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Tissue distribution, metabolism and excretion of 3, 3'-dichloro-4'-sulfooxy-biphenyl in the rat

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Abstract

Polychlorinated biphenyls (PCBs) with lower numbers of chlorine atoms exhibit a greater susceptibility to metabolism than their higher-chlorinated counterparts. Following initial hydroxylation of these lower chlorinated PCBs, metabolic sulfation to form PCB sulfates is increasingly recognized as an important component of their toxicology. Since procedures for the quantitative analysis of PCB sulfates in tissue samples have not been previously available, we have now developed an efficient, LC-ESI-MS/MS based, protocol for the quantitative analysis of 4-PCB 11 sulfate in biological samples. This procedure was used to determine the distribution of 4-PCB 11 sulfate in liver, kidney, lung, and brain, as well as its excretion profile, following its intravenous administration to male Sprague-Dawley rats. Following initial uptake of 4-PCB 11 sulfate, its concentration in these tissues and serum declined within the first hour following injection. Although biliary secretion was detected, analysis of 24 hour collections of urine and feces revealed recovery of less than 4% of the administered 4-PCB 11 sulfate. High-resolution LC-MS analysis of bile, urine, and feces showed metabolic products derived from 4-PCB 11 sulfate. Thus, 4-PCB 11 sulfate at this dose was not directly excreted in the urine, but was, instead, re-distributed to tissues and/or subjected to further metabolism.

Keywords

PCB; Polychlorinated Biphenyl; OHPCB; Sulfation; Sulfotransferase; Metabolism

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6. Supporting Information Available

Additional information summarizing the procedure and quality control for quantitative analysis of 4-PCB 11 sulfate from biological samples is available. The supplemental material also includes further data derived from the LC-ESI-HRMS analysis of rat urine and feces, including a list of predicted metabolites and a summary of chemical properties and LC-ESI-HRMS parameters for detected metabolites of 4-PCB 11 sulfate. This information is available free of charge via the internet at <http://pubs.acs.org>.

1. Introduction

The discovery that certain PCB congeners are unintentionally produced during industrial manufacturing processes, for example in paint and pigment production, and subsequently released into the environment by volatilization has raised public health concerns.^{1, 2} The most prominent example of these nonlegacy PCBs is 3,3'-dichlorobiphenyl (PCB 11), a lower-chlorinated congener that was detected in the air profiles of cities such as Chicago and Philadelphia, the Great Lakes area, and even in the Arctic region.² In Chicago air, PCB 11 was found to be among the most abundant congeners and was distributed throughout the metropolitan area with concentrations as high as 140 pg/ m³.^{3, 4} While outdoor air contamination represents a potential environmental hazard, indoor air levels of PCBs have been shown to exceed these concentrations by orders of magnitude and are thus of particular concern.^{5, 6} A recent study reporting detectable concentrations of PCB 11 in about 65 % of children and their mothers living in either East Chicago, Indiana or the Columbus Junction area of Louisa County, Iowa, emphasizes the need for additional studies addressing the toxicological significance of PCB 11 and other nonlegacy PCBs and their metabolites.⁷

Due to the semi-volatile characteristics of the lower-chlorinated PCBs (LC-PCBs), inhalation is the assumed major route of exposure in human populations.⁸ An initial exposure study indicated that [¹⁴C] labeled PCB 11 was readily absorbed in male Sprague-Dawley rats following acute intratracheal instillation.⁸ The radioactivity rapidly distributed in the animals within less than an hour and was detectable in various organs which included muscle, skin, liver, adipose tissue, and the brain. Moreover, the study indicated rapid metabolism of PCB 11. Unfortunately, precise information on the metabolites formed is lacking.

It is well-established that following absorption, LC-PCBs undergo hydroxylation catalyzed by cytochrome P450s.^{9, 10} Depending on the individual congener, PCBs and their hydroxylated metabolites (OHPCBs) are either retained in the body or they are subject to further metabolism mediated by sulfotransferases (SULTs), UDP-glucuronosyl transferases (UGTs) or glutathione *S*-transferases (GSTs).^{11, 12} Several studies have emphasized the role of sulfation as a primary metabolic pathway of OHPCBs *in vitro* and *in vivo*,^{11, 13-15} and a potential use of PCB sulfates as urinary biomarkers for PCB exposure has been hypothesized.¹¹

Among the most prevalent adverse health outcomes associated with PCB exposure are thyroid disrupting effects, primarily serum hypothyroxinemia.^{16, 17} We recently demonstrated the potential of PCB sulfates to bind with high-affinity to T₄ binding sites on the thyroid hormone transporter transthyretin, which may be a major contributing mechanism in environmental contaminant-mediated thyroid disruption.^{18, 19} Importantly, 4-PCB 11 sulfate was among the most potent ligands in these studies, and it bound with higher affinity than its respective OHPCB.

Despite this growing knowledge of the prevalence of lower chlorinated PCBs, surprisingly little is known about the metabolic fate and potential toxicities of these molecules following

exposure and absorption. This may be partially attributed to the lack of available procedures and analytical standards for the qualitative and quantitative analysis of many of these PCB metabolites in biological samples. In the past, quantitative analytical procedures have been developed and optimized for the routine screening of parent PCBs and OHPCBs in environmental and biological samples. However, considering the increased hydrophilicity associated with conjugation of PCBs with polar groups, these procedures are likely inappropriate for the analysis of metabolites such as PCB sulfates, glucuronides and mercapturic acid derivatives. A protocol for the extraction of sulfated metabolites of PCB 3 from rat serum, urine, and feces samples has been recently developed¹¹; however, the applicability of this method for the extraction of PCB sulfates from tissue samples and for different PCB congeners was not examined.

In the present study, we have explored the physiological fate of an individual PCB sulfate conjugate, 4-PCB 11 sulfate, following intravenous injection in male Sprague-Dawley rats. In particular, we utilized quantitative LC-ESI-MS/MS (LC-ESI-TQD) to determine the metabolite's tissue distribution and elimination profile within 24 hours of exposure. Since efficient procedures for the analysis of conjugated PCB metabolites in animal tissues were unavailable, we developed a rapid, quantitative extraction protocol for 4-PCB 11 sulfate from liver, kidney, brain and lung tissues. We further modified this procedure to extract 4-PCB 11 sulfate from serum, bile, urine and feces samples. In addition, we applied qualitative high-resolution mass spectrometry (LC-ESI-HRMS) using a hybrid quadrupole time-of-flight mass analyzer to identify additional metabolites of 4-PCB 11 sulfate and elucidate its metabolism in the rat.

2. Materials and methods

2.1 Chemicals

Ammonium salts of 3,3'-dichloro-4'-sulfooxy-biphenyl (4-PCB 11 sulfate) and 4'-chloro-3'-fluoro-4-sulfooxy-biphenyl (3-F, 4'PCB 3 sulfate) (structures shown in SI Figure S1) were provided by the Synthesis Core of Iowa Superfund Research Program and were synthesized and characterized as described elsewhere.^{11, 18} Both PCB derivatives were of high purity (>99%) as routinely determined by HPLC analysis. Dimethyl sulfoxide (DMSO), acetonitrile, triethylamine, acetic acid and formic acid were purchased from Fisher Scientific (Pittsburgh, PA). All reagents were at least ACS grade.

2.2 *In vivo* exposure study

All animal protocols were approved by the Institutional Animal Care and Use Committee. Male Sprague-Dawley rats were purchased from Harlan, Inc. (Indianapolis, IN). Following seven days of acclimatization, animals were injected via the tail vein with 2 mM 4-PCB 11 sulfate dissolved in 5% (v/v) DMSO in sterile saline. Injection volumes averaged $212 \pm 7 \mu\text{L}$ depending upon the weight of the individual animal. Intravenous injection was chosen as the route of administration due to the fact that exposure to 4-PCB 11 sulfate is most likely through *in vivo* sulfation of the corresponding hydroxylated PCB metabolite rather than by dietary, respiratory, or topical routes. The average weight of the animals at the time of the injections was $247 \pm 8 \text{ g}$ (mean $\pm$ standard deviation). The bodyweight adjusted dose was

575 µg/kg. This dose provided an estimated blood concentration of 25 µM and was based on measured serum concentrations of sulfated metabolites of PCB 3 in a previous *in vivo* study.¹¹ Six rats per control group received intravenous injections of the vehicle without 4-PCB 11 sulfate. Animals were euthanized by carbon dioxide asphyxiation and cervical dislocation approximately three minutes (n = 6), one hour (n = 6) or 24 hours (n = 6) post-injection. Four rats (1 control rat, 3 exposed rats) were individually housed in metabolism cages for 24 hours to allow for the collection of urine and feces. Following euthanasia, brains, lungs, kidneys and livers were collected and immediately frozen in liquid nitrogen. Blood was collected during the necropsies by cardiac puncture and transferred to BD vacutainers (BD, Franklin Lakes, NJ). Blood samples were allowed to coagulate before serum was prepared by centrifugation for 20 minutes at 3000 × g. Intestinal contents were collected from those rats where urine and feces had been obtained. In order to allow for quantification of 4-PCB 11 sulfate and other metabolites in bile, a separate set of four bile duct-cannulated male Sprague-Dawley rats weighing 321 (± 37) g were obtained from Charles River Laboratories (Roanoke, IL). Bile-cannulated rats were acclimatized for four days before they were injected via the tail vein with 4-PCB 11 sulfate (n = 3) or vehicle (n = 1) at concentrations as described above. Bile was collected through the surgically placed catheter during a one-hour time period immediately following injection. All tissues and samples were stored at -75°C until further use.

2.3 Extraction of 4-PCB 11 sulfate from biological samples

Sample extraction and cleanup for 4-PCB 11 sulfate from rat serum was conducted as previously described for the extraction of 4'-PCB 3 sulfate from rat serum.¹¹ The protocol was, however, modified in order to utilize a broader range of biological samples. In the modified procedure, tissues (brains (1.7 ± 0.1 g), kidneys (1.9 ± 0.2 g), and lungs (1.3 ± 0.2 g)), intestinal contents (9.5 ± 1.7 g) and feces (8.9 ± 1 g) were each homogenized in 4 volumes (w/v) of water in a Potter-Elvehjem homogenizer. Livers (10.7 ± 0.8 g) were homogenized in 4 volumes (w/v) of 20 % (v/v) acetonitrile in water, whereas urine (14.3 ± 9.2 ml) was used without initial dilution. Samples of homogenates of liver, brain, kidneys, lungs and urine (1 ml each), and 2 ml samples of homogenates of intestinal contents and fecal samples were transferred to glass test tubes. For analysis of bile, 400 µl samples were diluted by addition of 600 µl water to yield an initial sample volume of 1 ml. To each sample, 200 ng of the internal standard (3-F, 4'-PCB 3 sulfate) dissolved in 100 µl 33% (v/v) acetonitrile were added. Samples were thoroughly mixed and acidified by the addition of one volume of 1 % (v/v) formic acid, followed by the addition of three volumes of acetonitrile. Samples were maintained at 4°C for approximately 2 hours. Samples were then subjected to centrifugation for 30 minutes at 3000 × g and the supernatant fractions were transferred to new test tubes containing 50 mg of NaCl and 150 mg of MgSO₄. The extracts were thoroughly mixed and centrifuged for an additional 30 minutes at 3000 × g to promote phase separation of the aqueous and organic layers. The organic phase was collected and evaporated under a gentle stream of nitrogen to a volume of less than 2 ml. The concentrated organic phase was then transferred to supported liquid extraction cartridges (2 ml capacity, Biotage, Charlotte, NC) and allowed to interact with the column matrix for approximately 5 minutes. The analytes were eluted with 2 × 5 ml of ethyl acetate under vacuum according to

the manufacturer's protocol. The solvent was evaporated almost to dryness, and samples were reconstituted in 500 µl acetonitrile.

2.4 High-performance liquid chromatography

Recovery rates of 4-PCB 11 sulfate from all biological samples were initially determined by spiking the homogenates with either 10 or 20 µM of 4-PCB 11 sulfate instead of internal standard and by subjecting them to the extraction and cleanup steps outlined above. A 20 µl aliquot of each sample was analyzed by HPLC using a Shimadzu Model LC-20-AT liquid chromatograph with an SPD-20-AT UV/VIS detector according to our previously published method and normalized to an authentic standard at the appropriate concentration.¹⁸ However, the gradient (acetonitrile in 0.04 % (v/v) triethylammonium acetate, pH 7.5) was modified to provide shorter analysis times than in the original procedure (0-1 min: 15 % acetonitrile; 1-10 min: 15%-95%; 10-14 min: 95%; 14-15 min: 15%).

2.5 Extraction procedure for mass-spectrometric analysis of unknown PCB metabolites

In order to analyze bile, urine and feces samples for excreted metabolites of 4-PCB 11 sulfate, samples without added internal standard were subjected to both the formic acid acidification and the acetonitrile precipitation steps as outlined above. However, to avoid unintentional loss of certain metabolites during supported liquid extraction, this step was omitted. Instead, the acetonitrile layer was evaporated to the original sample volume and subsequently analyzed by LC-ESI-HRMS (see following paragraph).

2.6 Liquid Chromatography-Mass Spectrometry

The concentration of 4-PCB 11 sulfate in rat tissues, urine, bile and serum samples was determined by quantitative LC-ESI-MS/MS in negative ion mode. 3-F, 4'-PCB 3 sulfate served as the internal standard and due to its presence 4-PCB 11 sulfate concentrations were assumed to be corrected for potential analyte losses during the recovery process. All analyte separations were carried out on an Acquity UPLC BEH C18 column (1.7 µm, 2.1 × 100 mm) (Waters, Milford, MA) connected to an Acquity TQD ultraperformance liquid chromatograph (UPLC) with a coupled triple quadrupole mass analyzer (Waters, Milford, MA) using a linear acetonitrile gradient (0 - 2.38 min: 15 %; 2.38 - 11.38 min: 15% - 95%; 11.38 - 15.38 min: 95%; 15.38 - 15.48 min: 95% - 15% in 0.04 % v/v triethylammonium acetate , pH 7.5) at a flow rate of 0.2 ml/min. The MRM transitions used for quantitation were m/z 317 → m/z 237 for 4-PCB 11 sulfate and m/z 301 → m/z 221.

For the qualitative analysis of metabolites of 4-PCB 11 sulfate, the LC conditions were identical to the procedure described above, but the LC was coupled to a Waters Q-TOF Premier hybrid quadrupole time-of-flight mass analyzer in negative ion mode. Data were collected high resolution conditions using Leucine Enkephalin as a reference mass (lock mass).

2.7 Data Analysis, Quality Assurance, and Quality Control

For each experiment, a standard curve with known 4-PCB 11 sulfate concentrations ranging from 25 nM up to 20,000 nM was determined and utilized for the quantification of the analyte in the rat samples. Standard curves exhibited good and reproducible linearity with r^2

values ranging between 0.97 and 0.99. Sample analysis was performed using MassLynx 4.1 software (Waters, Milford, MA) and all quantitative data were above the software-derived limits of detection (LOD) and limits of quantitation (LOQ). In addition, we calculated the LOD (10 µg/L) by applying values from the standard curve to the equation $LOD = 3.3 \cdot (S_y/K)$. The LOQ (31 µg/L) was obtained by fitting those data to a second equation, $LOQ = 10 \cdot (S_y/K)$.²⁰ In both equations, K is the slope of the best linear fit, whereas S_y represents the standard deviation of each predicted y-value. The recovery of 4-PCB 11 sulfate from different tissues/ matrices was part of the method development for this study and is discussed in section 3.1 (“An efficient extraction for 4-PCB 11 sulfate from biological samples”) of this manuscript. Spiked quality control standards containing known concentrations of 4-PCB 11 sulfate and internal standard and sample blanks were routinely included in multiple-sample runs to assure consistent signal strength, and analyte and internal standard retention times. Quality control standards (317 µg/L) and internal standards (400 µg/L) indicated consistent results with little variation (317 ± 12 µg/L and 400 ± 16 µg/L, n = 8) (SI Figure S5). 4-PCB 11 sulfate and internal standard concentrations in blanks were below the LOD and LOQ.

3. Results

3.1 An efficient extraction for 4-PCB 11 sulfate from biological samples

In order to determine the extraction efficiencies of 4-PCB 11 sulfate from various rat tissues, serum, bile, urine, and feces, we modified the protocol previously published by Dhakal *et al.*¹¹ Samples and homogenates obtained from untreated male Sprague-Dawley rats were spiked with either 10 or 20 µM 4-PCB 11 sulfate and subsequently subjected to mild acidification with 0.5 % (v/v) formic acid, protein precipitation with acetonitrile and supported liquid extraction clean-up as outlined in SI Figure S1A. Subsequently, 4-PCB 11 sulfate concentrations in fully processed extracts were quantified by HPLC. Rates of recovery were determined by normalizing the obtained concentrations to an authentic standard in 33 % (v/v) acetonitrile at the respective concentrations in the original sample. Recovery rates were determined in triplicate and the determined standard deviations indicated overall good reproducibility (SI Figure S1B). The highest recovery rate was obtained from rat serum (92 ± 14 %). Recovery rates for urine (80 ± 1 %), brain (78 ± 5 %), liver (78 ± 4 %), lungs (77 ± 4 %), kidneys (62 ± 6 %), and bile (71 ± 11 %) were slightly lower. The lowest recovery was obtained for the extraction of 4-PCB 11 sulfate from rat feces samples (54 ± 4 %).

3.2 Tissue distribution and elimination of 4-PCB 11 sulfate

Analysis by LC-ESI-MS/MS was employed to determine the concentrations of 4-PCB 11 sulfate in various tissues and body fluids at time points up to 24 hours following administration (SI Figure S1C 1D). 4-PCB 11 sulfate concentration in tissues and fluids of control animals were all below the limit of detection and there were no significant differences in organ weights between control and exposed animals (see Table 1 for a detailed list of tissue and organ weights). Three minutes following injection, approximately 95 % of the 4-PCB 11 sulfate could be detected in the liver (47 ± 15 %), serum (35 ± 8.5 %), kidneys (8.7 ± 2.4 %), lungs (3.9 ± 1 %), and brain (0.6 ± 0.2 %). However, tissue levels of

4-PCB 11 sulfate decreased dramatically at one hour after injection to 2 ± 1 % of the original dose in the liver, 2.1 ± 1.2 % in serum, 1.1 ± 0.5 % in the kidneys, 0.4 ± 0.4 % in the lungs, and 0.7 ± 0.6 % in the brain. Interestingly in the brain, 4-PCB 11 sulfate was clearly detectable after 1 hour in three of the exposed animals (average 1.6 %), whereas the compound was below the LOD in the remaining animals at this time point. Bile samples were available only for the one hour time point and indicated about 24 ± 2.8 % biliary excretion of 4-PCB 11 sulfate within the first 60 minutes following exposure. Altogether, about two thirds of the 4-PCB 11 sulfate were not recoverable at this time point. After 24 hours of exposure, 4-PCB 11 sulfate concentrations in tissues and fluids examined were below the LOD. Only a small percentage (3.7 ± 1.8 %) was recovered from urine during the 24 hour collection period. This low recovery from urine was not due to hydrolysis during the sample collection period, since separate experiments (not shown) indicated that 4-PCB 11 sulfate was stable in rat urine for 24 hours at 25 °C. The intestinal contents and feces samples did not contain detectable levels of 4-PCB 11 sulfate at the 24 hour time point. Thus, only about 4 % of the initial dose was detectable after 24 hours of exposure, and this indicated that 96% was most likely biotransformed to other metabolites before elimination or was present in other tissues that were not examined.

3.3 LC-ESI-HRMS detection of metabolic products derived from 4'PCB 11 sulfate

The lack of a mass balance for 4-PCB 11 sulfate after 1 hour and 24 hours prompted us to analyze bile, urine and feces for the presence of metabolites of 4-PCB 11 sulfate. To maximize the recovery of a variety of potential metabolites, tissue homogenates were acidified and subjected to acetonitrile extraction. Identification of PCB metabolites by matching accurate masses and isotope patterns was achieved through analysis of the acetonitrile extracts by LC-ESI-HRMS. Bile samples contained a characteristic peak at a retention time of 8 minutes that corresponded to 4-PCB 11 sulfate (Figure 1). Mass spectral analysis confirmed the presence of 4-PCB 11 sulfate ($m/z = 316.94$) and its phenolic fragment ion ($m/z = 236.99$) (Figure 2C). By subsequent monitoring of the phenolic fragment ion, we identified two additional compounds with chromatographic retention times of approximately 6 and 10 minutes (Figure 2). The more polar of the two compounds was identified as a PCB 11 glucuronic acid conjugate, based on its retention time ($t_r=6.2$ min), mass-to-charge ratio ($m/z = 413.02$), isotope pattern, and fragment ion in the mass spectrum (Figure 2B), whereas the less polar compound was consistent with 4-OHPCB11, based on accurate mass of the (M-H) $^-$ ion ($m/z = 236.99$), isotope pattern and retention time ($t_r=10.33$ min) using an authentic standard as a reference (Figure 2E). In addition, we identified compounds with masses and isotope patterns consistent with a dihydroxylated PCB 11 metabolite ($m/z = 252.98$) and a mono-sulfated, mono-hydroxylated PCB 11 metabolite ($m/z = 332.94$) eluting after 6 and 8 minutes, respectively, (Figures 1, 2A, and 2D). Similarly, we identified 4-PCB 11 sulfate and 4-OHPCB 11 in urine samples (Figure 2F-G, SI Figure S2) as well as 4-OHPCB 11 and dihydroxylated PCB 11 in feces samples (Figure 2H-I, SI Figure S3). The masses, chemical structures and derived properties in LC-ESI-HRMS analysis (t_r and fragmentation pattern) are summarized in SI Table S2. It should be noted that mass spectrometric analysis does not provide any indications regarding the actual position of the additional substituent on either aryl ring. Consequently, the structures depicted for dihydroxylated and monohydroxylated, mono-sulfated PCB 11 metabolites in

Figures 1-3 should be considered as examples of potential regioisomers containing the indicated substituents on the biphenyl ring system.

Moreover, we examined all samples for evidence of more than 20 additional potential metabolites including intermediates of the mercapturic acid pathway, PCB 11 methyl sulfones, parent PCB 11 and glutathione conjugates (SI Figure S4, SI Table S1). However, none of these additional metabolites were detected in our samples.

4. Discussion

In this study, we examined the tissue distribution and elimination of an individual PCB sulfate following intravenous administration via the tail vein in male Sprague-Dawley rats. 4-PCB 11 sulfate was chosen as a model compound due to several key characteristics. First, the parent congener PCB 11 is an emerging environmental hazard that is abundant in urban air profiles.^{3, 21-23} The public health relevance of PCB 11 and other lower-chlorinated PCBs is also emphasized by the fact that they are actively released into the environment as chemical byproducts in the current production of commercial paints and pigments.² Importantly, PCB 11 has been reported as one of the most frequently detected PCB congeners in blood of children and their mothers in both rural and urban environments.⁷ In rats exposed to PCB 11 by inhalation, PCB 11 has been shown to undergo metabolism to its respective OHPCB metabolite.^{8, 24} While the authors conclude that OHPCB 11 is not retained in rat serum, they indicate its potential for further metabolism. Unfortunately, additional metabolites were not characterized in these studies. However, there is substantial experimental evidence supporting sulfation as a major metabolic pathway for lower-chlorinated PCBs.^{11, 13, 15, 18, 25, 26} Despite these factors, surprisingly little is known about the physiological fate and potential toxicities of PCB sulfates. In beginning to address this gap in our knowledge, we have previously identified PCB sulfates as high-affinity ligands for human transthyretin^{18, 19}, and 4-PCB 11 sulfate was identified as one of the PCB sulfates with an affinity comparable to the physiological ligand, thyroxine. Such binding of PCB sulfates to transthyretin may have implications for thyroid hormone disruption as has been suggested for hydroxylated metabolites of PCBs.²⁷⁻²⁹

While studies on *in vivo* disposition of PCB 3 and PCB 11 have been published^{8, 11, 24, 30}, none of these have focused on the uptake or distribution of individual metabolites. Our observation of the rapid tissue distribution of 4-PCB 11 sulfate following intravenous injection, primarily to the liver and to a lesser extent to the kidneys, lungs, and brain, was consistent with previous findings on the distribution of radioactivity in male Sprague-Dawley rats exposed to [¹⁴C]-PCB 11.^{8, 24} The finding that 4-PCB 11 sulfate concentrations rapidly decreased in all tissues and serum within one hour following injection, and after 24 hours the metabolite was undetectable, was also consistent with those previous studies that demonstrated that PCB 11 and its metabolites were cleared from tissues with half-lives ($t_{1/2}$) of approximately two hours (e.g., lungs: $t_{1/2}$ = 1.9 h; serum: $t_{1/2}$ = 1.8 h; liver: $t_{1/2}$ = 2.1 h).^{8, 24} While only a small percentage of the initial dose was recovered in urine ($4.2 \pm 2\%$) after 24 hours in our study, 4-PCB 11 sulfate was undetectable in feces and intestinal contents after 24 hours. However, the fact that a majority of the initial dose rapidly

concentrated in the liver and that we were able to detect almost one third in bile within the first hour of exposure points towards biliary excretion as a significant excretory route.

An important implication of our results is that they emphasize congener dependent-differences in routes of elimination. While our studies on 4-PCB 11 sulfate suggest a significant uptake and metabolism in tissues as well as biliary excretion, sulfated metabolites of PCB 3 were previously found to be primarily excreted in the urine.³¹ Consequently, our findings indicate that it is not likely that all PCB sulfates could be utilized as universal urinary biomarkers for PCB exposure. In fact, suitable urinary PCB sulfates for use as biomarkers will likely have to be identified and evaluated on an individual congener basis.

The rapid decline of observable 4-PCB 11 sulfate levels to about one third of the initial dose after one hour, and to less than 4 % after 24 hours, can potentially be explained by extensive metabolism, intestinal re-absorption and/ or uptake by tissues not examined in this study. Involvement of either systemic metabolism following intestinal absorption of the PCB sulfate or direct metabolism by intestinal microflora would be consistent with our finding that roughly a third of the dose of 4-PCB 11 sulfate was excreted into the bile within an hour, but it was not present in the fecal matter within the entire exposure duration of 24 hours. The accessibility of positions on both aryl rings of 4-PCB 11 sulfate for enzymatic conversion certainly supports this explanation. By applying mass spectrometry techniques, we were able to detect and identify additional metabolites of 4-PCB 11 sulfate, including a glucuronide conjugate as well as mono- and di-hydroxylated compounds (Figure 3). A search by LC-ESI-HRMS for 14 potential metabolites derived from 4-PCB 11 sulfate did not indicate any additional compounds in the samples examined. However, while the possibility that additional metabolites of 4-PCB 11 sulfate have been formed cannot be entirely excluded, our findings indicate that there are four major metabolites being formed. The structures and proposed pathways for formation of these metabolites are shown in Figure 3.

Our findings indicate that hydrolysis of 4-PCB 11 sulfate to 4-OHPCB 11 can occur *in vivo*, presumably catalyzed by sulfatases. The 4-OHPCB 11 thus formed would be subject to further biotransformation catalyzed by CYPs, SULTs and UGTs. From a toxicological perspective the presence of a dihydroxylated PCB 11 species is particularly significant, since such structures could imply the formation of catechols and toxic quinone intermediates. Besides further metabolism and subsequent excretion, it is possible that 4-PCB 11 sulfate may have partially deposited in other compartments such as adipose tissues or the skin, as was previously demonstrated for [¹⁴C]-PCB 11 following intratracheal administration to rats.⁸

Of particular interest is the detection of 4-PCB 11 sulfate in brain tissue which suggests the metabolite's ability to cross the blood brain barrier. This observation is consistent with previous studies reporting the presence of PCBs and/or metabolites in rat brains^{8, 32}, and deserves further scientific attention in a future study. Interestingly, we observed an increase in the concentration of 4-PCB 11 sulfate in brain tissues from 0.6 ± 0.2 % to 1.6 ± 0.4 % in three of the six exposed animals, whereas in the other three animals, 4-PCB 11 sulfate was cleared from the brain within one hour. This observation is likely reflective of the overall

low analyte concentration in the rat brains, with 4-PCB 11 sulfate levels approaching the LOQ.

The initial dose of 4-PCB 11 sulfate (estimated at 25 μ M in blood) was chosen based on previously determined concentrations of 4-PCB 3 sulfate in the serum of rats exposed to PCB 3.¹¹ While our current study used a single dose that was much lower than that commonly used for examination of the toxicological effects of PCBs^{33, 34}, studies at these concentrations provide important information on the metabolism and distribution of low levels of PCB sulfates. Such low concentrations may be particularly relevant when considering exposures to airborne PCBs. It should also be noted that the scope of this study was strictly focused on the metabolism and physiological fate of a single dose of one PCB sulfate congener. However, populations exposed to PCB 11 and other airborne PCBs, such as children in contaminated school buildings, typically have continuous exposure to complex mixtures containing various PCB congeners and other classes of environmental contaminants. Consequently, continuous exposure to mixtures resembling air profiles, preferably by inhalation, will be needed to develop models appropriate for risk assessment purposes.

In summary, we present an efficient protocol for the extraction and quantification of 4-PCB 11 sulfate from various rat tissues and other biological samples. Using this methodology, we determined the tissue distribution and elimination of 4-PCB 11 sulfate following a bolus intravenous injection in male Sprague-Dawley rats. Importantly, 4-PCB 11 sulfate rapidly distributed into the liver, the kidneys, the lungs and the brain. Within an hour following exposure, the metabolite was undetectable in most tissues but significant amounts were recovered in collected bile samples, indicating uptake and biliary excretion of the PCB sulfate. The low concentrations of 4-PCB 11 sulfate found in the feces, however, suggest that either intestinal reabsorption and/or metabolism may be occurring. Using LC-ESI-HRMS, we elucidated the metabolism of 4-PCB 11 sulfate to a variety of species including OH-PCB sulfates, PCB glucuronides and dihydroxylated PCB metabolites. Thus, these studies provide a firm analytical basis for future determinations of the roles of sulfation in the metabolism, disposition, and toxicology of sulfate esters derived from lower-chlorinated PCBs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

5. Acknowledgements

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7. References

1. Grossman E. Nonlegacy PCBs: pigment manufacturing by-products get a second look. *Environ Health Perspect.* 2013; 121:A86–93. [PubMed: 23454657]
2. Hu D, Hornbuckle KC. Inadvertent polychlorinated biphenyls in commercial paint pigments. *Environ Sci Technol.* 2010; 44:2822–2827. [PubMed: 19957996]
3. Hu D, Lehmler HJ, Martinez A, Wang K, Hornbuckle KC. Atmospheric PCB congeners across Chicago. *Atmos Environ* (1994). 2010; 44:1550–1557. [PubMed: 21918637]
4. Hu D, Martinez A, Hornbuckle KC. Discovery of non-aroclor PCB (3,3'-dichlorobiphenyl) in Chicago air. *Environ Sci Technol.* 2008; 42:7873–7877. [PubMed: 19031874]
5. Frederiksen M, Meyer HW, Ebbehøj NE, Gunnarsen L. Polychlorinated biphenyls (PCBs) in indoor air originating from sealants in contaminated and uncontaminated apartments within the same housing estate. *Chemosphere.* 2012; 89:473–479. [PubMed: 22763332]
6. Heinzow B, Mohr S, Ostendorf G, Kerst M, Korner W. PCB and dioxin-like PCB in indoor air of public buildings contaminated with different PCB sources--deriving toxicity equivalent concentrations from standard PCB congeners. *Chemosphere.* 2007; 67:1746–1753. [PubMed: 17258273]
7. Marek RF, Thorne PS, Wang K, Dewall J, Hornbuckle KC. PCBs and OH-PCBs in serum from children and mothers in urban and rural U.S. communities. *Environ Sci Technol.* 2013; 47:3353–3361. [PubMed: 23452180]
8. Hu X, Adamcakova-Dodd A, Thorne PS. The fate of inhaled (14)C-labeled PCB11 and its metabolites in vivo. *Environ Int.* 2014; 63:92–100. [PubMed: 24275706]
9. Safe S. Polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs): biochemistry, toxicology, and mechanism of action. *Crit Rev Toxicol.* 1984; 13:319–395. [PubMed: 6091997]
10. Kaminsky LS, Kennedy MW, Adams SM, Guengerich FP. Metabolism of dichlorobiphenyls by highly purified isozymes of rat liver cytochrome P-450. *Biochemistry.* 1981; 20:7379–7384. [PubMed: 6798990]
11. Dhakal K, He X, Lehmler HJ, Teesch LM, Duffel MW, Robertson LW. Identification of sulfated metabolites of 4-chlorobiphenyl (PCB3) in the serum and urine of male rats. *Chem Res Toxicol.* 2012; 25:2796–2804. [PubMed: 23137097]
12. Tampal N, Lehmler HJ, Espandiari P, Malmberg T, Robertson LW. Glucuronidation of hydroxylated polychlorinated biphenyls (PCBs). *Chem Res Toxicol.* 2002; 15:1259–1266. [PubMed: 12387623]
13. Ekuase EJ, Liu Y, Lehmler HJ, Robertson LW, Duffel MW. Structure-activity relationships for hydroxylated polychlorinated biphenyls as inhibitors of the sulfation of dehydroepiandrosterone catalyzed by human hydroxysteroid sulfotransferase SULT2A1. *Chem Res Toxicol.* 2011; 24:1720–1728. [PubMed: 21913674]
14. Liu Y, Apak TI, Lehmler HJ, Robertson LW, Duffel MW. Hydroxylated polychlorinated biphenyls are substrates and inhibitors of human hydroxysteroid sulfotransferase SULT2A1. *Chem Res Toxicol.* 2006; 19:1420–1425. [PubMed: 17112228]
15. Liu Y, Smart JT, Song Y, Lehmler HJ, Robertson LW, Duffel MW. Structure-activity relationships for hydroxylated polychlorinated biphenyls as substrates and inhibitors of rat sulfotransferases and modification of these relationships by changes in thiol status. *Drug Metab Dispos.* 2009; 37:1065–1072. [PubMed: 19196841]
16. Patrick L. Thyroid disruption: mechanism and clinical implications in human health. *Altern Med Rev.* 2009; 14:326–346. [PubMed: 20030460]
17. Pearce EN, Braverman LE. Environmental pollutants and the thyroid. *Best Pract Res Clin Endocrinol Metab.* 2009; 23:801–813. [PubMed: 19942155]
18. Grimm FA, Lehmler HJ, He X, Robertson LW, Duffel MW. Sulfated metabolites of polychlorinated biphenyls are high-affinity ligands for the thyroid hormone transport protein transthyretin. *Environ Health Perspect.* 2013; 121:657–662. [PubMed: 23584369]
19. Grimm FA, Lehmler HJ, He X, Robertson LW, Duffel MW. Modulating inhibitors of transthyretin fibrillogenesis via sulfation: Polychlorinated biphenyl sulfates as models. *Chem Biol Interact.* 2015; 228C:1–8. [PubMed: 25595224]

20. Stavova J, Stahl DC, Seames WS, Kubatova A. Method development for the characterization of biofuel intermediate products using gas chromatography with simultaneous mass spectrometric and flame ionization detections. *J Chromatogr A*. 2012; 1224:79–88. [PubMed: 22245174]
21. Persoon C, Peters TM, Kumar N, Hornbuckle KC. Spatial distribution of airborne polychlorinated biphenyls in Cleveland, Ohio and Chicago, Illinois. *Environ Sci Technol*. 2010; 44:2797–2802. [PubMed: 20384374]
22. Guo J, Capozzi SL, Kraeutler TM, Rodenburg LA. Global distribution and local impacts of inadvertently generated polychlorinated biphenyls in pigments. *Environ Sci Technol*. 2014; 48:8573–8580. [PubMed: 24965012]
23. Shang H, Li Y, Wang T, Wang P, Zhang H, Zhang Q, Jiang G. The presence of polychlorinated biphenyls in yellow pigment products in China with emphasis on 3,3'-dichlorobiphenyl (PCB 11). *Chemosphere*. 2014; 98:44–50. [PubMed: 24231041]
24. Hu X, Lehmler HJ, Adamcakova-Dodd A, Thorne PS. Elimination of inhaled 3,3'-dichlorobiphenyl and the formation of the 4-hydroxylated metabolite. *Environ Sci Technol*. 2013; 47:4743–4751. [PubMed: 23582014]
25. Grimm FA, Hu D, Kania-Korwel I, Lehmler HJ, Ludewig G, Hornbuckle KC, Duffel MW, Bergman A, Robertson LW. Metabolism and metabolites of polychlorinated biphenyls. *Crit Rev Toxicol*. 2015; 45:245–272. [PubMed: 25629923]
26. James MO, Ambadapadi S. Interactions of cytosolic sulfotransferases with xenobiotics. *Drug Metab Rev*. 2013; 45:401–414. [PubMed: 24188364]
27. Bergman A, Klasson-Wehler E, Kuroki H. Selective retention of hydroxylated PCB metabolites in blood. *Environ Health Perspect*. 1994; 102:464–469. [PubMed: 8593850]
28. Brouwer A, Morse DC, Lans MC, Schuur AG, Murk AJ, Klasson-Wehler E, Bergman A, Visser TJ. Interactions of persistent environmental organohalogenes with the thyroid hormone system: mechanisms and possible consequences for animal and human health. *Toxicol Ind Health*. 1998; 14:59–84. [PubMed: 9460170]
29. Kodavanti PR, Curras-Collazo MC. Neuroendocrine actions of organohalogenes: thyroid hormones, arginine vasopressin, and neuroplasticity. *Front Neuroendocrinol*. 2010; 31:479–496. [PubMed: 20609372]
30. Dhakal K, Uwimana E, Adamcakova-Dodd A, Thorne PS, Lehmler HJ, Robertson LW. Disposition of phenolic and sulfated metabolites after inhalation exposure to 4-chlorobiphenyl (PCB3) in female rats. *Chem Res Toxicol*. 2014; 27:1411–1420. [PubMed: 24988477]
31. Dhakal K, Adamcakova-Dodd A, Lehmler HJ, Thorne PS, Robertson LW. Sulfate conjugates are urinary markers of inhalation exposure to 4-chlorobiphenyl (PCB3). *Chem Res Toxicol*. 2013; 26:853–855. [PubMed: 23713983]
32. Ness DK, Schantz SL, Hansen LG. PCB congeners in the rat brain: selective accumulation and lack of regionalization. *J Toxicol Environ Health*. 1994; 43:453–468. [PubMed: 7990170]
33. Chang SC, Thibodeaux JR, Eastvold ML, Ehresman DJ, Bjork JA, Froehlich JW, Lau C, Singh RJ, Wallace KB, Butenhoff JL. Thyroid hormone status and pituitary function in adult rats given oral doses of perfluorooctanesulfonate (PFOS). *Toxicology*. 2008; 243:330–339. [PubMed: 18063289]
34. Morse DC, Wehler EK, Wesseling W, Koeman JH, Brouwer A. Alterations in rat brain thyroid hormone status following pre- and postnatal exposure to polychlorinated biphenyls (Aroclor 1254). *Toxicol Appl Pharmacol*. 1996; 136:269–279. [PubMed: 8619235]

Figure 1.

Identification of PCB 11 metabolites in rat bile.

Ion chromatograms were determined by LC-ESI-HRMS and indicate the approximate retention times of all five identified PCB 11 metabolites (**PCB 11 glucuronide**, $t_r=6.22$ min; **PCB 11 sulfate**, $t_r=7.96$ min; **mono-hydroxy PCB 11 sulfate**, $t_r=6.11$ min; **di-hydroxylated PCB 11**, $t_r=8.20$ min; **mono-hydroxylated PCB 11**, $t_r=10.33$ min).

Structures of mono-hydroxylated and mono-sulfated PCB 11 metabolites are representative examples, since mass spectral data will not provide evidence regarding the exact positions of the hydroxyl, sulfate, and glucuronic acid substituents

Figure 2.

LC-ESI-HRMS analysis of the 4-PCB 11 sulfate metabolome in the rat.

Qualitative LC-ESI-HRMS identification of metabolites of 4-PCB 11 sulfate in bile (**A-E**), urine (**F, G**) and feces (**H, I**) samples. Five classes of metabolites were identified: 4-PCB 11 sulfate (**C**, $t_r=7.96$ min; **D**, $t_r=8.20$ min; **F**, $t_r=8.09$ min); PCB 11 glucuronide (**B**, $t_r=6.22$ min); mono-hydroxylated PCB 11 (**E**, $t_r=10.33$ min; **G**, $t_r=10.35$ min; **I**, $t_r=10.35$ min); di-hydroxylated PCB 11 (**D**, $t_r=8.20$ min; **H**, $t_r=8.22$ min); mono-hydroxy PCB 11 sulfate (**A**, $t_r=6.11$ min). Structures of mono-hydroxylated and mono-sulfated PCB 11 metabolites are representative examples, since mass spectral data will not provide evidence regarding the exact positions of the hydroxyl, sulfate, and glucuronic acid substituents [G.A. = Glucuronic Acid]

Figure 3.

Identified urinary, biliary and fecal metabolites of 4-PCB 11 sulfate (**A**) and a proposed model for its metabolism in male Sprague-Dawley rats (**B**). Structures of mono-hydroxylated and mono-sulfated PCB 11 metabolites are representative examples, since mass spectral data will not provide evidence regarding the exact positions of the hydroxyl, sulfate, and glucuronic acid substituents [CYP = cytochrome P450; STS = sulfatase; SULT = sulfotransferase; UGT = UDP-glucuronosyl transferase]

Table 1 Tissue distribution and elimination of 4-PCB 11 sulfate in the rat following a single intravenous dose.^a

Control Animals					Exposed Animals			
	Fluid or Tissue (ml or g)		µg	% of total dose	Fluid or Tissue (ml or g)		µg	% of total dose ^b
Serum	9.34 ± 0.27	3min	< LOD	0	9.27 ± 0.36	3min	50 ± 12	35 ± 8.5
		1 hour	< LOD	0		1 hour	3.0 ± 1.6	2.1 ± 1.2
		24 hours	< LOD	0		24 hours	< LOD	0
Kidneys	1.92 ± 0.14	3min	< LOD	0	1.86 ± 0.16	3min	12 ± 3.4	8.7 ± 2.4
		1 hour	< LOD	0		1 hour	1.6 ± 0.7	1.1 ± 0.5
		24 hours	< LOD	0		24 hours	0.1 ± 0.1	0
Brain	1.66 ± 0.07	3min	< LOD	0	1.69 ± 0.07	3min	0.8 ± 0.3	0.6 ± 0.2
		1 hour	< LOD	0		1 hour	0.9 ± 0.9 ^c	0.7 ± 0.6 ^c
		24 hours	< LOD	0		24 hours	< LOD	0
Lungs	1.31 ± 0.16	3min	< LOD	0	1.3 ± 0.18	3min	5.6 ± 1.4	3.9 ± 1
		1 hour	< LOD	0		1 hour	0.6 ± 0.6	0.4 ± 0.4
		24 hours	< LOD	0		24 hours	< LOD	0
Liver	10.9 ± 0.73	3min	< LOD	0	10.6 ± 0.94	3min	67 ± 21	47 ± 15
		1 hour	< LOD	0		1 hour	2.9 ± 1.4	2 ± 1
		24 hours	< LOD	0		24 hours	< LOD	0
Bile	2.35	3min	N/A	N/A	1.52 ± 0.13	3min	N/A	N/A
		1 hour	< LOD	0		1 hour	34 ± 3.9	24 ± 2.8
		24 hours	N/A	N/A		24 hours	N/A	N/A
Urine	8	3min	N/A	N/A	16.3 ± 10	3min	N/A	N/A
		1 hour	N/A	N/A		1 hour	N/A	N/A
		24 hours	< LOD	0		24 hours	5.3 ± 2.5	3.7 ± 1.8
Feces	8.85	3min	N/A	N/A	9.15 ± 0.98	3min	N/A	N/A
		1 hour	N/A	N/A		1 hour	N/A	N/A

Control Animals				Exposed Animals					
Fluid or Tissue (ml or g)		µg	% of total dose	Fluid or Tissue (ml or g)		µg	% of total dose ^b		
		24 hours	< LOD	0			24 hours	< LOD	0
Intestinal Contents	9.9	3min	N/A	N/A	9.38 ± 2.08	3min	N/A	N/A	
		1 hour	N/A	N/A		1 hour	N/A	N/A	
		24 hours	< LOD	0		24 hours	< LOD	0	

^aConcentrations in bile were determined at 1 hour post-injection, and concentrations in urine, intestinal contents, and feces were determined only at 24 hours post-injection. The numbers of animals used for these determinations are provided in the *Materials and Methods* section. N/A = not available.

^bPercentage assuming an average total dose of 142 µg per animal, based on a target concentration of 575 µg/kg and an average weight of 247 g per animal at the time of the injection.

^c4-PCB 11 sulfate was detectable only in three samples