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Authors

Zhang, Chun-Yun
Klocke, Carolyn R
Lein, Pamela J
et al.

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Rapid Report

Disposition of PCB 11 in Mice Following Acute Oral Exposure

Chun-Yun Zhang, Carolyn R. Klocke, Pamela J. Lein, and Hans-Joachim Lehmler*

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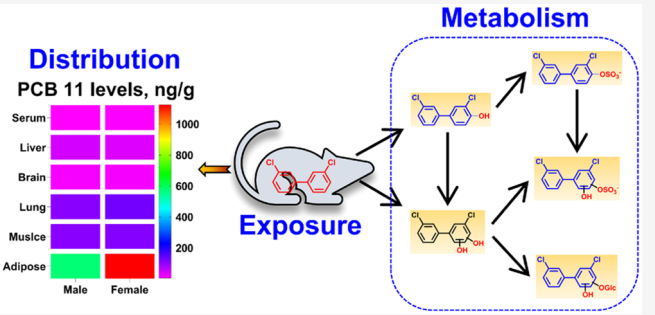
ABSTRACT: PCB 11 (3,3′-dichloro-biphenyl) is an emerging environmental contaminant that represents a public health concern. Here, we investigated the distribution of PCB 11 and its metabolites in mice exposed orally to PCB 11. PCB 11 tissue levels followed the rank order adipose > lung ∼ muscle > liver > brain > blood 4 h after PCB 11 exposure, which varied from the rank order predicted with a composition-based model. We detected hydroxylated and sulfate metabolites in the liver and sulfate and glucuronide metabolites in serum. These findings lay the groundwork for future toxicity studies with PCB 11.



Unintentionally produced polychlorinated biphenyls (PCBs), particularly PCB 11 (3,3′-dichlorobiphenyl), represent an emerging public health concern.[1] PCB 11 is not present in PCB mixtures produced under the trade name Aroclor in the United States, but is present as a major PCB congener in air and a predominant nonlegacy PCB congener in human blood. Humans can be exposed to PCB 11 via the diet or by inhalation. Like other lower chlorinated PCB congeners, PCB 11 is readily metabolized to hydroxylated, sulfated, glucuronidated, and methoxylated metabolites *in vitro* and *in vivo*. PCB 11 and its metabolites are linked to toxic outcomes *in vitro*. For example, PCB 11 and its hydroxylated and sulfated metabolites increase axonal and dendritic growth in primary neurons.[1] However, the disposition of PCB 11 and its metabolites in mice, an important model for *in vivo* PCB toxicity studies, has not been characterized.

The objective of the present study was to investigate the distribution of PCB 11 in male and female mice following acute oral exposure, one of the major routes of PCB exposure, and to identify metabolites present in target tissues to lay the groundwork for future toxicity studies. Male and female C57Bl/6J mice (Jackson Laboratories, Sacramento, CA; 8 weeks of age; N = 6/sex/group) were exposed via the diet to 1.56 mg/kg bodyweight of PCB 11 in peanut oil/peanut butter. Control animals were given peanut oil/peanut butter alone. Similar PCB 95 doses cause adverse outcomes in developmental neurotoxicity studies.[2] Animals were euthanized 4 h after PCB 11 exposure to obtain serum and tissues.

PCB 11 and its metabolites were extracted with acetonitrile and cleaned up with a dispersive solid-phase extraction (dSPE)-based spin filter. A phospholipid SPE cartridge was used to clean up the extract from a pooled (2 g) liver sample instead of the dSPE cartridge. PCB 11 was quantified by gas chromatography−mass spectrometry (GC-MS). Subsequently, PCB 11 metabolites were identified in the same extracts by nontarget-high resolution mass spectrometry (Nt-HRMS) using a subject list.[3] Briefly, selected extracts were analyzed by liquid chromatography-quadrupole time-of-flight mass spectrometry (LC-QTof MS), and metabolites were identified based on their accurate mass and isotope pattern. Samples were subsequently analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) to quantify metabolites identified by Nt-HRMS.

The PCB 11 tissue levels following oral exposure to PCB 11 followed the rank order adipose > lung ∼ muscle > liver > brain > blood, based on the GC-MS analysis (Figure 1A). Similar rank orders in PCB tissue levels have been observed in other disposition studies following acute to chronic oral exposure to higher chlorinated PCB congeners.[4] This rank order reflects the initial distribution to highly perfused organs, such as the muscle, and the blood to tissue partitioning of PCBs based on the lipid content of the tissue. PCB 11 levels showed no sex differences, which is consistent with other disposition studies.[4]

Predictions with composition-based models can approximate the theoretical distribution of chemicals *in vivo*, irrespective of biological uptake effects.[5] Here, PCB 11 tissue levels were predicted from the experimental serum PCB 11 levels and the published content of the sorptive components (i.e., proteins, lipids, and water) of the tissues investigated. The theoretical tissue levels were in good agreement with experimental levels for most tissues, except for the brain and muscle (Figure 1B).

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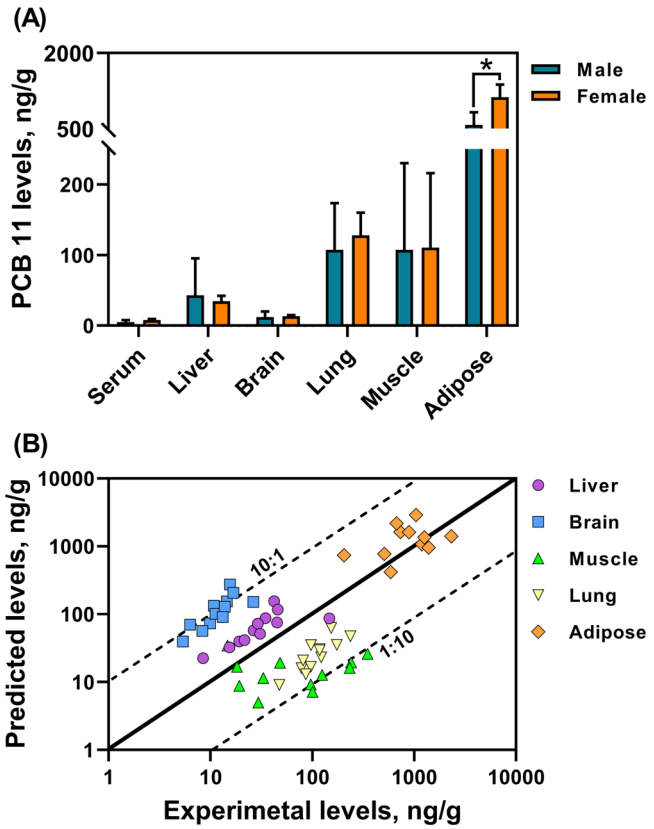

Figure 1. (A) Levels of PCB 11 in selected tissues from male and female mice exposed orally to PCB 11 and (B) comparison of PCB 11 levels predicted using a composition-based model with experimental data. Statistical analyses were performed with a two-tailed student's *t* test with equal variance [$p < 0.05$ (*)]. For more details about the quality assurance/quality control data in PCB 11 analysis, see Table S1.

The observation that PCB 11 levels in the brain are lower than expected is consistent with earlier work.[5] It is likely a result of the different brain lipid composition compared with other tissues. The predicted PCB 11 rank order in the liver, lung, and muscle (liver > lung ~ muscle) reversed the experimental rank order likely because PCB 11 was rapidly biotransformed in the liver. Similarly, the composition-based model most likely does not correctly account for the tissue composition in muscle tissue, thus underestimating PCB tissue levels. These findings suggest that PCB 11 tissue levels can be approximated from serum levels, which may be useful for toxicity studies in *in vivo* models where tissues may not be available for chemical measurements.

Nt-HRMS analyses with LC-QTof MS revealed the presence of several PCB 11 metabolites in serum and liver (Figure 2) based on their accurate mass and the isotope pattern of their molecular ion. We detected 4-PCB 11 sulfate, two hydroxylated isomers of PCB 11 sulfates (OH-PCB 11 sulfate), and a hydroxylated PCB 11 glucuronide in serum. In a large, pooled liver sample, we observed 4-OH-PCB 11 (3,3′-dichlorobiphenyl-4-ol), 4-PCB 11 sulfate (3,3′-dichloro-4-sulfooxybiphenyl), and two OH-PCB 11 sulfate isomers. The hydroxylated (OH−)PCB 11 glucuronide was not detected in the liver sample. The formation of 4-OH-PCB 11 and 4-PCB 11 sulfate was confirmed with authentic standards. No PCB 11 metabolites were observed in other tissues.

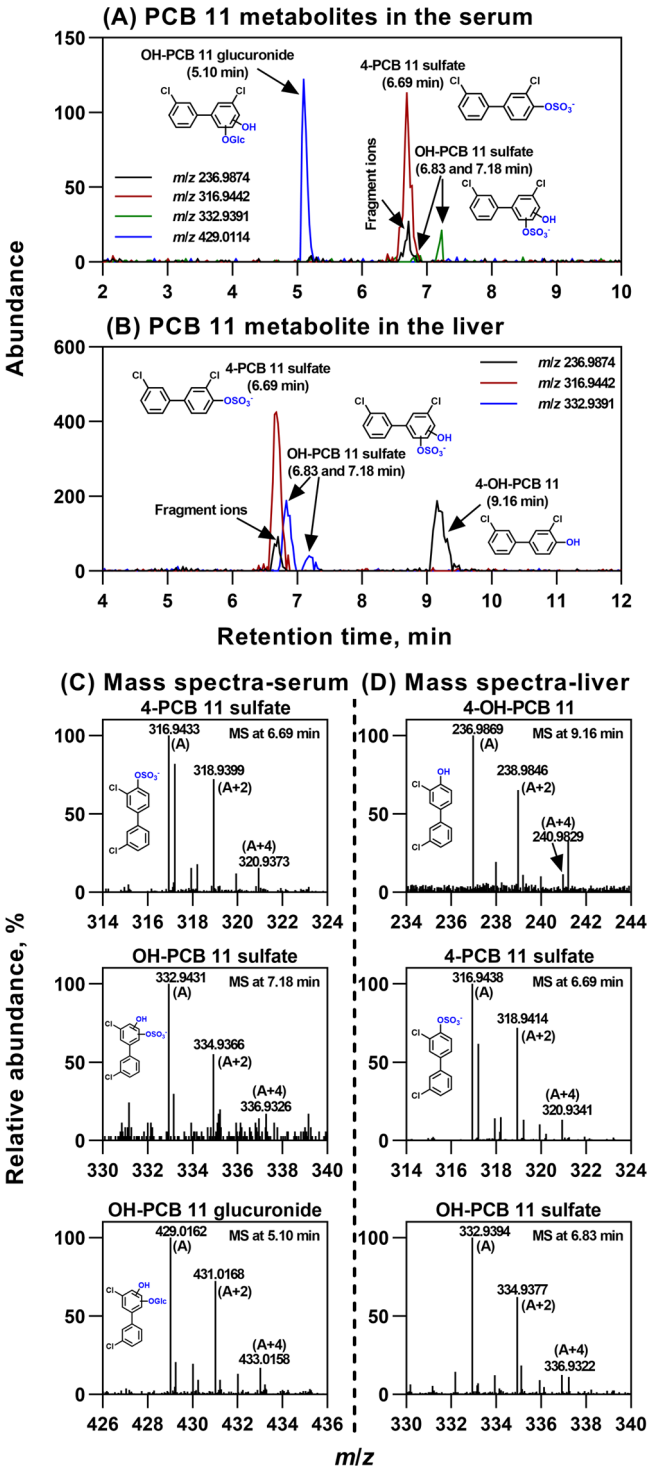

Figure 2. Representative extracted ion chromatograms (EICs) showing the presence of PCB 11 metabolites (i.e., 4-OH-PCB 11, 4-PCB 11 sulfate, OH-PCB 11 sulfate, and OH-PCB 11 glucuronide) in (A) a serum and (B) a pooled liver sample. The presence of these metabolites in (C) the serum and (D) the liver was confirmed on the basis of their accurate mass and the isotope pattern of the molecular ion. LC-QTof MS performed analyses in the negative mode. 4-OH-PCB 11 and 4-PCB 11 sulfate were identified with authentic standards. The EICs are extracted based on the calculated accurate masses of each metabolite class (mass window of 0.1 Da). A, isotopic ion with two ^{35}Cl; A+2, isotopic ion with one ^{35}Cl and one ^{37}Cl; A+4, isotopic ion with two ^{37}Cl. For a list of PCB 11 metabolites detected in mice serum and liver, see Table S2.

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Several of the metabolites were also detected in earlier studies.[3,6] 4-OH-PCB 11 was observed in rats exposed by inhalation to PCB 11 and a small number of human serum samples. 4-PCB 11 sulfate was detected in human serum samples and is a major PCB 11 metabolite formed by human HepG2 liver cells. An OH-PCB 11 sulfate was formed in rats exposed intravenously to 4-PCB 11 sulfate and in HepG2 cells exposed to PCB 11. OH-PCB 11 glucuronides have not been detected previously *in vivo* but are formed by HepG2 cells from PCB 11. Analogous to the metabolites observed in metabolism studies with HepG2 cells, the hydroxylated PCB 11 glucuronide is likely a glucuronidated PCB 11 catechol metabolite.

The number of metabolites detected in the serum and liver from mice is much smaller than the 30 metabolites observed in PCB 11 metabolism studies in HepG2 cells.[3] For example, methoxylated-hydroxylated and methoxylated-dihydroxylated metabolites of PCB 11 and the corresponding sulfate and glucuronide conjugates were not detected in the present study. We hypothesize that these metabolites are rapidly eliminated with the urine or the feces and do not enter the systemic circulation. Consistent with this expectation, an analysis of the fecal PCB metabolite profile detected many PCB metabolites, including diverse methoxylated PCB metabolites.[6] It is also possible that the levels of metabolites were below the limit of detection of the LC-QTof MS system used in this study.

Targeted LC-MS/MS analyses confirmed the metabolites identified in the Nt-HRMS analysis (Figure 3). Serum levels of 4-PCB 11 sulfate were higher than liver levels. The levels of 4-PCB 11 sulfate and the summed OH-PCB 11 sulfates tended to be higher in the serum from male than female mice. No sex differences were observed for 4-PCB 11 sulfate and the summed OH-PCB 11 sulfate levels in the liver and for the OH-PCB 11 glucuronide in the serum. The level of one OH-PCB 11 sulfate isomer eluting at 4.47 min is higher in the serum than in the liver. In contrast, the other isomer eluting at 4.76 min is higher in the liver than serum, indicating the isomer-specific disposition of this metabolite.

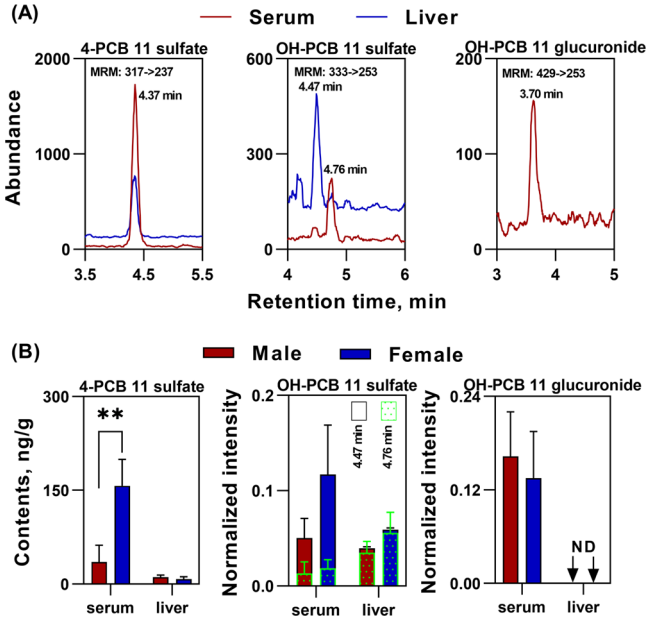

Figure 3. (A) Chromatograms and (B) absolute or relative levels confirm the presence of PCB 11 metabolites in the serum or liver from male and female mice and indicate significant sex differences in the 4-PCB 11 sulfate levels in serum. Samples (n = 3 or 4) were analyzed by tandem MS in the multiple reaction monitoring mode. Statistical analyses were performed with two-tailed student's t test with equal variance [$p < 0.05$ (*) and $p < 0.01$ (**)].

Overall, we detected several PCB 11 metabolites in the serum and liver but not in other tissues of mice exposed orally to PCB 11. This study provides important insights into the disposition of an emerging environmental contaminant in a toxicologically relevant animal model.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.chemrestox.1c00067.

Chemicals and materials; description of the animal study; details regarding the chemical analysis and quality assurance/quality control data; PCB 11 metabolites detected in the Nt-HRMS analysis; and matrix effect in the quantitative analysis (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Hans-Joachim Lehmler − *Department of Occupational and Environmental Health, College of Public Health, University of Iowa, Iowa City, Iowa 52242-5000, United States;* orcid.org/0000-0001-9163-927X; Phone: +1-319-335-4981; Email: hans-joachim-lehmler@uiowa.edu

Authors

Chun-Yun Zhang − *Department of Occupational and Environmental Health, College of Public Health, University of Iowa, Iowa City, Iowa 52242-5000, United States*

Carolyn R. Klocke − *Department of Molecular Biosciences, School of Veterinary Medicine, University of California Davis, Davis, California 95616, United States*

Pamela J. Lein − *Department of Molecular Biosciences, School of Veterinary Medicine, University of California Davis, Davis, California 95616, United States;* orcid.org/0000-0001-7665-7584

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.chemrestox.1c00067

Author Contributions

All authors contributed to the manuscript's writing and have approved the final version of the manuscript.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

PCB	polychlorinated biphenyls
PCB 11	3,3′-dichlorobiphenyl
dSPE	dispersive solid phase extraction
GC-MS	gas chromatography−mass spectrometry
Nt-HRMS	nontarget-high resolution mass spectrometry
LC-QTof MS	liquid chromatography-quadrupole time-of-flight mass spectrometry
LC-MS/MS	liquid-chromatography tandem mass spectrometry
4-OH-PCB 11	3,3′-dichlorobiphenyl-4-ol
4-PCB 11 sulfate	3,3′-dichloro-4-sulfooxy-biphenyl
OH-PCB 11 sulfate	hydroxylated PCB 11 sulfate

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