

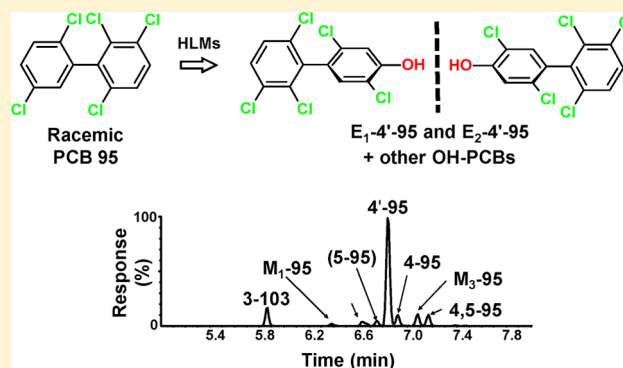
## 2,2',3,5',6-Pentachlorobiphenyl (PCB 95) Is Atropselectively Metabolized to para-Hydroxylated Metabolites by Human Liver Microsomes

Eric Uwimana,<sup>1</sup> Xueshu Li, and Hans-Joachim Lehmler\*<sup>2</sup>

Interdisciplinary Graduate Program in Human Toxicology and Department of Occupational and Environmental Health, University of Iowa, Iowa City, Iowa 52242, United States

### S Supporting Information

**ABSTRACT:** Exposure to neurotoxic, chiral PCBs has been associated with neurodevelopmental disorders, but their metabolism in humans remains unexplored. We investigated the enantioselective metabolism of PCB 95 by human liver microsomes (HLMs) to potentially neurotoxic, hydroxylated metabolites (OH-PCBs). OH-PCB profiles formed in experiments with HLMs differed from metabolite profiles reported for rodent species. The second eluting atropisomer of 2,2',3,5',6-pentachlorobiphenyl-4'-ol, the major metabolite, was preferentially formed by all HLM preparations investigated. Differences in metabolite formation rates were observed with single donor HLMs. The metabolism of PCBs and its role in PCB-mediated neurodevelopmental disorders need to be further characterized.



PCBs represent a human health concern because they are detected in environmental samples and human tissues.<sup>1,2</sup> Epidemiological studies suggest negative associations between developmental exposures to PCBs and neurophysiological function in infancy and childhood.<sup>3</sup> Animal studies demonstrate that developmental exposures to PCBs, such as PCB 95, cause behavioral deficits in rodents due to the disruption of calcium homeostasis.<sup>3</sup> Higher levels of PCB 95 have been observed in the brain from individuals diagnosed with a neurodevelopmental disorder compared to that in neurotypical individuals.<sup>2</sup> PCB 95 and its hydroxylated metabolites (OH-PCBs) are potent sensitizers of ryanodine receptors,<sup>4</sup> calcium release channels implicated in the developmental neurotoxicity of PCBs.<sup>3</sup> PCB 95 and its metabolites exist as two stable rotational isomers or atropisomers that are nonsuperimposable mirror images of each other. PCB 95 is enantioselectively metabolized by P450 enzymes, thus resulting in atropisomerically enriched PCB 95 in brain tissue.<sup>5,6</sup> Although PCBs atropselectively alter end points implicated in PCBs' developmental neurotoxicity,<sup>1,3</sup> the oxidation of PCB 95 by HLMs has not been investigated previously.

Initial microsomal metabolism studies with pooled HLMs (pHLM; pool of 50, mixed gender; Xenotech, Lenexa KS, USA) were carried out to identify hydroxylated PCB 95 metabolites potentially formed in humans (Figure 1 and Figures S1 to S14).<sup>7</sup> Analysis by GC/time-of-flight MS after extraction and derivatization with diazomethane revealed the formation of six mono- and two dihydroxylated metabolites (as methylated derivatives). The identification of the metabolites was based on accurate mass determination and the isotope

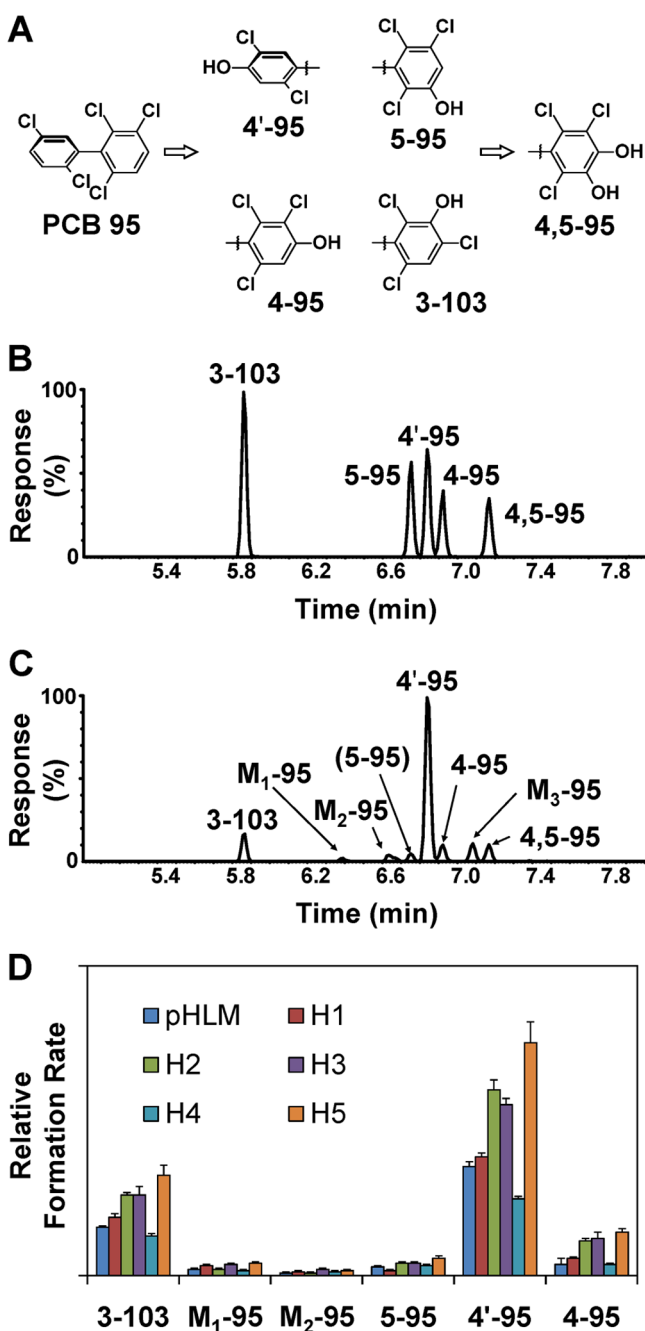
patterns of the  $[M]^+$  ion (expected abundance ratio: 1:1.6:1.0:0.3). The fragmentation patterns of the derivatized mono- or dihydroxylated metabolites were consistent with meta- or para-methoxylated pentachlorobiphenyl derivatives, with characteristic fragments such as  $[M-CH_3]^+$  and  $[M-CH_3-CO-Cl_2]^+$ ; see Supporting Information.<sup>8</sup> The proposed chemical structures and abbreviations of the metabolites are shown in Figure 1A. The formation of 3-103 (a 1,2-shift product), 5-95, 4'-95, 4-95, and 4,5-95 was further confirmed by comparing relative retention times and MS data of the metabolites to those of authentic standards.

GC with electron capture detection (ECD; see Supporting Information) was used to determine levels and relative formation rates<sup>7</sup> of the OH-PCB metabolites in incubations with HLMs (Figure 1D; Tables S1 to S5). Incubation conditions for the determination of formation rates were optimized for time, protein, NADPH, and PCB 95 concentrations (i.e., 50  $\mu$ M PCB 95; 5 min incubation at 37  $^{\circ}$ C; 0.1 mg/mL microsomal protein; and 1 mM NADPH).<sup>9</sup> The para position of the 2,5-dichloro substituted phenyl ring was the major site of PCB 95 metabolism, with 4'-95 accounting for 73% of total OH-PCBs formed. Metabolite formation rates in experiments with pHLMs followed the rank order 4'-95  $\gg$  4-95  $\sim$  3-103  $>$  5-95  $\gg$  4,5-95. M<sub>1</sub>-95, M<sub>2</sub>-95, and M<sub>3</sub>-95 were minor metabolites.

Similar to our findings with PCB 95 reported herein, PCB 52 (2,2',5,5'-tetrachlorobiphenyl) and PCB 101 (2,2',4,5,5'-

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**Figure 1.** Metabolism of PCB 95 by pHLMs results in the formation of hydroxylated metabolites (analyzed as methylated derivatives), with para-hydroxylated metabolites being the major metabolites. (A) Metabolism scheme showing the chemical structure and abbreviations of PCB 95 metabolites (only one atropisomer is shown for clarity reasons). Gas chromatograms of (B) PCB 95 metabolite standards and (C) metabolites formed in an incubation with pHLMs (50  $\mu$ M PCB 95, 90 min, 37  $^{\circ}$ C, 0.3 mg/mL protein, and 1 mM NADPH). (D) Relative formation rates of OH-PCB metabolites (relative peak area/nmol P450/min  $\times 10^2$ ) in incubations with pHLMs and single donor HLMs (H1 to H5). Data are the mean  $\pm$  standard deviation,  $n = 3$ .

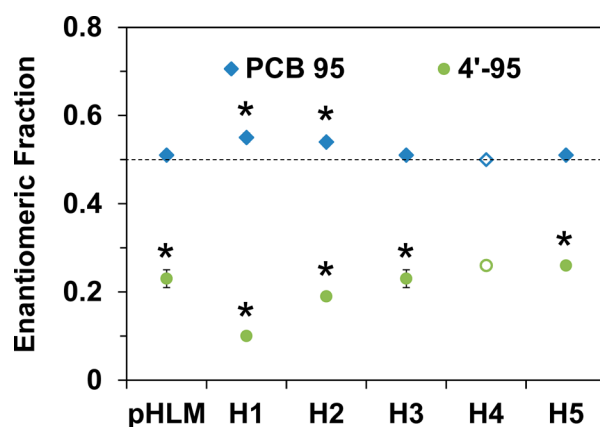
pentachlorobiphenyl) are preferentially metabolized in the para position by human P450 enzymes.<sup>10,11</sup> These earlier studies identified P450 2A6 as the primary P450 isoform involved in the para-oxidation of a 2,5-dichloro substituted phenyl ring. In contrast, PCB 136 (2,2',3,3',6,6'-hexachlorobiphenyl), a symmetrical congener with two 2,3,6-trichloro substituted phenyl

rings, is oxidized by pHLMs to both para- and meta-hydroxylated metabolites.<sup>7,12</sup> Metabolism studies with recombinant enzymes suggest a role of P450 2B6 in the metabolism of chiral PCBs with a 2,3,6-trichloro substitution.<sup>13</sup>

Studies using rodent models reveal different OH-PCB profiles *in vitro* and *in vivo*, without the predominant formation of 4'-95 observed with HLMs. Briefly, PCB 95 is preferentially metabolized to 5-95 and an unidentified OH-PCB by rat P450 2B1<sup>14</sup> and rat liver microsomes from rats pretreated with phenobarbital.<sup>15</sup> The unidentified metabolite does not correspond to 4'-95 and likely is 2,2',3,5',6-pentachlorobiphenyl-3'-ol (3'-95).<sup>15</sup> However, 4'-95 is present in the blood and liver from rats orally exposed to PCB 95.<sup>5</sup> Metabolism studies with PCB 95 revealed the formation of 5-95 as a major metabolite in liver tissue slices from phenobarbital-treated mice.<sup>16</sup> Studies in pregnant mice exposed to PCB 95 during gestation and lactation showed no detectable levels of 4'-95 in the blood and liver of the dams.<sup>6</sup>

To explore interindividual differences in the metabolism of PCB 95, incubations with HLMs from five randomly selected individual donors were performed (Table S1). All microsomal preparations yielded similar metabolite profiles after a 5 min incubation time, with an approximate ratio of 3-103:5-95:4'-95:4-95 = 2:1:20:4. Irrespective of the incubation conditions, these four OH-PCBs and 4,5-95 accounted for <5% of the total PCB added to each incubation (Figure 1D; Table S1). Depending on the OH-PCB congener, the OH-PCB formation rates differed 2- to 4-fold between individual donor HLMs. In general, the metabolism rates followed the rank order H5 > H1 > H2 ~ H3 > pHLM > H4 (Tables S2–S5).

The atropisomeric enrichment, expressed as an enantiomeric fraction (EF), of PCB 95 and 4'-95 was assessed as described previously (Figure 2, Table S6, and Figures S15 and S16).<sup>9</sup>



**Figure 2.** Metabolism of racemic PCB 95 in incubations with different HLMs results in the atropselective formation of  $E_2$ -4'-95 and for HLMs from donors H1 and H2, a depletion of  $E_2$ -PCB 95. Incubations were performed for 120 min; Table S6). The dotted line indicates the EF value of the racemic standards. Data are the mean  $\pm$  standard deviation. Open symbols indicate  $n = 1$ . \*Significantly different from the racemic standard ( $p < 0.05$ ).

Long-term incubations were performed with several HLM preparations to obtain nonracemic chiral signatures of the parent PCB. A significant enrichment (EF = 0.55) of the PCB 95 atropisomer eluting first on the GC column ( $E_1$ -PCB 95) was observed in incubations with two HLM preparations. No enrichment was observed with most HLM experiments because

the excess of racemic PCB 95 masked the atropisomeric enrichment due to a low conversion of the parent PCBs. The 4'-95 atropisomer eluting second on the GC column ( $E_2$ -4'-95) was significantly enriched under these conditions in all HLM preparations investigated. Apparent EF values of 4'-95 ranged from 0.10 to 0.26. HLM incubations with a 15 min incubation time also revealed an enrichment of  $E_2$ -4'-95 (Table S6). These observations are consistent with a preferential metabolism of  $E_2$ -PCB 95 to  $E_2$ -4'-95 by human P450 enzymes.

Our results suggest that interindividual differences in the metabolism of PCB 95 are one factor that modulates chiral signatures of PCB and their OH-PCB metabolites in humans. Further studies are needed to identify the P450 isoforms involved in the metabolism of PCB 95 in humans, determine if PCB 95 metabolites are present in human target tissues and, ultimately, assess how their atropisomers affect end points implicated in the neurodevelopmental toxicity of PCBs.

## ■ ASSOCIATED CONTENT

### ■ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.chemrestox.6b00371.

Experimental details of microsomal incubations and chemical analysis; levels and formation rates of OH-PCB metabolites; enantiomeric fractions; and mass spectra of OH-PCB derivatives (PDF)

## ■ AUTHOR INFORMATION

### Corresponding Author

\*Phone: 319-335-4310. E-mail: [hans-joachim-lehmler@uiowa.edu](mailto:hans-joachim-lehmler@uiowa.edu).

### ORCID

Eric Uwimana: 0000-0003-0653-1155

Hans-Joachim Lehmler: 0000-0001-9163-927X

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### Notes

The authors declare no competing financial interest.

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## ■ ABBREVIATIONS

3-103, 2,2',4,5',6-pentachlorobiphenyl-3-ol; 4-95, 2,2',3,5',6-pentachlorobiphenyl-4-ol; 4'-95, 2,2',3,5',6-pentachlorobiphenyl-4'-ol; 5-95, 2,2',3,5',6-pentachlorobiphenyl-5-ol; 4,5-95, 4,5-dihydroxy-2,2',3,5',6-pentachlorobiphenyl;  $E_1$ , atropisomer eluting first on the enantioselective GC column;  $E_2$ , atropisomer eluting second on the enantioselective GC column; EF, enantiomeric fraction; HLM, human liver microsomes; OH-PCBs, hydroxylated polychlorinated biphenyls; PCB 52, 2,2',5,5'-pentachlorobiphenyl; PCB 95, 2,2',3,5',6-pentachlorobiphenyl; PCB 101, 2,2',4,5,5'-pentachlorobiphenyl; PCB 136, 2,2',3,3',6,6'-hexachlorobiphenyl; pHLM, pooled human liver microsomes

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