204; 99.9% purity), and 2′,3,3′,4,5,5′-hexachlorobiphenyl-4′-ol (4′-159, 100% purity) were purchased from AccuStandard (New Haven, CT, USA). 3-Methoxy-2,2′,4,5′,6-pentachlorobiphenyl (3-103), 2,2′,3,5′,6-pentachlorobiphenyl-4-ol (4-95), 2,2′,3,5′,6-pentachlorobiphenyl-4′-ol (4′-95), 2,2′,3,5′,6-pentachlorobiphenyl-5-ol (5-95), and 4,5-dimethoxy-2,2′,3,5′,6-pentachlorobiphenyl (4,5-95) were synthesized to >95% purity as described previously.[30,31]

Animal Experiments. Animals were part of a larger developmental neurotoxicity study and were treated humanely and with regard for alleviation of suffering according to protocols approved by the Institutional Animal Care and Use Committee of the University of California, Davis. Female C57BL/6 mice (8 weeks old) were purchased from Charles Rivers Laboratories (Hollister, CA) and dosed daily for a total of 56 days with three different doses of PCB 95 (0.1, 1.0, or 6.0 mg/kg body weight/day [bw/d], which corresponds to 5.6, 56, or 336 mg/kg bw total dose) in peanut butter/peanut oil or vehicle alone according to previously described protocols.[32] After 1 week of acclimatization to the vehicle and 2 weeks of PCB 95 dosing, exposed and control dams were mated over a period of 3 days with congenic male mice. Dosing continued during mating and throughout gestation and lactation. Animals were euthanized on day 56 after beginning PCB 95 dosing. Litters were culled to 7−8 pups on postnatal day 2 (PND 2), weaned at PND 21, and euthanized at PND 30−33. Dams (n = 32) and neonates (n = 38) used for PCB and OH-PCB analysis were euthanized by cervical dislocation followed by thoracotomy. Blood was collected by cardiac puncture and placed into glass vials. Brain, liver, and abdominal adipose tissues were excised, wrapped in aluminum foil, and snap frozen in liquid nitrogen. All tissues were stored at −80 °C until analysis.

Tissue Extraction. The analytical methods were described in detail previously.[32] In short, adipose, brain, and liver tissue samples (tissue masses used for extraction are provided in Table S1, Supporting Information) were extracted by pressurized liquid extraction (ASE 200, Thermo Fisher Sci., Waltham, MA) with hexane/dichloromethane/methanol (48:43:9, v/v/v). Blood samples were denaturated with 2-propanol, followed by liquid−liquid extraction with hexane-methyl-*tert*-butyl ether (1:1, v/v). All extracts were concentrated, derivatized with diazomethane, and further cleaned up with sulfuric acid. Laboratory blank samples, containing only the reagents used in the extraction procedure, and tissues from animals exposed to vehicle were analyzed in parallel as part of the quality control/quality assurance (detection limits are detailed in Table S1, Supporting Information).

Gas Chromatographic Analyses. The quantification of tissue levels of PCB 95 and OH-PCBs (as methylated derivatives) was achieved in a single analysis using Agilent 7870A or Agilent 6890 gas chromatographs equipped with a ^{63}Ni microelectron capture detector and SPB 1 or Equity-1 columns (60 m length × 0.25 mm inner diameter × 0.25 μm film thickness; Supelco, St. Louis, MO, USA). The injector and the detector temperatures were 280 and 300 °C, respectively. The oven temperature was programmed at 50 °C for 1 min, followed by 30 °C/min to 200 °C, 1 °C/min to 260 °C, 10 °C/min to 280 °C, and hold for 2 min. Helium was used as a carrier gas with a flow of 2 mL/min. Levels of PCB 95 and OH-PCBs were determined using PCB 204 as internal standard (volume corrector).[33] Detailed results are presented in Tables S3−S8, Supporting Information.

Enantiomeric fractions (EFs) were determined on the same Agilent systems, using Chirasil-Dex (CD, 2,3,6-tri-*O*-methyl-β-cyclodextrin, 30 m length × 250 μm inner diameter × 0.39 μm film thickness, Agilent, Santa Clara, CA; resolved PCB 95, R_s = 0.67 ± 0.16 and 4,5−95, R_s = 0.56 ± 0.05), ChiralDex B-DM (BDM; 2,3-di-O-methyl-6-*tert*-butylsilyl-β-cyclodextrin, 30 m length × 250 μm inner diameter × 0.12 μm film thickness, Supelco, St. Louis, MO; resolved PCB 95, R_s = 0.75 ± 0.12), or BGB-172 columns (BGB; 20% *tert*-butyldimethyl-silyl-β-cyclodextrin, 30 m length × 250 μm inner diameter × 0.25 μm film thickness, BGB Anlytics, Boecten, Switzerland; resolved 4-95, R_s = 1.1 ± 0.1). Although the atropisomers of 5-95 resolved well on the CD column, determination of its EF value was not possible due to coelution of its second eluting atropisomer with 4-95. The following short temperature program was used to determine EF values of PCB 95 in extracts from adipose and brain tissues: 90 °C for 1 min, 30 °C/min to 160 °C, hold for 20 min, 1 °C/min to 170 °C, hold for 7 min, 1 °C/min to 190 °C, hold for 20 min and 10 °C/min to 200 °C, and hold for 20 min.[34] A longer temperature program was used for blood and liver extracts to simultaneously determine EF values for PCB 95 and its metabolites when their presence was observed during short analyses. The long oven program started at 35 °C, followed by 10 °C/min increase to 140 °C, hold for 420 min, 10 °C/min to 200 °C, and hold for 15 min. The injector and the detector temperatures were set to 250 °C, and the helium flow was 3 mL/min. The enantiomeric fractions were calculated from the formula EF = $A_1/(A_1 + A_2)$, where A_1 and A_2 are the peak area of the first (E_1) and the second (E_2) eluting atropisomers, respectively.

Quality Control/Quality Assurance. Instrument detection limits and detector linear ranges were published previously.[32] The recoveries of the surrogate standards, added before extraction to each sample,

DOI: 10.1021/acs.chemrestox.5b00241
Chem. Res. Toxicol. XXXX, XXX, XXX−XXX

were 100 ± 12% in blood and 104 ± 15% in the remaining tissues for PCB 117 and 93 ± 19% in blood and 80 ± 23% in liver for 4′-159.

Statistical Analysis. All data, unless otherwise specified, are presented as mean ± standard deviation. The differences in tissue levels and EFs between tissue and dose treatment groups were studied using ANOVA with Bonferroni's *posthoc* test. The differences between dams and adult mice (data from age-matched congenic female mice were previously published by Kania-Korwel et al.;[32] summary tables are presented in Tables S3A–S6A, Supporting Information) were tested using two-sample, two-tailed *t*-test, with *p*-values adjusted for multiple tests. The difference of EF from racemic was tested using one-sample *t*-test against the actual value of racemic standard (0.49 ± 0.01 for PCB 95 on BDM and CD columns, 0.50 (n = 2) for 4-95 on BGB column, and 0.49 ± 0.01 for 4,5-95 on CD column). The differences in EF values between tissues from different treatment groups were tested with two-sample, two-tailed *t*-test. The differences were considered to be statistically significant for $p < 0.05$. R software (version 3.0.2; www.r-project.org) was used for statistical analyses.

RESULTS

General Toxicity. Gestational and lactational exposure to PCB 95 did not cause any general toxicity in female C57BL/6 mice. Animals gained on average 7.9 g during the whole study, with an average weight gain of 18 g during pregnancy, close to half of which was lost after giving birth (11 g on average). No treatment-related differences in body weights were observed throughout the study. Similarly, the major organ weights (liver and brain) did not change as a result of PCB treatment at any of the doses investigated (Table S2, Supporting Information).

Tissue Levels of PCB 95. The levels of PCB 95 were determined in adipose, blood, brain, and liver tissues. Adipose tissue had significantly higher levels of the parent compound compared to that in all other tissues, with tissues levels following the rank order adipose ≫ liver > brain > blood (Figure 1 and Table S3A, Supporting Information). Depending on the dose, PCB 95 levels in adipose tissue were 1 to 2 orders of magnitude higher compared to those in the other three tissues investigated. This observation is in agreement with published data in the literature showing that adipose is a storage site for lipophilic compounds like PCBs.[35,36] Moreover, there was a clear, dose-dependent increase in PCB 95 tissue levels that approximately followed the increase in the dose. For example, the 10-fold increase in dose from the low (0.1 mg/kg bw/d) to medium dose (1 mg/kg bw/d) resulted in a 12-fold increase in the PCB 95 level in adipose tissue, an 8-fold increase in brain and liver, and a 5-fold increase in blood. Similarly, the 6-fold increase from the medium to high dose (6 mg/kg bw/d) corresponded to a 6-fold increase in PCB 95 levels in adipose tissue, a 7-fold increase in liver, a 5-fold increase in brain, and a 4-fold increase in blood. When the PCB 95 levels were expressed as a percent of the total dose,[37] we were able to account for only a minor percentage of the total dose, with <0.3% of the total dose being present in the four tissues investigated (data not shown). The adipose tissue contained the highest percent of the total dose (0.24–0.27%).

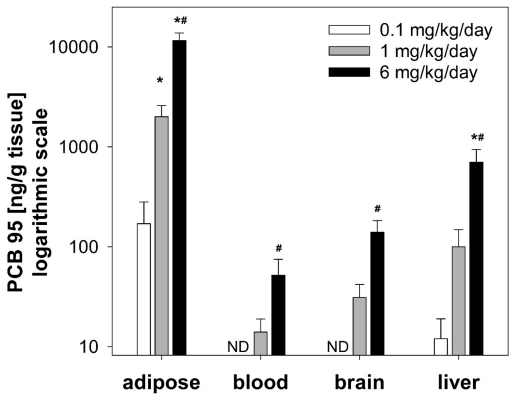


Figure 1. Levels of PCB 95 in tissues from dams exposed to racemic PCB 95 during gestation and lactation increased with increasing dose. Data presented as mean ± standard deviation. *Significantly higher than 0.1 mg/kg bw/d treatment group, $p < 0.05$. #Significantly higher than 1 mg/kg bw/d treatment group. ND, below detection limit; for limits of detection, see Table S1, Supporting Information.

Tissue Levels of Hydroxylated PCB 95 Metabolites. PCB 95 is metabolized by cytochrome P450 enzymes to several hydroxylated metabolites in mice[32] and other mammalian species.[15,38,39] In the present study, the levels of five PCB 95 metabolites, including 3-103 (1,2 shift product of PCB 95),[40] 4-95, 4′-95, 5-95, and 4,5-95, were quantified in tissues. Only three of these metabolites, 4-95, 5-95, and 4,5-95, were detected in blood and liver. Together, these metabolites accounted for approximately 0.02% of the total dose (data not shown). Levels of all OH-PCBs investigated were below the detection limit in brain and adipose tissue samples (for detection limits see Table S1, Supporting Information). Similarly, OH-PCBs were not detected in brain and adipose tissues from an earlier PCB 95 disposition study in adult female mice.[32] In addition, we identified X′-95 in blood and liver, a metabolite previously found in rat liver microsomal incubations[15] and a PCB 95 disposition study in adult female mice;[32] however, this metabolite could not be quantified because an analytical standard was not available. Additional metabolites, which may have been present in tissues based on an earlier study,[39] were not analyzed due to the unavailability of authentic standards.

The sum of the hydroxylated metabolites (∑OH-PCBs) in blood was 4–6 times higher than the concentration of the parent compound (Table S4A, Supporting Information). However, ∑OH-PCB levels in liver were 2–3 times lower than the PCB 95 levels in the liver. Blood ∑OH-PCB levels of the dams were slightly higher than liver levels; however, this difference did not reach statistical significance. Similar to PCB 95 levels, ∑OH-PCB and individual OH-PCB levels increased with increasing dose. Specifically, the 10-fold increase from the low to medium dose resulted in an 8-fold increase in ∑OH-PCB in both blood and liver. The 6-fold increase from the medium to high dose resulted in 3- and 6-fold increases in ∑OH-PCB in blood and liver, respectively.

There was a clear difference in the metabolite profiles between blood and liver in the dams. The levels of hydroxylated metabolites in blood generally followed the order 4-95 ≫ 4,5-95 > 5-95 at all doses (Figure 2 and Table S4A, Supporting Information). 4-95 was also the major metabolite in the liver; however, the levels of the remaining two metabolites followed the order 5-95 > 4,5-95. The 5-95:4-95 ratios differed considerably between both compartments. For example, the 5-95:4-95 ratios in the highest PCB 95 dose group were approximately 0.03 and 0.58 in blood and liver, respectively. Interestingly, the 5-95:4-95 ratios slightly decreased from 0.58 in the high-dose group to 0.50 in the low-dose group.

Atropisomeric Enrichment of PCB 95. PCB 95 and other chiral PCBs undergo atropisomeric enrichment in animal models and humans.[11] To study the extent of the atropisomeric enrichment in dams dosed throughout gestation and lactation with racemic PCB 95, the EF values of PCB 95 in tissues were

DOI: 10.1021/acs.chemrestox.5b00241
Chem. Res. Toxicol. XXXX, XXX, XXX–XXX

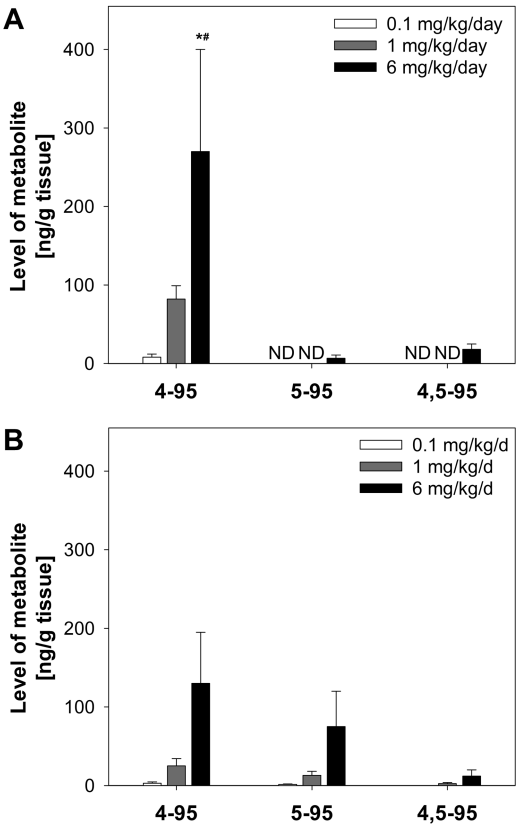


Figure 2. Levels of hydroxylated metabolites of PCB 95 in (A) blood and (B) liver from dams exposed to racemic PCB 95 during gestation and lactation increased with increasing dose. Data presented as mean ± standard deviation. *Significantly higher than 0.1 mg/kg bw/d treatment group, $p < 0.05$. #Significantly higher than 1 mg/kg bw/d treatment group. ND, below detection limit; for limits of detection, see Table S1, Supporting Information.

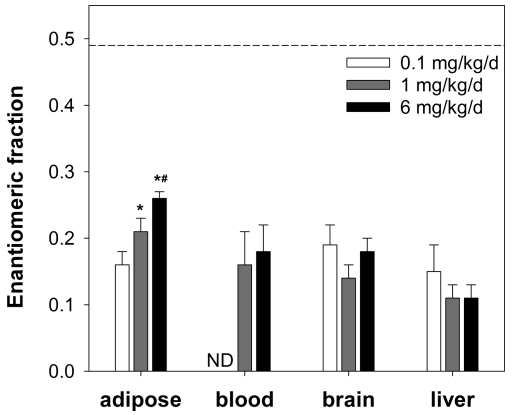


Figure 3. Atropisomeric enrichment of PCB 95 in tissues from dams exposed to racemic PCB 95 during gestation and lactation was tissue-dependent, with the liver presenting the most pronounced enrichment. With exception of the adipose tissue, the atropisomeric enrichment in tissues appeared to be independent of the dose. The dashed line corresponds to the EF of racemic PCB 95 (EF = 0.49 ± 0.01). Analyses were performed on a CD column (for details see Experimental procedures). Data presented as mean ± standard deviation. *Significantly higher than 0.1 mg/kg bw/d treatment group, $p < 0.05$. #Significantly higher than 1 mg/kg bw/d treatment group. ND, below detection limit; for limits of detection, see Table S1, Supporting Information.

determined using two different enantioselective columns (Figure 3 and Table S5A, Supporting Information). There was a good agreement between the results obtained on both the BDM and CD columns, and statistical analysis revealed no statistically significant differences. The second eluting peak of PCB 95 (E_2-PCB95) was enriched in all tissues at all doses investigated.

The most pronounced atropisomeric enrichment was observed in the liver, with an average EF value across all dose levels of 0.11. This EF value was significantly different from the EF values in all other tissues investigated. The EF values were also similar across all three doses in blood (EF ∼ 0.17) and brain (EF ∼ 0.16). The least pronounced atropisomeric enrichment was observed in adipose tissue, with an average EF value across all dose levels of 0.22. The EF values in adipose tissue showed some dose-dependent increase; however, this difference reached statistical significance only for EF values determined on the BDM column.

Atropisomeric Enrichment of PCB 95 Metabolites. Several studies have demonstrated that, like their parent compounds, OH-PCBs are atropisomerically enriched *in vivo*.[30,32] Consistent with these earlier studies, a considerable atropisomeric enrichment of several hydroxylated PCB 95 metabolites was observed in the liver (Figure 4A–C) and blood (Figure 4D, see also Table S6A, Supporting Information). An enrichment of the second eluting atropisomer was observed for 4-95 (E_2-4-95, Figure 4A,C) and 4,5-95 (E_2-4,5-95, Figure 4A,B). An enrichment of the first eluting atropisomer of the unidentified metabolite X′-95 (E_1-X′-95) was observed in the liver (Figure 4B). E_2-4-95 was also enriched in blood (Figure 4D). The enrichment E_2-4-95 appeared to be more pronounced in blood than in liver, whereas the enrichment of E_2-4,5-95 was similar in blood and liver (Table S6A, Supporting Information).

Levels and Atropisomeric Enrichment of PCB 95 and Metabolites in Pups. Tissues from a small number of pups were analyzed for a preliminary assessment of the transfer of PCB 95 to the offspring exposed via the dam. Overall, pups euthanized on PND 30–33 showed low but detectable tissue levels of PCB 95. Liver PCB 95 levels in the pups were 2- to 4-fold higher than blood levels (Table S7, Supporting Information). The levels of OH-PCBs were mostly below or close to the limit of detection (Table S8, Supporting Information). Similar to the corresponding dams, 4-95 was the major metabolite in blood. Liver samples from the offspring in the high-dose group contained comparable levels of 4-95 and 5-95. The determination of the EF values for PCB 95 was challenging due to the low PCB tissue levels; however, the direction of the atropisomeric enrichment of PCB 95 was the same as in dams, with E_2-PCB 95 being enriched in all samples. Moreover, the atropisomeric enrichment in the offspring appeared to be more pronounced compared to dams, with EF values < 0.03.

Atropisomeric Enrichment of PCB 95 in Postmortem Human Brain Samples. Because only limited information is available about chiral signatures of PCB 95 and other chiral PCB congeners in the general human population (Table 1), we determined the chiral signatures for PCB 95 and other chiral PCBs in four postmortem brain samples from an earlier study[8] to gain additional insights into chiral signatures in human brain tissue. In contrast to mouse brain (Figure 3), EF values of PCB 95 in all human brain samples were racemic. Similarly, chiral signatures of PCB 136 ($n = 1$) and PCB 149 ($n = 3$) were racemic in these extracts (Table S9, Supporting Information).

DOI: 10.1021/acs.chemrestox.5b00241
Chem. Res. Toxicol. XXXX, XXX, XXX–XXX

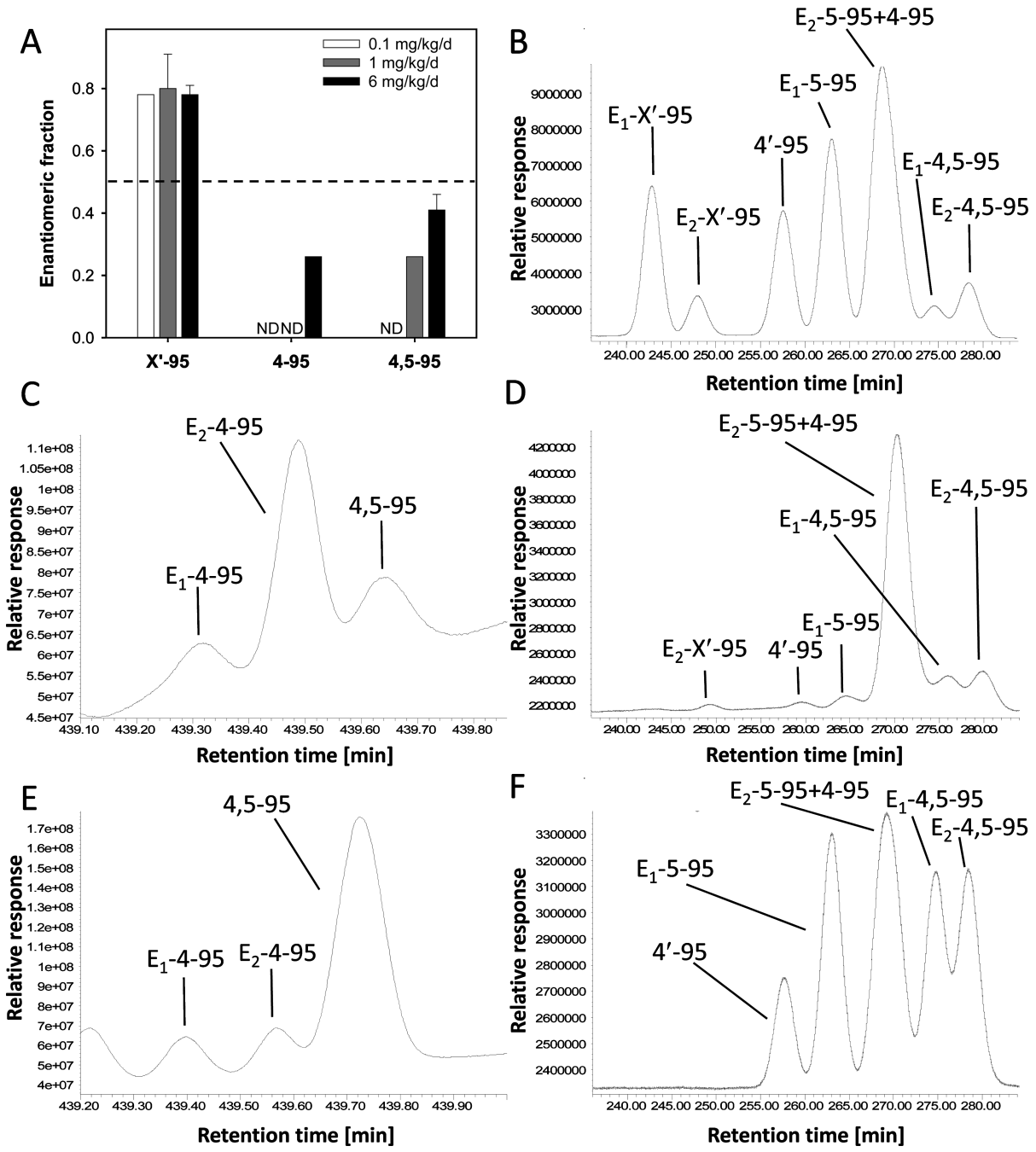


Figure 4. Hydroxylated PCB 95 metabolites display considerable atropisomeric enrichment in liver and blood from dams exposed to racemic PCB 95 throughout gestation and lactation. (A) Summary of EF values of X′-95, 4-95, and 4,5-95 in the liver. The dashed line indicates the EF value of a racemic mixture (note that the EF values were not 0.50 for all racemic standards; see text for details). Representative chromatograms showing the atropisomeric enrichment of (B) X′-95 and 4,5-95 in the liver (BDM column), (C) 4-95 in the blood (BGB column), and (D) 4,5-95 in blood (BDM column). (E, F) Representative chromatograms of the corresponding racemic standards analyzed on the BGB and BDM columns, respectively. Enantioselective analyses were performed as described in the Experimental Procedures. #Significantly higher than 1 mg/kg bw/d treatment group. ND, not detected. Data in panel (A) are presented as mean ± standard deviation. The enantiomeric fractions were calculated from the formula EF = $A_1/(A_1 + A_2)$, where A_1 and A_2 are the peak area of the first (E_1) and the second (E_2) eluting atropisomers, respectively.

DISCUSSION

Female mice received cumulative doses of 0.15, 1.4, and 8.2 mg (0.1, 1, and 6 mg/kg/day bw, respectively) of racemic PCB 95 throughout lactation and gestation. Because only four tissues and a limited number of PCB metabolites were quantified in the present study, it was not possible to obtain a complete mass balance of PCB 95. Adipose tissue, a well-documented storage tissue of PCBs,[32,37,41] accounted for approximately 0.24−0.27% of the total PCB 95 dose, which is considerably lower compared to earlier disposition studies with PCB 136 in mice.[33,37,41] All other tissues investigated accounted for only minute amounts of the total PCB 95 dose (<0.05% of the total dose). Moreover, OH-PCBs in blood and liver represented only a tiny percentage (~0.02% of the total dose) of the total PCB 95 dose. Taken together, these observations are consistent with

DOI: 10.1021/acs.chemrestox.5b00241
Chem. Res. Toxicol. XXXX, XXX, XXX−XXX

Table 1. Comparison of the Atropisomeric Enrichment of PCB 95 in Human and Mouse Samples Reveals Mice Display a More Pronounced Atropisomeric Enrichment

species	tissue	range	ref
human	breast milk (n = 10)	0.51−0.58	Blanch et al., 1999[60]
human	liver (n = 11)	0.51−0.75	Chu et al, 2003[59]
human	brain (n = 1)	0.50	Chu et al, 2003[59]
human	kidney (n = 3)	0.50−0.57	Chu et al, 2003[59]
human	muscle (n = 3)	0.51−0.53	Chu et al, 2003[59]
human	feces (n = 10)	0.42−0.50	Harrad et al., 2006[1]
human	breast milk (n = 11)	0.45−0.60	Bordajandi et al, 2008[61]
human	brain (n = 4)	0.50	current project[a]
human	serum (n = 30)	0.41−0.91	Megson et al, 2015[62]
mouse, adult	brain (n = 13)	0.12−0.20	Kania-Korwel, 2012[32]
mouse, adult	liver (n = 14)	0.07−0.19	Kania-Korwel, 2012[32]
mouse, dam	brain (n = 10)	0.11−0.20	current project[a]
mouse, dam	liver (n = 21)	0.07−0.19	current project[a]
mouse, pup	liver (n = 31)	0.00−0.03	current project[a]

[a]For details of sample origin and analysis, see Supporting Information. The enantiomeric fractions were calculated from the formula EF = A_1/(A_1 + A_2), where A_1 and A_2 are the peak area of the first (E_1) and the second (E_2) eluting atropisomers, respectively

the rapid elimination of chiral PCBs, such as PCB 95, in rodents.[32,42,43]

The levels of PCB 95 and its hydroxylated metabolites were within the range of PCB 95 levels reported in a few studies investigating populations with known PCB exposure. For example, PCB 95 blood levels were <5 ng/g blood (limit of detection, see Table S1, Supporting Information) and 14 ng/g blood for the low and medium PCB 95 treatment groups, respectively. In comparison, PCB 95 levels were ≤1.3 ng/g plasma in anglers from Wisconsin (data collected 1993−1995)[44] and 6.8−7.9 ng/g plasma in Swedish construction workers removing PCB-contaminated sealants (data from 2002).[45] A recent analysis of postmortem brain samples revealed higher PCB 95 levels in individuals with a neurodevelopmental disorder compared to those in neurotypical controls, with brain PCB 95 levels reaching 1.9 ng/g tissue.[8] These human brain levels are close to the PCB 95 levels (3.7 ng/g tissue) observed in the brain of dams from the low-dose treatment group. It is important to emphasize that PCB levels in the environment and in humans have decreased considerably since their production ended in the 1970s. A recent follow up study in Great Lakes anglers showed a decrease of PCB levels in humans of 3.5% per year from 1994−1995 to 2001−2005.[46]

Unlike the parent PCB, the levels of hydroxylated metabolites of PCB 95 have not been determined in human or environmental samples, partly because authentic standards are not readily available. However, the ∑OH-PCB levels found in dams in the current study (11 ng/g wet weight for the low dose group) were of the same order of magnitude as OH-PCB levels observed in human biomonitoring studies. For example, the ∑OH-PCB levels of Chukchi and Canadian Inuits were ≤12 ng/g wet weight.[47,48] These comparisons suggest that the PCB 95 doses used in the present study result in maternal PCB 95 and OH-PCB levels that are similar to levels found in human populations.

While they are comparable to human tissue levels, the PCB 95 tissue levels in the present study were significantly lower compared to levels observed previously in PCB 95-treated adult female mice (see Table 3A, Supporting Information).[32] Pregnancy-associated changes in lipid and lipoprotein disposition[49−52] explain, at least in part, differences in the disposition of PCB 95 between pregnant vs nonpregnant mice. Briefly, endogenous lipids and PCB 95 are mobilized from adipose tissue during lactation and, ultimately, eliminated via the breast milk.[49] Moreover, exogenous lipids and lipid-soluble PCB 95 are redirected to the mammary gland during pregnancy and, thus, effectively eliminated via the breast milk.[22] These changes in lipid disposition likely contributed to the elimination of a significant percentage of PCB 95 from the mouse dams examined in this study, thus resulting in lower PCB 95 levels compared to those in nonpregnant mice. In addition, some PCB 95 administered antepartum was likely eliminated with the fetuses at birth; however, the percentage of the total PCB 95 dose eliminated by this route is probably small because fetal PCB levels are typically low.[19,20] The present study was not designed to assess PCB 95 levels in fetuses and neonates; however, the PCB 95 levels in weanlings at PND 30−33 were low, which suggests that PCB 95 is also rapidly eliminated from weanling mice dosed throughout gestation and lactation via the dam.

In addition to the efficient elimination of PCB 95 via the breast milk, the oxidation of PCB 95 by P450 enzymes made a significant contribution to the elimination PCB 95 in mice.[12−15,43] The OH-PCB metabolite profiles in tissues from pregnant mice were consistent with our earlier *in vivo* study in nonpregnant mice,[32] with 4-95 being the major metabolite detected in blood and liver. 4-95 was also a major metabolite in liver and blood from weaned pups on PND 30−33 at the higher doses. OH-PCBs were below the detection limit in the adipose and brain tissue from pregnant mice as well as their offspring. 4-95 and 5-95 are also major metabolites formed in other mammalian species.[12,15,39] Similar to PCB 95 levels, the OH-PCB levels in dams were lower compared to OH-PCB levels observed in nonpregnant mice;[32] however, these differences were not as pronounced as with the parent compound. Taken together, these observations suggest that pregnancy-associated physiological changes result in lower OH-PCBs levels, which is not surprising considering the lower levels of the parent PCB 95. At the same time, pregnancy did not alter the profiles of potentially neurotoxic OH-PCB metabolites in the dams and their offspring.

The P450 enzyme-mediated metabolism of PCB 95 not only results in its rapid elimination and the formation of OH-PCBs but also explains the atropisomeric enrichment of PCB 95 and its metabolites in pregnant mice from this study. The direction and rank order of the enrichment of PCB 95 in different tissues of dams (i.e., adipose > blood ≈ brain > liver) was similar to other studies investigating the atropisomeric enrichment of PCB 95 in nonpregnant adult mice (see Table 5A, Supporting Information).[32] The direction of the atropisomeric enrichment of PCB 95 in PND 30−33 pups was the same as in the corresponding dams. Interestingly, the effect of the PCB dose on EF values in dams differed considerably from the trends observed in nonpregnant adult mice.[32] In the present study, the enantiomeric enrichment of PCB 95 in the adipose tissue was less pronounced in the higher dose treatment groups, whereas the dose had no significant effect on the EF values of PCB 95 in all other tissues investigated. In contrast, a more pronounced

DOI: 10.1021/acs.chemrestox.5b00241
Chem. Res. Toxicol. XXXX, XXX, XXX−XXX

enantiomeric enrichment was observed previously with increasing dose in tissues from nonpregnant adult mice.[32] An atropisomeric enrichment of E_2-4-95 and E_2-4,5-95 was detected in blood and liver from both dams and nonpregnant adult mice.[32] However, there were slight differences in the extent of the atropisomeric enrichment of PCB 95, 4-95, and 4,5-95 in pregnant vs nonpregnant mice (see Table 5A and 6A, Supporting Information) as well as in pregnant mice vs their offspring. These differences in the EF values between studies in part reflect differences in the internal PCB 95 dose (i.e., PCB tissue levels), with higher PCB tissue levels (e.g., in the adipose tissue from dams) masking an enantiomeric enrichment. Although this was not investigated in the present study, it is likely that the hepatic PCB 95 levels in the present study were too low to significantly alter P450 activities in the liver of dams and, thus, affect PCB 95's own enantioselective metabolism. In agreement with this interpretation, we have shown that the extent of the atropisomeric enrichment of PCB 136 increases with decreasing dose in mice treated with a single oral dose.[33] In contrast, the induction of P450 enzymes due to the high internal PCB 95 dose is thought to explain the more pronounced enantiomeric enrichment of PCB 95 in nonpregnant adult mice.[32]

In addition to differences in the internal dose, pregnancy-associated physiological changes, such as changes in P450 enzyme composition[50−52] and/or lipid disposition,[49] may contribute to small differences in the OH-PCB metabolite profile and/or the chiral signatures of PCB 95 and its metabolites. For example, changes in the hepatic P450 enzyme composition have been shown to alter OH-PCB profiles in *in vitro* metabolism studies[53,54] and affect chiral signatures of other chiral environmental pollutants, such as chlordanes.[55,56] Several animal studies have shown that physiological changes during pregnancy alter the enantioselective disposition of chiral bronchodilators[25] or antidepressants.[26] Moreover, PCBs absorbed from the diet may avoid the atropselective hepatic first-pass metabolism due to differences in the lipoprotein transport during pregnancy,[49] which could also contribute to differences in EF values. It is also possible that PCBs are enantioselectively transported across the placental barrier, thus affecting the enantiomeric enrichment in the dams. Consistent with this explanation, there is indication that some chiral persistent organic pollutants, such as α-hexachlorocyclohexane, can enantioselectively cross the blood−brain barrier.[57]

Our results demonstrate that pregnancy-associated changes in P450 enzyme composition and lipid disposition have only a small, if any, effect of the direction and extent of the atropisomeric enrichment of PCB 95 as well as the profiles and chiral signatures of its OH-PCB metabolites. Because PCB 136 and other chiral PCBs do not appear to be subject to enantioselective active transport, for example, by multidrug resistance transporters mdr1a/1b,[41] it is also unlikely that chiral signatures of PCB 95 are significantly altered by enantioselective transplacental transport; however, the possibility of active transplacental transport of PCBs cannot be completely dismissed because of the small differences in the EF values between dams and their pups. The findings from the present study, together with earlier studies,[32,35] provide additional evidence suggesting that disposition studies in nonpregnant adult animals predict PCBs' chiral signatures throughout all developmental stages of mice and, possibly, other species following oral exposure. Consistent with this hypothesis, a recent study investigating the maternal transfer of chiral PCBs in chicken also reported that the direction and extent of the atropisomeric enrichment of PCBs in the hen, egg, and chicks were also comparable for several PCBs, including PCB 95.[58] PCB 135 was an exception because the direction of the atropisomeric enrichment of this congener was different between hens and chicks. Additional studies investigating the maternal transfer of different chiral PCBs are needed, therefore, to fully understand chiral signatures observed in wildlife, laboratory studies, and humans following developmental PCB exposure.

While there is experimental evidence that chiral PCBs undergo considerable atropisomeric enrichment in mice and other species, few studies have investigated the atropisomeric enrichment of PCBs in the human population in general and in pregnant and/or nursing women in particular.[11] Limited evidence suggests that the atropisomeric enrichment of PCB 95 in humans appears to be much less pronounced and more variable compared to that in mice (Table 1).[27] A number of studies report an enrichment of E_1-PCB 95 in several human postmortem tissue samples[59] and breast milk.[60,61] A modest, if any, enrichment of E_2-PCB 95 has been reported for a few human breast milk and feces samples.[1,61] As reported in this study, the atropselective analysis of brain tissue extracts from the study by Mitchell et al.[8] also revealed racemic signatures of PCB 95 in brain tissue. A notable exception is a recent study of serum of transformer dismantling workers.[62] Most of the workers exhibited clear enrichment of E_1-PCB 95 in serum, with EF values as high as 0.91 (Table 1). Similarly, we have shown that E_1-PCB 95 undergoes considerable enantiomeric enrichment in rats exposed orally to racemic PCB 95. In contrast, E_2-PCB 95 is significantly enriched in tissues and excreta from mice exposed orally to racemic PCB 95 in this and other studies.[32,36]

Several factors may contribute to differences between EF values observed in human and laboratory-generated mouse tissue samples. *In vitro* studies with liver microsomes demonstrate significant species differences in the atropselective metabolism of PCB 136, with pooled human liver microsomes and mouse liver microsomes metabolizing PCB 136 atropisomers with different selectivity.[63] Since pure PCB atropisomers interfere with each other's biotransformation,[38,64] complex human exposures may also result in chiral signatures that are different from chiral signatures in animal studies with individual, racemic PCB congeners. It is also possible that interindividual differences in the activity of P450 enzymes result in differences in chiral signatures of PCB congeners in humans. Indeed, the activity of CYP2B6, a P450 enzyme involved in the metabolism of chiral PCBs,[12] depends on age,[65] is highly inducible by environmental and dietary factors,[66] and is affected by genetic polymorphisms.[66] Last but not least, humans may be exposed to atropisomerically enriched PCB 95 via the diet or air. Because chiral PCB 95 signatures in the diet and air are typically racemic,[1,4,67] the less pronounced atropisomeric enrichment in human samples suggests that near-racemic PCB 95 signatures reported for most human samples may reflect a recent, ongoing exposure to PCBs. Further studies are needed, therefore, to advance our understanding of the complex processes contributing to species differences in the disposition of PCB atropisomers, especially in light of the growing evidence demonstrating that the developmental neurotoxicity of chiral PCBs is atropselective.[16−18]

DOI: 10.1021/acs.chemrestox.5b00241
Chem. Res. Toxicol. XXXX, XXX, XXX−XXX

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.chemrestox.5b00241.

Method detection limits and background tissue levels in control animals for PCB 95 and hydroxylated metabolites, body and organ weights of dams subchronically exposed to PCB 95, levels and enantiomeric fractions of PCB 95 and hydroxylated metabolites in various tissues of dams, comparison of levels and enantiomeric fractions of PCB 95 and hydroxylated metabolites in various tissues between dams and nonpregnant age-matched congenic female mice, levels and enantiomeric fractions of PCB 95 and hydroxylated metabolites in various tissues of pups exposed to PCB 95 through gestation and lactation, and description of the analysis of human brain samples and the enantiomeric fractions of chiral PCBs in human brain samples (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*Phone: (319) 335-4310. Fax: (319) 335-4290. E-mail: hans-joachim-lehmler@uiowa.edu.

Funding

This work was supported by NIEHS grant nos. ES05605 (H.-J.L.), ES013661 (H.-J.L.), ES014901 (P.J.L.), and ES017425 (H.-J.L. and P.J.L.). Christopher Barnhart was supported by predoctoral fellowships provided by NIEHS training grant ES007059, UC Davis Superfund Research Program (ES04699), and the ARCS Foundation.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We would like to thank Drs. Sudhir N. Joshi, Sandhya M. Vyas, Yang Song, and Huimin Wu for the synthesis of analytical standards and Adriana Jarosz and Alex O'Donnell for help with the chemical analyses. We thank Dr. Janine LaSalle (UC Davis) and Dr. Paul Kostyniak (SUNY at Buffalo) for providing human brain extracts. Human tissues were obtained from the Autism Tissue Program and the Harvard Brain and Tissue Resource Center.

■ ABBREVIATIONS

3-103, 3-methoxy-2,2′,4,5′,6-pentachlorobiphenyl; 4-95, 2,2′,3,5′,6-pentachlorobiphenyl-4-ol; 4′-159, 2′,3,3′,4,5,5′-hexachlorobiphenyl-4′-ol; 4′-95, 2,2′,3,5′,6-pentachlorobiphenyl-4′-ol; 5-95, 2,2′,3,5′,6-pentachlorobiphenyl-5-ol; X′-95, unidentified monohydroxylated metabolite of PCB 95; 4,5-95, 2,2′,3,5′,6-pentachlorobiphenyl-4,5-diol; BDM, ChiralDex B-DM enantioselective column, 2,3-di-O-methyl-6-*tert*-butyl-silyl-*β*-cyclodextrin; BGB, BGB-172 enantioselective column, 20% *tert*-butyldimethyl-silyl-*β*-cyclodextrin; CD, Chirasil-Dex enantioselective column, 2,3,6-tri-*O*-methyl-*β*-cyclodextrin; E_1, first eluting atropisomer; E_2, second eluting atropisomer; EF, enantiomeric fraction; ND, below limit of detection; OH-PCBs, hydroxylated metabolites of polychlorinated biphenyls; PCB, polychlorinated biphenyls; PCB 95, 2,2′,3,5′,6-pentachlorobiphenyl; PCB 117, 2,3,4′,5,6-pentachlorobiphenyl; PCB 204, 2,2′,3,4,4′,5,6,6′-octachlorobiphenyl; PND, postnatal day; R_s, resolution

■ REFERENCES

(1) Harrad, S., Ren, J., Hazrati, S., and Robson, M. (2006) Chiral signatures of PCB#s 95 and 149 in indoor air, grass, duplicate diets and human faeces. *Chemosphere 63*, 1368−1376.

(2) Schecter, A., Colacino, J., Haffner, D., Patel, K., Opel, M., Papke, O., and Birnbaum, L. (2010) Perfluorinated compounds, polychlorinated biphenyl, and organochlorine pesticide contamination in composite food samples from Dallas, Texas. *Environ. Health Perspect. 118*, 796−802.

(3) Garner, C. E., Demeter, J., and Matthews, H. B. (2006) The effect of chlorine substitution on the disposition of polychlorinated biphenyls following dermal administration. *Toxicol. Appl. Pharmacol. 216*, 157−167.

(4) Jamshidi, A., Hunter, S., Hazrati, S., and Harrad, S. (2007) Concentrations and chiral signatures of polychlorinated biphenyls in indoor and outdoor air and soil in a major UK conurbation. *Environ. Sci. Technol. 41*, 2153−2158.

(5) Hu, X., Adamcakova-Dodd, A., Lehmler, H. J., Hu, D., Hornbuckle, K., and Thorne, P. S. (2012) Subchronic inhalation exposure study of an airborne polychlorinated biphenyl mixture resembling the Chicago ambient air congener profile. *Environ. Sci. Technol. 46*, 9653−9662.

(6) Thomas, K., Xue, J., Williams, R., Jones, P., and Whitaker, D. (2012) *Polychlorinated Biphenyls (PCBs) in School Buildings: Sources, Environmental Levels, and Exposures*, United States Environmental Protection Agency, Office of Research and Development, National Exposure Research Laboratory, Las Vegas, NV, http://www.epa.gov/pcbsincaulk/pdf/pcb_EPA600R12051_final.pdf.

(7) Megson, D., O'Sullivan, G., Comber, S., Worsfold, P. J., Lohan, M. C., Edwards, M. R., Shields, W. J., Sandau, C. D., and Patterson, D. G., Jr (2013) Elucidating the structural properties that influence the persistence of PCBs in humans using the National Health and Nutrition Examination Survey (NHANES) dataset. *Sci. Total Environ. 461−462*, 99−107.

(8) Mitchell, M. M., Woods, R., Chi, L. H., Schmidt, R. J., Pessah, I. N., Kostyniak, P. J., and LaSalle, J. M. (2012) Levels of select PCB and PBDE congeners in human postmortem brain reveal possible environmental involvement in 15q11-q13 duplication autism spectrum disorder. *Environ. Mol. Mutagen. 53*, 589−598.

(9) Mariussen, E., and Fonnum, F. (2006) Neurochemical targets and behavioral effects of organohalogen compounds: an update. *Crit. Rev. Toxicol. 36*, 253−289.

(10) Pessah, I. N., Cherednichenko, G., and Lein, P. J. (2010) Minding the calcium store: Ryanodine receptor activation as a convergent mechanism of PCB toxicity. *Pharmacol. Ther. 125*, 260−285.

(11) Lehmler, H. J., Harrad, S. J., Hühnerfuss, H., Kania-Korwel, I., Lee, C. M., Lu, Z., and Wong, C. S. (2010) Chiral polychlorinated biphenyl transport, metabolism and distribution: A review. *Environ. Sci. Technol. 44*, 2757−2766.

(12) Warner, N. A., Martin, J. W., and Wong, C. S. (2009) Chiral polychlorinated biphenyls are biotransformed enantioselectively by mammalian cytochrome P-450 isozymes to form hydroxylated metabolites. *Environ. Sci. Technol. 43*, 114−121.

(13) Lu, Z., Xue, M., Shen, G., Li, K., Li, X., Wang, X., and Tao, S. (2011) Accumulation dynamics of chlordanes and their enantiomers in cockerels (Gallus gallus) after oral exposure. *Environ. Sci. Technol. 45*, 7928−7935.

(14) Wu, X., Kania-Korwel, I., Chen, H., Stamou, M., Dammanahalli, K. J., Duffel, M., Lein, P. J., and Lehmler, H. J. (2013) Metabolism of 2,2′,3,3′,6,6′-hexachlorobiphenyl (PCB 136) atropisomers in tissue slices from phenobarbital or dexamethasone-induced rats is sex-dependent. *Xenobiotica 43*, 933−947.

(15) Kania-Korwel, I., Duffel, M. W., and Lehmler, H. J. (2011) Gas chromatographic analysis with chiral cyclodextrin phases reveals the

DOI: 10.1021/acs.chemrestox.5b00241
Chem. Res. Toxicol. XXXX, XXX, XXX−XXX

enantioselective formation of hydroxylated polychlorinated biphenyls by rat liver microsomes. *Environ. Sci. Technol. 45*, 9590−9596.
(16) Pessah, I. N., Lehmler, H. J., Robertson, L. W., Perez, C. F., Cabrales, E., Bose, D. D., and Feng, W. (2009) Enantiomeric specificity of (−)-2,2′,3,3′,6,6′-hexachlorobiphenyl toward ryanodine receptor types 1 and 2. *Chem. Res. Toxicol. 22*, 201−207.
(17) Lehmler, H.-J., Robertson, L. W., Garrison, A. W., and Kodavanti, P. R. S. (2005) Effects of PCB 84 enantiomers on [^{3}H] phorbol ester binding in rat cerebellar granule cells and $^{45}Ca^{2+}$-uptake in rat cerebellum. *Toxicol. Lett. 156*, 391−400.
(18) Yang, D., Kania-Korwel, I., Ghogha, A., Chen, H., Stamou, M., Bose, D. D., Pessah, I. N., Lehmler, H. J., and Lein, P. J. (2014) PCB 136 atropselectively alters morphometric and functional parameters of neuronal connectivity in cultured rat hippocampal neurons via ryanodine receptor-dependent mechanisms. *Toxicol. Sci. 138*, 379−392.
(19) Masuda, Y., Kagawa, R., Kuroki, H., Tokudome, S., and Kuratsune, M. (1979) Transfer of various polychlorinated biphenyls to the foetuses and offspring of mice. *Food Cosmet. Toxicol. 17*, 623−627.
(20) Vodicnik, M. J. (1986) The effect of pregnancy and lactation on the disposition of [2,4,2′,4′-14C]tetrachlorobiphenyl in the mouse. *Fundam. Appl. Toxicol. 6*, 53−61.
(21) Masuda, Y., Kagawa, R., Tokudome, S., and Kuratsune, M. (1978) Transfer of polychlorinated biphenyls to the foetuses and offspring of mice. *Food Cosmet. Toxicol. 16*, 33−37.
(22) Vodicnik, M. J., and Lech, J. J. (1980) The transfer of 2,4,5,2′,4′,5′-hexachlorobiphenyl to fetuses and nursing offspring. I. Disposition in pregnant and lactating mice and accumulation in young. *Toxicol. Appl. Pharmacol. 54*, 293−300.
(23) Lucier, G., McDaniel, O., Schiller, C., and Matthews, H. (1978) Structural requirements for the accumulation of chlorinated biphenyl metabolites in the fetal rat intestine. *Drug Metab. Dispos. 6*, 584−590.
(24) Meerts, I. A., Assink, Y., Cenijn, P. H., Van Den Berg, J. H., Weijers, B. M., Bergman, A., Koeman, J. H., and Brouwer, A. (2002) Placental transfer of a hydroxylated polychlorinated biphenyl and effects on fetal and maternal thyroid hormone homeostasis in the rat. *Toxicol. Sci. 68*, 361−371.
(25) Boulton, D. W., and Fawcett, J. P. (1996) Enantioselective disposition of albuterol in humans. *Clin. Rev. Allergy Immunol. 14*, 115−138.
(26) Sit, D. K., Perel, J. M., Helsel, J. C., and Wisner, K. L. (2008) Changes in antidepressant metabolism and dosing across pregnancy and early postpartum. *J. Clin. Psychiatry 69*, 652−658.
(27) Kania-Korwel, I., Lehmler, H.-J. (2015) Toxicokinetics of chiral polychlorinated biphenyls across different species—a review. *Environ. Sci. Pollut. Res.* DOI: 10.1007/s11356-015-4383-0.
(28) Wayman, G. A., Yang, D., Bose, D. D., Lesiak, A., Ledoux, V., Bruun, D. A., Pessah, I. N., and Lein, P. J. (2012) PCB 95 promotes dendritic growth via ryanodine receptor-dependent mechanisms. *Environ. Health Perspect. 120*, 997−1002.
(29) Yang, D., Kim, K. H., Phimister, A., Bachstetter, A. D., Ward, T. R., Stackman, R. W., Mervis, R. F., Wisniewski, A. B., Klein, S. L., Kodavanti, P. R., Anderson, K. A., Wayman, G., Pessah, I. N., and Lein, P. J. (2009) Developmental exposure to polychlorinated biphenyls interferes with experience-dependent dendritic plasticity and ryanodine receptor expression in weanling rats. *Environ. Health Perspect. 117*, 426−435.
(30) Kania-Korwel, I., Vyas, S., Song, Y., and Lehmler, H. J. (2008) Gas chromatographic separation of methoxylated polychlorinated biphenyl atropisomer. *J. Chromatogr. A 1207*, 146−154.
(31) Joshi, S. N., Vyas, S. M., Duffel, M. W., Parkin, S., and Lehmler, H. J. (2011) Synthesis of sterically hindered polychlorinated biphenyl derivatives. *Synthesis 7*, 1045−1054.
(32) Kania-Korwel, I., Barnhart, C. D., Stamou, M., Truong, K. M., El-Komy, M. H., Lein, P. J., Veng-Pedersen, P., and Lehmler, H. J. (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.
(33) Kania-Korwel, I., Hornbuckle, K. C., Robertson, L. W., and Lehmler, H. J. (2008) Dose-dependent enantiomeric enrichment of 2,2′,3,3′,6,6′-hexachlorobiphenyl in female mice. *Environ. Toxicol. Chem. 27*, 299−305.
(34) Bordajandi, L. R., Ramos, L., and Gonzalez, M. J. (2005) Chiral comprehensive two-dimensional gas chromatography with electron-capture detection applied to the analysis of chiral polychlorinated biphenyls in food samples. *J. Chromatogr. A 1078*, 128−135.
(35) Kania-Korwel, I., Xie, W., Hornbuckle, K. C., Robertson, L. W., and Lehmler, H.-J. (2008) Enantiomeric enrichment of 2,2′,3,3′,6,6′-hexachlorobiphenyl (PCB 136) in mice after induction of CYP enzymes. *Arch. Environ. Contam. Toxicol. 55*, 510−517.
(36) Kania-Korwel, I., El-Komy, M. H. M. E., Veng-Pedersen, P., and Lehmler, H. J. (2010) Clearance of polychlorinated biphenyl atropisomers is enantioselective in female C57Bl/6 mice. *Environ. Sci. Technol. 44*, 2828−2835.
(37) Kania-Korwel, I., Shaikh, N., Hornbuckle, K. C., Robertson, L. W., and Lehmler, H.-J. (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.
(38) Lu, Z., Kania-Korwel, I., Lehmler, H. J., and Wong, C. S. (2013) Stereoselective formation of mono- and dihydroxylated polychlorinated biphenyls by rat cytochrome P450 2B1. *Environ. Sci. Technol. 47*, 12184−12192.
(39) Sundström, G., and Jansson, B. (1975) The metabolism of 2,2′,3,5′,6-pentachlorobiphenyl in rats, mice and quails. *Chemosphere 4*, 361−370.
(40) Guroff, G., Daly, J. W., Jerina, D. M., Renson, J., Witkop, B., and 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.
(41) Milanowski, B., Lulek, J., Lehmler, H.-J., and Kania-Korwel, I. (2010) Assesment of disposition of chirl polychlorinated biphenyls in female mdr 1a/b knockout versus wild-type mice using multivariate analyses. *Environ. Int. 36*, 884−892.
(42) Birnbaum, L. S. (1983) Distribution and excretion of 2,3,6,2′,3′,6′- and 2,4,5,2′,4′,5′-hexachlorobiphenyl in senescent rats. *Toxicol. Appl. Pharmacol. 70*, 262−272.
(43) Wu, X., Barnhart, C., Lein, P. J., and Lehmler, H.-J. (2015) Hepatic Metabolism Affects the Atropselective Disposition of 2,2′,3,3′,6,6′-Hexachlorobiphenyl (PCB 136) in Mice. *Environ. Sci. Technol. 49*, 616−625.
(44) DeVoto, E., Fiore, B., Millikan, R., Anderson, H., Sheldon, L., Sonzogni, W., and Longnecker, M. (1997) Correlations among human blood levels of specific PCB congeners and implications for epidemiologic studies. *Am. J. Ind. Med. 32*, 606−613.
(45) Wingfors, H., Seldén, A., Nilsson, C., and Haglund, P. (2005) Identification of markers for PCB exposure in plasma from Swedish construction workers removing old elastic sealants. *Ann. Occup. Hyg. 50*, 65−73.
(46) Knobeloch, L., Turyk, M., Imm, P., Schrank, C., and Anderson, H. (2009) Temporal changes in PCB and DDE levels among a cohort of frequent and infrequent consumers of Great Lakes sportfish. *Environ. Res. 109*, 66−72.
(47) Sandanger, T. M., Dumas, P., Berger, U., and Burkow, I. C. (2004) 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. 6*, 758−765.
(48) Sandau, C. D., Ayotte, P., Dewailly, D., Duffe, J., and Norstrom, R. J. (2000) Analysis of hydroxylated metabolites of PCBs (OH-PCBs) and other chlorinated phenolic compunds in whole blood from Canadian Inuit. *Environ. Health Persp. 108*, 611−616.
(49) Spindler-Vomachka, M., and Vodicnik, M. J. (1984) Distribution of 2,4,5,2′,4′,5′-hexachlorobiphenyl among lipoproteins during pregnancy and lactation in the rat. *J. Pharmacol. Exp. Ther. 230*, 263−268.

DOI: 10.1021/acs.chemrestox.5b00241
Chem. Res. Toxicol. XXXX, XXX, XXX−XXX

(50) Lambert, G. H., Lietz, H. W., and Kotake, A. N. (1987) Effects of pregnancy on the cytochrome P-450 system in mice. *Biochem. Pharmacol. 36*, 1965−1971.
(51) Koh, K. H., Xie, H., Yu, A. M., and Jeong, H. (2011) Altered cytochrome P450 expression in mice during pregnancy. *Drug Metab. Dispos. 39*, 165−169.
(52) Shuster, D. L., Bammler, T. K., Beyer, R. P., Macdonald, J. W., Tsai, J. M., Farin, F. M., Hebert, M. F., Thummel, K. E., and Mao, Q. (2013) Gestational age-dependent changes in gene expression of metabolic enzymes and transporters in pregnant mice. *Drug Metab. Dispos. 41*, 332−342.
(53) Wu, X., Duffel, M., and Lehmler, H.-J. (2013) Oxidation of polychlorinated biphenyls by liver tissue slices from phenobarbital-preatreated mice is congener-specific and atropselective. *Chem. Res. Toxicol. 26*, 1642−1651.
(54) Wu, X., Pramanik, A., Duffel, M. W., Hrycay, E. G., Bandiera, S. M., Lehmler, H. J., and Kania-Korwel, I. (2011) 2,2′,3,3′,6,6′-Hexachlorobiphenyl (PCB 136) is enantioselectively oxidized to hydroxylated metabolites by rat liver microsomes. *Chem. Res. Toxicol. 24*, 2249−2257.
(55) Kania-Korwel, I., and Lehmler, H.-J. (2013) Chlordane and heptachlor are metabolized enantioselectively by rat liver microsomes. *Environ. Sci. Technol. 47*, 8913−8922.
(56) Bondy, G. S., Coady, L., Doucet, J., Armstrong, C., Kriz, R., Liston, V., Robertson, P., Norstrom, R., and Moisey, J. (2005) Enantioselective and gender-dependent depletion of chlordane compounds from rat tissues. *J. Toxicol. Environ. Health, Part A 68*, 1917−1938.
(57) Ulrich, E. M., Willett, K. L., Caperell-Grant, A., Bigsby, R. M., and Hites, R. A. (2001) Understanding enantioselective processes: a laboratory rat model for α-hexachlorocyclohexane accumulation. *Environ. Sci. Technol. 35*, 1604−1609.
(58) Zheng, X.-B., Luo, X.-J., Zeng, Y.-H., Wu, J.-P., and Mai, B.-X. (2015) Chiral polychlorinated biphenyls (PCBs) in bioaccumulation, maternal transfer, and embryo development of chicken. *Environ. Sci. Technol. 49*, 785−791.
(59) Chu, S., Covaci, A., and Schepens, P. (2003) Levels and chiral signatures of persistent organochlorine pollutants in human tissues from Belgium. *Environ. Res. 93*, 167−176.
(60) Blanch, G. P., Glausch, A., and Schurig, V. (1999) Determination of the enantiomeric ratios of chiral PCB 95 and 149 in human milk samples by multidimensional gas chromatography with ECD and MS(SIM) detection. *Eur. Food Res. Technol. 209*, 294−296.
(61) Bordajandi, L. R., Abad, E., and Gonzales, M. J. (2008) Occurrence of PCBs, PCDD/Fs, PBDEs and DDTs in Spanish breast milk: Enantiomeric fraction of chiral PCBs. *Chemosphere 70*, 567−575.
(62) Megson, D., Focant, J.-F., Patterson, D. G., Robson, M., Lohan, M. C., Worsfold, P. J., Comber, S., Kalin, R., Reiner, E., and O'Sullivan, G. (2015) Can polychlorinated biphenyl (PCB) signatures and enantiomer fractions be used for source identification and to age date occupational exposure? *Environ. Int. 81*, 56−63.
(63) Wu, X., Kammerer, A., and Lehmler, H.-J. (2014) Microsomal oxidation of 2,2′,3,3′,6,6′-hexachlorobiphenyl (PCB 136) results in species-dependent chiral signatures of the hydroxylated metabolites. *Environ. Sci. Technol. 48*, 2436−2444.
(64) Lu, Z., and Wong, C. S. (2011) Factors affecting phase I stereoselective biotransformation of chiral polychlorinated biphenyls by rat cytochrome P-450 2B1 isozyme. *Environ. Sci. Technol. 45*, 8298−8305.
(65) Croom, E. L., Stevens, J. C., Hines, R. N., Wallace, A. D., and Hodgson, E. (2009) Human hepatic CYP2B6 developmental expression: The impact of age and genotype. *Biochem. Pharmacol. 78*, 184−190.
(66) Zanger, U. M., Klein, K., Saussele, T., Blievernicht, J., H Hofmann, M., and Schwab, M. (2007) Polymorphic CYP2B6: molecular mechanisms and emerging clinical significance. *Pharmacogenomics 8*, 743−759.
(67) Robson, M., and Harrad, S. (2004) Chiral PCB signatures in air and soil: implications for atmospheric source apportionment. *Environ. Sci. Technol. 38*, 1662−1666.

DOI: 10.1021/acs.chemrestox.5b00241
Chem. Res. Toxicol. XXXX, XXX, XXX−XXX