Published in final edited form as:

Environ Int. 2017 January ; 98: 120–128. doi:10.1016/j.envint.2016.10.023.

Identification of a sulfate metabolite of PCB 11 in human serum

Fabian A. Grimm^{1,2}, Hans-Joachim Lehmler^{1,3}, Wen Xin Koh^{1,4}, Jeanne DeWall³, Lynn M. Teesch⁵, Keri C. Hornbuckle^{1,4}, Peter S. Thorne^{1,3}, Larry W. Robertson^{1,3}, and Michael W. Duffel^{1,2}

¹Interdisciplinary Graduate Program in Human Toxicology, The University of Iowa, Iowa City, Iowa, USA

²Department of Pharmaceutical Sciences and Experimental Therapeutics, College of Pharmacy, The University of Iowa, Iowa City, Iowa, USA

³Department of Occupational and Environmental Health, College of Public Health, The University of Iowa, The University of Iowa, Iowa City, Iowa, USA

⁴Department of Civil and Environmental Engineering, College of Engineering, The University of Iowa, Iowa City, USA

⁵High Resolution Mass Spectrometry Facility, The University of Iowa, Iowa City, USA

Abstract

Despite increasing evidence for a major role for sulfation in the metabolism of lower-chlorinated polychlorinated biphenyls *in vitro* and *in vivo*, and initial evidence for potential bioactivities of the resulting sulfate ester metabolites, the formation of PCB sulfates in PCB exposed human populations had not been explored. The primary goal of this study was to determine if PCB sulfates, and potentially other conjugated PCB derivatives, are relevant classes of PCB metabolites in the serum of humans with known exposures to PCBs. In order to detect and quantify dichlorinated PCB sulfates in serum samples of 46 PCB-exposed individuals from either rural or urban communities, we developed a high-resolution mass spectrometry-based protocol using 4-PCB 11 sulfate as a model compound. The method also allowed the preliminary analysis of these 46 human serum extracts for the presence of other metabolites, such as glucuronic acid conjugates and hydroxylated PCBs. Sulfate ester metabolites derived from dichlorinated PCBs were detectable and quantifiable in more than 20 % of analyzed serum samples. Moreover, we were able to utilize this method to detect PCB glucuronides and hydroxylated PCBs, albeit at lower frequencies than PCB sulfates. Altogether, our results provide initial evidence for the presence of PCB sulfates in human serum. Considering the inability of previously employed analytical protocols for PCBs to extract these sulfate ester metabolites and the concentrations of these

Address correspondence to Michael W. Duffel, Ph.D., Department of Pharmaceutical Sciences and Experimental Therapeutics, College of Pharmacy, The University of Iowa, 115 S. Grand Ave, S325, Iowa City, IA, 52246. Telephone: 319-335-8840. Fax: 319-335-8766. michael-duffel@uiowa.edu.

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Competing financial interests declaration

The authors declare that there are no competing financial interests.

metabolites observed in our current study, our data support the hypothesis that total serum levels of PCB metabolites in exposed individuals may have been underestimated in the past.

Keywords

Polychlorinated Biphenyls; PCB; Metabolism; Exposure Assessment; Sulfation

1. Introduction

Polychlorinated biphenyls (PCBs), a class of hazardous and environmentally persistent industrial chemicals, were mass-produced globally from 1929 until their production was banned, initially in the United States in 1979 (Kaiser and Enserink 2000). Traditionally, PCBs were manufactured and distributed as complex mixtures such as the Aroclors, and these mixtures were often predominantly composed of higher-chlorinated PCBs (HC-PCBs), i.e., those containing more than four chlorine atoms (Erickson and Kaley 2011). These congeners are fairly resistant towards degradation reactions, and, due to their comparably high lipophilicity, HC-PCBs and their hydroxylated metabolites have a significant potential for bioaccumulation in serum and adipose tissues (Barron et al. 1994; Troisi et al. 2001).

Lower-chlorinated PCBs (LC-PCBs) are increasingly recognized as emerging environmental contaminants that are detected in both indoor and outdoor air (Ampleman et al. 2015; Carpenter 2015; Grossman 2013; Herrick et al. 2004; Hu et al. 2010; Lehmann et al. 2015; Wethington and Hornbuckle 2005). In addition, there is accumulating evidence that certain LC-PCBs such as PCB 11 (3,3'-Dichlorobiphenyl, CAS #: 2050-67-1, see figure S1), a byproduct in commercial paint and pigment production, are still actively released into the environment, thereby representing a continuous public health hazard (Hu et al. 2008; Hu and Hornbuckle 2010). Unfortunately, research data regarding human respiratory exposures, the metabolic fates, and potential toxicities of LC-PCBs remain limited (Hu et al. 2008; Lehmann et al. 2015).

The metabolism of PCBs to conjugated metabolites and the potential toxicities of those conjugates remain poorly characterized. LC-PCBs exhibit a high potential for metabolic conversion (Grimm et al. 2015b; Robertson and Ludewig 2011). There is long-standing evidence for the ability of PCBs to be metabolized to their hydroxylated metabolites (OH-PCBs) by hepatic cytochrome P450 monooxygenases (Safe 1984), and the susceptibility of individual OH-PCBs to act as substrates for conjugation reactions mediated by sulfotransferases (SULTs) or UDP-glucuronosyl transferases (UGTs) has also been described (Dhakai et al. 2012; Kester et al. 2000; Liu et al. 2006; Sacco et al. 2008; Tampal et al. 2002; Wang et al. 2005; Wang et al. 2006). Recent *in vitro* and *in vivo* studies revealed SULT-catalyzed sulfation of OH-PCBs as a major reaction in the metabolism of LC-PCBs in the rat (Dhakai et al. 2012; Ekuase et al. 2011; Liu et al. 2011). *In vivo* concentrations of sulfate conjugates were significantly higher than those of glutathione conjugate derivatives and glucuronide metabolites in serum of rats exposed to PCB 3 (Dhakai et al. 2012). Considering the recent evidence for the metabolic conversion of OH-PCBs to PCB sulfates and potentially other conjugates *in vivo*, these metabolites have gained increasing scientific

attention (Dhakal et al. 2012; Dhakal et al. 2013; Grimm et al. 2015b). Moreover, the finding that PCB sulfates are competitive ligands for T₄ binding sites on the thyroid hormone binding protein transthyretin (TTR) *in vitro* indicates their bioactivity and potential toxicological relevance (Grimm et al. 2013, 2015c). However, while sulfation has been suggested as a major metabolic pathway for lower chlorinated PCBs, serum concentrations in exposed human populations had not been previously determined.

Quantitative analytical procedures have not previously been developed for the detection of PCB sulfates in human serum, and concentrations of PCB sulfates in human populations have not been evaluated, despite their reported thyroid disrupting potential as well as their utility as biomarkers of PCB exposure in rats (Dhakal et al. 2013; Dhakal et al. 2014).

Conjugation with a polar functional group is generally expected to result in a decreased octanol/water partition coefficient (logP value) and thus in enhanced hydrophilicity of the resulting PCB metabolites (James 2001). Considering that current standard protocols for the analysis of PCBs in biological samples have been optimized for the extraction of parent PCBs and OH-PCBs into an organic solvent layer (e.g. hexane (Hx), diethyl ether (DEE), methyl-*tert*-butyl ether (MTBE)), the fate of the more hydrophilic metabolites, such as PCB sulfates and PCB glucuronides in these procedures has remained elusive (Burse et al. 1990; Frenich et al. 2000; Hovander et al. 2000; Kania-Korwel et al. 2008). Most extraction protocols include an initial acidification step as well as a lipid cleanup step utilizing concentrated sulfuric acid during the later stages of processing the sample (Bergman et al. 1994; Dirtu and Covaci 2010; Dirtu et al. 2010; Fangstrom et al. 2005b). Should PCB conjugates be co-extracted alongside PCBs and OH-PCBs, their stability under strongly acidic conditions is doubtful. Thus, the possibility that PCB sulfates exhibit poor extraction efficiencies and the potential for acid-mediated conversion of PCB sulfates to their respective OH-PCBs signifies the need to study their behavior in these procedures in order to validate current protocols and to evaluate the requirement for novel procedures to analyze PCB sulfates in biological samples.

In this study, we hypothesized that PCB sulfates are a previously underestimated class of PCB metabolites in the sera of humans exposed to PCBs. To test this hypothesis, we initially evaluated the acid stability and solvent extraction efficiency of sulfate metabolites of OH-PCBs, and demonstrate that while they appear to be stable, they would not be co-extracted, and thus effectively neglected, in conventional methods for PCB and OH-PCB analysis in biological samples. As a feasible alternative we applied an LC-MSMS (UPLC/TQD) approach to quantify dichlorinated PCB sulfates in human serum samples obtained from 46 PCB exposed individuals (Ampleman et al. 2015; Koh et al. 2015; Koh et al. 2016b; Marek et al. 2013; Marek et al. 2014). Structural confirmation of the quantified analytes was achieved using high resolution mass spectrometry (UPLC/QTOF). In addition to detecting and quantitating a sulfate metabolite derived from PCB 11, our findings also provided evidence for the formation of other sulfate and glucuronic acid derivatives of LC-PCBs.

2. Methods

2.1 Chemicals and biologicals

PCBs [4-chlorobiphenyl (PCB 3) and 3,3'-dichlorobiphenyl (PCB 11)], hydroxylated PCBs [4'-hydroxy-4-chlorobiphenyl (4'-OH-PCB 3) and 4-hydroxy-3,3'-dichloro-biphenyl (4-OH-PCB 11)] and PCB sulfates [4-chloro-4'-sulfooxy-biphenyl (4'-PCB 3 sulfate) and 3,3'-dichloro-4'-sulfooxy-biphenyl (4-PCB 11 sulfate)] were synthesized and characterized as described previously (Supplemental Material, Figure S1) (Grimm et al. 2013; Lehmler and Robertson 2001a, b; Li et al. 2010). 4-chloro-3-fluoro-4'-sulfooxy-biphenyl (3-F,4'-PCB 3 sulfate) was synthesized and characterized as reported recently (Dhakal et al. 2012).

Acetonitrile, triethylamine, hexane, acetone, dichloromethane (DCM), acetic acid, formic acid, hydrochloric acid and sulfuric acid were purchased from Fisher Scientific (Pittsburgh, PA). Potassium chloride (KCl), methyl-tert-butyl ether and diethyl ether were obtained from Sigma-Aldrich (St. Louis, MO). All reagents were at least ACS grade. Human serum samples for initial method development were from anonymous male donors obtained from a blood bank in Chicago, IL. These sera were selected as controls due to their analytically proven absence of the analytes of interest. (Norstrom et al. 2010)

46 serum samples from mothers and their daughters were provided by the AESOP Study and originated from samples collected as part of the urban (East Chicago) and rural (Columbus Junction area in East Iowa) cohort studies to characterize population exposure levels to PCBs. Blood collection and serum preparation strictly occurred after informed consent of all study participants (Ampleman et al. 2015; Koh et al. 2015; Koh et al. 2016b; Marek et al. 2013; Marek et al. 2014).

2.2 HPLC separation of PCBs, OH-PCBs and PCB sulfates

The HPLC-based separation and quantification of PCBs, OH-PCBs and PCB sulfates was conducted using a modification of our previously published procedure (Grimm et al. 2013). Briefly, PCB metabolites were separated at a flow rate of 1.5 ml/min on a C18 AQ 5 μ m HPLC column (4.6 $\times$ 250 mm; Grace, Deerfield, IL) using a linear acetonitrile gradient (0–10 min: 15 %; 10–30 min: 15% – 95%; 30–40 min: 95%; 40–45 min: 15%) with triethylammonium acetate (0.04 % v/v, pH 7.5) as the mobile phase (Supplemental Material, Figure S2 A–B). For the determination of the recovery of 4-PCB 11 sulfate and 3-F,4'-PCB 3 sulfate (internal standard) from human serum samples, a more rapid HPLC protocol (0–1 min: 15 % acetonitrile; 1–10 min: 15% – 95%; 10–14 min: 95%; 14–15 min: 15%) was applied with similar resolution. All separations were conducted at room temperature using a Shimadzu LC-20-AT HPLC instrument and analytes were monitored by absorbance measurements at 254 nm using a Shimadzu SPD-20-AT UV/VIS detector.

2.3 Acid stability tests of PCB sulfates

In order to determine the stability of 4'-PCB 3 sulfate and 4-PCB 11 sulfate against acid-mediated hydrolysis, we incubated 20 μ M samples of each standard dissolved in 33 % v/v acetonitrile for 0, 15 and 30 minutes in either 1 M hydrochloric acid (Hovander et al. 2000; Koh et al. 2015; Marek et al. 2013; Marek et al. 2014), 6.25 M (33 % v/v) sulfuric acid (Dirinck et al. 2016; Dirtu et al. 2013), 0.14 M (0.5 % v/v) formic acid or 12 M (44 % v/v)

formic acid (Dhakal et al. 2012; Dhakal et al. 2014; Dirtu et al. 2010). Subsequently, 20 μ l samples were analyzed and quantified by HPLC as described above. Compound stability was then determined by normalizing obtained concentrations to a 20 μ M analytical standard in 33% acetonitrile (representing 100 % recovery).

2.4 Solvent extraction efficiencies of PCB sulfates added to human serum

To evaluate the recovery of PCB sulfates under the extraction protocols used in the analysis of PCBs and hydroxylated PCBs, we spiked 4-PCB 11 sulfate, 4-OH-PCB 11, and PCB 11 in human serum and extracted it using 1:1 (v/v) Hx/MTBE, 3:1 (v/v) Hx/acetone and 1:1 (v/v) Hx/DEE as the extraction solvents. In addition, we included the more polar solvent DCM in our analysis to test the influence of the polarity on the recovery rate of 4-PCB 11 sulfate.

We evaluated the recovery of PCB sulfates using a method optimized for the extraction and analysis of PCBs and OH-PCBs (Hovander et al. 2000; Marek et al. 2013). We spiked 4 g serum samples with 40 nmol of three compounds: PCB 11, 4-OH-PCB 11 and 4-PCB 11 sulfate (dissolved in 33% (v/v) acetonitrile in water). The spiked chemical masses greatly exceeded concentrations of the PCBs and OH-PCBs previously reported for these samples (Koh et al. 2015; Marek et al. 2014). The extracts were acidified with 1 ml of 6 M HCl prior to the addition of 6 ml 2-propanol. Samples were inverted for 5 min, followed by centrifugation for 30 min at 3000 $\times$ g. The aqueous layer was removed and re-extracted three times with either a nonpolar organic solvent, i.e. 1:1 (v/v) Hx/MTBE, 3:1 (v/v) Hx/acetone or 1:1 (v/v) Hx/DEE, or DCM. The solvent was evaporated to a final sample volume of approximately 4 ml, and spiked authentic standards of PCB metabolites were analyzed in 20 μ l samples by HPLC as described above. Recovery of standards was calculated by normalizing obtained concentrations to a 20 μ M analytical standard after subtracting background signals using post extraction serum controls.

2.5 Extraction and cleanup of 4-PCB 11 sulfate from human serum

We evaluated the presence of native 4-PCB 11 sulfate in 46 human serum samples. Samples of human serum (1.0 ml each) were spiked with 40 ng of internal standard, 3-F,4'-PCB 3 sulfate, before they were acidified by the addition of one volume of 1% (v/v) formic acid and extracted with three additional volumes of acetonitrile (Dhakal et al. 2013; Grimm et al. 2015a). The samples were thoroughly mixed and incubated for 2 hours at 4°C, followed by 30 min centrifugation at 3000 $\times$ g. The supernatant was then transferred to a new test tube containing approximately 50 mg of NaCl and 150 mg of MgSO₄. The solution was mixed thoroughly using a vortex mixer for 20 seconds and centrifuged for 30 min at 3000 $\times$ g. The organic layer was collected and evaporated under a stream of nitrogen to a final volume of 0.5 ml.

2.6 Liquid Chromatography-Mass Spectrometry

Analysis of PCB metabolites in human serum samples was accomplished using a combined Liquid Chromatography-Mass Spectrometry approach (Grimm et al. 2015a). Quantitation of 4-PCB 11 sulfate was achieved using an Acquity TQD ultraperformance liquid chromatograph coupled to a triple quadrupole mass analyzer (UPLC/TQD, Waters, Milford,

MA) in negative ion mode. The analyte and internal standard were separated by reversed-phase LC on an Acquity UPLC BEH C18 column (1.7 μ m, 2.1 $\times$ 100 mm) (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 injection volume for each sample was 10 μ l. The column was re-calibrated in 15% ACN in 0.04 % (v/v) triethylammonium acetate, pH 7.5 for 5 min between runs. The ESI capillary voltage was 2.6 kV and source sampling cone was 35 V. The cone gas flow was 100 L/hr. The ESI source temperature was set at 150°C. Nitrogen is used as the desolvation gas on the UPLC/TQD. The desolvation gas flow was set to 600 L/hr at a temperature of 350°C. Retention times for 4-PCB 11 sulfate (analyte) and 3-F,4'-PCB 3 sulfate (internal standard) were 8.36 – 8.43 min and 8.00 – 8.07 min, respectively. Quantitation of 4-PCB 11 sulfate was based on the following MRM transitions: m/z 316.94 $\rightarrow$ m/z 236.98 (4-PCB 11 sulfate) and m/z 301.1 $\rightarrow$ m/z 221.1 (3-F,4'-PCB 3 sulfate). The collision energy used for both MRM transitions was 20 eV and the cone voltage was as listed above in the ESI source parameters. Argon is used as the collision gas on the UPLC/TQD.

To confirm the presence of 4-PCB 11 sulfate, 3-F,4'-PCB 3 sulfate, and other potential PCB metabolites present in human serum, high-resolution mass spectra ranging from 100–1000 m/z ratio were recorded for all samples using a Waters Q-TOF Premier high resolution (HR) mass spectrometer (UPLC/Q-TOF). For all samples, full scan mass spectra were collected over the mass range 100–1000 Da in reflectron mode at an instrument resolution of 10,000 (FWHM). The (M-H)⁻ ion of m/z 554.2615 of Leucine Enkephalin served as a reference (lock) mass in this study. A reference mass spectrum was collected every two minutes throughout each HR LC/MS experiment to ensure mass accuracy. The ESI capillary voltage was set to 3.0 kV and cone voltage was 40 V. Nitrogen gas was used as the desolvation gas, and the cone and desolvation gas flows were set to 30 and 650 L/hr, respectively. The source temperature and desolvation gas temperature were 110°C and 450°C, respectively. Conditions for chromatographic separation were identical to those described for quantitative UPLC/TQD.

2.7 Mass Spectrometry Data Analysis and Quality Control

In order to be consistent in the analysis, we defined a peak as 4-PCB 11 sulfate, when it was within the determined range of observed retention times (t_r) for analytical standards (t_r = 8.35 – 8.43 min for multiple reaction monitoring using UPLC/TQD, t_r = 8.13 – 8.18 min for UPLC/Q-TOF) and the peak height was at least fivefold higher than the noise between 5 min and 12 min. In addition, we evaluated both the mass accuracy of m/z values and the isotopic distribution observed, i.e. the presence and estimated 9:6:1 ratios of A, A+2, and A+4 peaks for dichlorinated chemicals, to confirm the identities of the peaks (A is used to designate the isotopic ion with two ³⁵Cl, A+2 is the isotope with one ³⁵Cl and one ³⁷Cl, and A+4 is the isotope with two ³⁷Cl). 4-PCB 11 sulfate was quantified in serum samples using standard curves of known analyte and internal standard concentrations in 33 % (v/v) acetonitrile, ranging from 0.3 ng/ml – 80 ng/ml. In order to account for matrix effects for each serum sample, quantitation was based on ratios of authentic 4-PCB 11 sulfate standards (or analyte peaks in human serum samples) and 3-F,4'-PCB 3 sulfate, which served as the internal

surrogate recovery standard as described previously (Dhakal et al. 2012; Dhakal et al. 2014). Standard curves exhibited good linearity with r^2 values of 0.96 – 0.99, and a theoretical limit of quantitation (LOQ) of 4 ng/ml (12 nM). The LOQ was determined by fitting the data to the equation $LOQ = 10 \cdot (S_y/K)$ (Stavova et al. 2012). In this equation, K represents the slope of the best linear fit of the standard curve and S_y represents the standard deviation of each predicted y-value. To assure the quality of the recorded mass spectra and MRM chromatograms, we routinely included quality control standards in multi-sample runs. Negative controls included blanks (33% acetonitrile in water), positive controls consisted of 100 nM 4-PCB 11 sulfate authentic standard (100 nM) with added internal standard (40 ng/ml). Both blanks and analytical standards were randomly injected between sample runs in LC-MSMS screenings of human serum extracts to assure the consistency of the recorded signals.

3. Results

3.1 Acid stability of PCB sulfates

The stability of authentic 4'-PCB 3 sulfate and 4-PCB 11 sulfate standards towards acid treatments used in conventional PCB extraction protocols that reflected either the initial pre-extraction treatment with hydrochloric or formic acid, or the post-solvent extraction lipid cleanup step with concentrated sulfuric acid, was evaluated. PCB sulfate concentrations remained unaltered for at least 30 minutes following incubation in either 1 M hydrochloric acid or 0.5 % (v/v) formic acid (Supplemental Material, Figure S2C–D). Interestingly, both model PCB sulfates were even stable upon treatment with up to 44 % (v/v) formic acid. The recovery rates for all of these relatively mild acid treatments were directly comparable to the solvent control, 33 % (v/v) acetonitrile. By contrast, treatment with 33 % (v/v) sulfuric acid resulted in the rapid disappearance of the PCB sulfate (Supplemental Material, Figure S4). Instead, a chromatographic peak corresponding to the respective OH-PCB appeared in these samples, indicating the hydrolysis of PCB sulfates to their OH-PCBs.

3.2 Extraction efficiencies of 4-PCB 11 sulfate from human serum

Recovery of PCB 11 and 4-OH-PCB 11 (n=3) from human serum using Hx/MTBE, Hx/DEE, or DCM as extraction solvents ranged from 89 ± 2 % (DCM) to 100 ± 11 % (Hx/DEE), and from 88 ± 8 % (Hx/acetone) to 108 ± 12 % (Hx/DEE), respectively (Supplemental Material, Figure S2E). By contrast, PCB 11 and 4-OH-PCB 11 exhibited relatively poor recovery rates (77 ± 6 % and 41 ± 2 %, respectively) following their extraction from human serum using Hx/acetone (3:1) (Supplemental Material, Figure S2E). Recovery of 4-PCB 11 sulfate in extractions was poor in all cases, with non-detectable levels in Hx/acetone and Hx/DEE samples. Extraction with Hx/MTBE yielded roughly 15 ± 1 % of 4-PCB 11 sulfate, whereas the highest recovery of this PCB sulfate was obtained upon extraction with DCM (34 ± 2 %). Attempts to extract 4-PCB 11 sulfate from this aqueous waste layer yielded only poor recoveries of 13 ± 2 % (data not shown), likely due to the analyte being trapped in denatured protein. Altogether, these results demonstrate that conventional methods for the extraction of PCBs and OH-PCBs were not effective for the PCB sulfates.

Considering the incompatibility of standard protocols for PCB/OH-PCB analysis in human serum samples, we modified a previously published extraction protocol for PCB sulfates from rat tissues to develop a rapid and efficient analytical procedure for PCB sulfates from human serum samples (Dhakal et al. 2012; Grimm et al. 2015a). Initially, we spiked human serum with 10 μ M 4-PCB 11 sulfate. Utilizing an acetonitrile precipitation followed by aqueous and organic phase separation using NaCl and MgSO_4 , we used HPLC to determine the recovery of 4-PCB 11 sulfate ($83 \pm 6\%$) and of the internal standard, $80 \pm 2\%$ for 3-F, 4'-PCB 3 sulfate, in the organic layer.

3.3 Extraction and mass-spectrometric analysis of human serum samples

We analyzed 46 serum samples obtained from mothers and their children from East Chicago, IN or the Columbus Junction area of Louisa County, IA (Marek et al. 2013). Mass-spectrometric analysis consisted of initial quantification of 4-PCB 11 sulfate by UPLC/TQD, followed by full-scan, high-resolution UPLC/Q-TOF analysis. Quality controls, i.e. spiked samples with known concentrations of 4-PCB 11 sulfate and internal standard consistently indicated excellent technical replicability (average recoveries of $100 \pm 4.7\%$ and $100 \pm 3.9\%$, $n=4$) with little to no variation in the observed analyte/internal standard ratios (Supplemental Material, Figure S3). Likewise, 4-PCB 11 sulfate concentrations in blank samples ($n=20$) were consistently below the LOD.

A summary of the findings is found in Table 1. 4-PCB 11 sulfate was detectable in 10 out of the 46 serum samples. Five samples (# 11, 22, 27, 33, and 45) had 4-PCB 11 sulfate concentrations above the LOQ, ranging between 6.6 and 61 nM. In addition to the quantitative analysis of 4-PCB 11 sulfate, we also analyzed full scan mass spectra of human serum extracts for the presence of mono-hydroxylated ($m/z = 236.98$), mono-hydroxylated and mono-sulfated ($m/z = 332.94$), di-hydroxylated ($m/z = 252.98$) and glucuronidated ($m/z = 236.98$) metabolites of dichlorinated PCBs, based on metabolites previously detected in PCB sulfate-exposed male Sprague-Dawley rats (Grimm et al. 2015a). While none of the samples contained detectable levels of dihydroxylated PCBs or mono-hydroxylated PCB sulfates, mono-hydroxylated PCBs and glucuronic acid conjugates of OH-PCBs could be identified in several samples (Table 1, Figures 1–3). The retention time for a dichlorinated OH-PCB ($t_r = 10.40 - 10.42$ min), which was detectable in three of the human serum samples, correlated with the analytical standard for 4-OH-PCB 11. A potential glucuronic acid conjugate derived from PCB 11 was detectable in six samples. The molecular ion ($m/z = 413.02$) was detectable at a retention time of $t_r = 6.57 - 6.63$ min (Figure 1, Figure 3). In addition, mass spectra indicated the presence of a dichlorinated OH-PCB ($m/z = 236.98$), a result of in-source fragmentation of the glucuronic acid derivative, at the same retention time. Further analysis of these peaks confirmed their high-resolution m/z and chlorine isotope patterns (Figure 2A). In the majority of human serum sample extracts both conjugated (sulfate and glucuronic acid) metabolites (samples 11, 17, 22, 28, 33, and 45) were detectable; however, there were also four samples containing sulfated PCB sulfates without any evidence for the glucuronide conjugates (# 3, 27, 29, 42) (Table 1). In addition to these differences, the ratios between peak areas for sulfated and glucuronidated PCB metabolites differed between samples, with some samples indicating higher concentrations of either the glucuronide (samples 11, 45) or sulfate (sample 29) (Figure 3). There was no

correlation in PCB sulfate concentrations observable between the geographic origin of the serum samples or the age (mother vs child) of the participants.

4. Discussion

Even though standard procedures for the routine extraction and analysis of PCBs and OH-PCB metabolites have been developed and widely applied (Burse et al. 1990; Hovander et al. 2000; Kania-Korwel et al. 2008; Pauwels et al. 1999), the assessment of the potential formation of PCB sulfate metabolites has not been possible with these procedures. Based on their chemical properties, we hypothesized that PCB sulfates were overlooked either due to poor extraction efficiencies in standard protocols or as a result of hydrolysis to their OH-PCB precursors during one of the acid treatment steps.

In order to exclude the potential for acid-mediated hydrolysis of the sulfate ester to its corresponding phenol, and therefore to exclude the possibility that PCB sulfates were incorrectly quantified as OH-PCBs, we tested the stability of 4'-PCB 3 sulfate and 4-PCB 11 sulfate under these conditions. (Dhakal et al. 2012; Hovander et al. 2000; Marek et al. 2013; Pauwels et al. 1999). The results of our acid stability study clearly indicated that PCB sulfates are not being hydrolyzed to their OH-PCB precursors in this initial mild acidification step (Supplemental Material, Figure S2 C–D). Consequently, PCB sulfates, if present in the original sample, are not destroyed prior to the solvent extraction step.

We subsequently determined the partitioning of 4-PCB 11 sulfate from human serum into commonly applied solvents: 1:1 (v/v) Hx/MTBE, 3:1 (v/v) Hx/acetone, 1:1 (v/v) Hx/DEE and in the more polar solvent, DCM (Burse et al. 1990; Dirtu and Covaci 2010; Frenich et al. 2000; Hovander et al. 2000). In these experiments, 4-OH-PCB 11 and PCB 11 were quantitatively extracted from human serum into all solvents, whereas 4-PCB 11 sulfate was not quantitatively extracted by the traditionally utilized organic solvent combinations (Supplemental Material, Figure S2E). As expected, a more polar solvent such as DCM marginally improved the recovery of 4-PCB 11 sulfate, but only to $34 \pm 2\%$, thereby providing further evidence that conventional solvent extraction methods are not suitable for the quantitative analysis of PCB sulfates.

The low extraction efficiency of PCB sulfates from human serum indicates that PCB sulfates, if present in the original samples, will likely be discarded in the aqueous layer along with precipitated protein, which would be in agreement with their increased hydrophilicity as compared to hydroxylated and parent PCBs (James 2001). However, attempts to extract 4-PCB 11 sulfate from this aqueous waste layer yielded only poor recoveries of $13 \pm 2\%$ (data not shown). Based on these findings, we could not conclude that workup of the aqueous layer from conventional extraction of PCBs and OH-PCBs represents a viable option for the analysis of PCB sulfates. Due to the low extraction efficiencies of PCB sulfates in conventional procedures for analysis of PCBs and OH-PCBs, it is also unlikely that hydrolysis of PCB sulfates in the sulfuric acid treatments utilized during the later lipid cleanup steps will yield significant additional quantities of OH-PCBs.

As a result of these findings, we modified a recently developed procedure for the extraction and purification of PCB sulfates from rat tissues and fluids for the routine screening of human serum samples. Using initial sample volumes of 1000 μ l, we found that a combination approach utilizing protein precipitation with acetonitrile followed by subsequent separation of aqueous and acetonitrile layers in the presence of NaCl and MgSO_4 results in quantitative extraction of the analyte into the organic phase.

Moreover, the resulting samples were compatible with quantitative liquid chromatography tandem mass spectrometry. Considering a calculated LOQ of approximately 4 ng/ml (12 nM), our procedure will be capable of detecting PCB sulfates at concentrations comparable to those determined for some higher-chlorinated parent PCBs and OH-PCBs (Fangstrom et al. 2005a; Hovander et al. 2006) but not at concentration ranges previously determined for PCB methyl sulfones which are typically found to be two or more orders of magnitude lower than their respective parent PCBs (Grimm et al. 2015b). Previous studies in rats indicated that concentrations of sulfated metabolites of PCB 3 significantly exceeded those determined for its hydroxylated precursor and for the parent PCBs (Dhakal et al. 2012). Thus, the analytical procedure developed for the current studies may be capable of detecting a variety of relevant PCB sulfates in exposed human populations. However, considering that we utilized (dichlorinated) 4-PCB 11 sulfate as a model compound, rigorous examination of the extraction efficiencies of higher-chlorinated (tri-, tetra-) PCB sulfates will be required for future quantitation of these sulfated congeners.

Using our approach for extraction and high resolution UPLC/Q-TOF, we analyzed 46 serum samples from mothers and their daughters from East Chicago and the Columbus Junction area of Louisa County, IA. The rationale for the sample selection was that these individuals' exposures to PCBs have been established, and serum samples were available due to their participation in the Iowa Superfund Research Program's AESOP Study (Ampleman et al. 2015; Koh et al. 2015; Marek et al. 2013; Marek et al. 2014). AESOP Study participants have mean (std dev) Σ_{209} PCB levels of 0.5050 (0.5164) ng/g f.w. (Koh et al. 2016b) and Σ_{64} OH-PCB levels of 0.0637 (0.0463) ng/g f.w. (Koh et al. 2016a).

In more than 20 % of the samples (n=10), the dichlorinated PCB 11 sulfate could be clearly identified based on retention time, accurate mass determination, and chlorine isotope pattern. Quantitative analysis of these samples indicated PCB sulfate concentrations in the low nanomolar range. However, it should be noted that there were an additional nine samples in which peaks with retention times and m/z ratio corresponding to the PCB sulfate were detected. However, these nine chromatographic peaks were below the defined LOD, and in most cases the isotope pattern could not be determined due to suppression of the signal of the A+2 and A+4 peaks by other interfering compounds. Altogether, these findings demonstrate that one or more dichlorinated PCB sulfates with a retention time matching PCB 11 sulfate was present in at least 20 %, and potentially up to 40 %, of all analyzed samples. The numbers agree well with previously observed detection frequencies (~40%) of parent PCB 11 in AESOP study serum samples (Koh et al. 2015). PCB sulfate levels were not found to be correlated between mothers and daughters or geographic origin, a finding that agrees well with previous quantitative analyses for OH-PCBs and parent PCB congeners

(Marek et al. 2014), even though more recent evidence reports moderate correlation of detectable PCB and OH-PCB serum levels and community of residence (Koh et al. 2016b).

Our results also indicate the need to further increase the sensitivity, i.e. decrease LODs and LOQs, of the UPLC/TQD procedure to be able to detect and quantify suspected sulfated PCB metabolites in additional samples. In fact, it is possible that the current methodology is only capable of measuring PCB sulfates in those samples with relatively high total PCB concentrations, as PCB levels in human serum samples were previously shown to vary quite significantly (Koh et al. 2015; Marek et al. 2013; Marek et al. 2014). Future studies are therefore required to improve the sample extraction process to decrease the LOD and LOQ to picomolar concentrations and to identify relevant metabolites derived from mono-, and higher-chlorinated PCB congeners.

In addition to sulfate conjugates of OH-PCBs, LC-MS analysis of human serum extracts resulted in the identification of glucuronic acid derivatives of dichlorinated OH-PCBs. Thus, our results clearly indicate that conjugates of lower-chlorinated PCBs are relevant metabolites in exposed human populations. However, the lack of quantitative LC-MSMS data for PCB glucuronides is due to the unavailability of authentic analytical standards. Synthesis of glucuronic acid derivatives of PCBs could potentially aid the development of quantitative procedures for these conjugates.

From a public health perspective, our findings indicate that LC-PCBs may not be just transient congeners that are rapidly excreted, but that metabolism plays an important role in their yet-to-be determined physiologic fate. The concentration range at which we were able to detect dichlorinated PCB sulfates in human serum suggests that conjugated metabolites of LC-PCBs could significantly contribute to the overall PCB concentration, a finding that challenges current estimates of the total body burden of PCBs in exposed individuals. As a result, the findings presented herein demand further research into analytical method development to investigate the role and contribution of LC-PCBs and their metabolism and metabolites, as well as the associated potential for bioaccumulation and/or excretion, in PCB-exposed populations. Likewise, in order to appropriately represent monitoring data to risk assessors, a future challenge will be the development of novel procedures for comprehensive metabolite profiling in the routine monitoring of PCBs in exposed human populations. Similarly, more research is required to expand our current knowledge about potential bioactivities associated with these sulfate metabolites. Previous studies have clearly indicated the potential of PCB sulfates to interact with two major serum transport proteins, albumin (Rodriguez et al. 2016) and TTR (Grimm et al. 2013, 2015c) *in vitro*. Binding affinities to both proteins suggest potential mechanisms for the retention and inter-tissue transport of PCB metabolites. In the case of TTR, binding of certain PCB sulfates, including 4-PCB 11 sulfate, to the high-affinity binding site occurred with dissociation constants in the low nanomolar range, and consistent with previous reports on their hydroxylated precursors and the natural ligand, L-thyroxine (Ferguson et al. 1975; Purkey et al. 2004). Considering PCB sulfate serum concentrations were detectable at nanomolar concentrations, it can be reasonably speculated that these metabolites will be capable of binding to the high-affinity binding site on human TTR as well as binding sites on human serum albumin. However, considering human serum concentrations of these transport proteins in the micromolar

range, competitive interactions with the natural ligand L-thyroxine, which is a contributing factor in chemical-induced thyroid disruption, will depend on the cumulative sum of all xenobiotic ligands available in the blood of an exposed individual, an analysis that is outside the scope of this study (Porterfield 2000).

Altogether, their potential for inter-tissue transport, further metabolism, and additional biological activities indicates a need to better understand the physiologic fate and potential toxicities associated with LC-PCBs and their metabolites.

5. Conclusion

In summary, we demonstrate the inapplicability of current standard procedures for the routine assessment of PCBs and OH-PCB metabolites when applied to the determination of sulfated metabolites of OH-PCBs. This is due to the inability to quantitatively extract these metabolites from biological samples using previous protocols. Thus, we successfully adapted an alternative extraction procedure and high-resolution mass spectrometry for the qualitative and quantitative evaluation of sulfate metabolites derived from dichlorinated OH-PCBs in human serum. Analysis of these dichlorinated PCB 11 sulfates in serum samples of 46 PCB-exposed individuals from both rural and urban communities revealed detectable and quantifiable metabolite concentrations in the low nanomolar range in more than 20 % of the analyzed serum samples. The method also allowed the preliminary analysis of these 46 human serum extracts for the presence of other metabolites, such as glucuronic acid conjugates and hydroxylated PCBs. Semi-quantitative comparison of PCB metabolites in serum obtained from multiple individuals revealed inter-individual variability in their metabolic profiles. Thus, our findings provide initial evidence concerning the role of PCB sulfates and glucuronic acid derivatives as relevant metabolites in serum of PCB-exposed individuals and support the hypothesis that total serum levels of PCBs and their various metabolites in exposed individuals may have been underestimated in the past.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We would like to thank participants in the AESOP Study and Study staff (Barb Mendenhall and Nancy Morales). We acknowledge Dr. Xueshu Li of the Synthesis Core of the Iowa Superfund Research Program for providing us with authentic standards of several PCB metabolites. This work was supported by NIH grant P42 ES013661 from the National Institute of Environmental Health Sciences and the University of Iowa Environmental Health Sciences Research Center (NIH P30 ES005605).

List of Abbreviations

DCM	Dichloromethane
DEE	diethyl ether
Hx	Hexane
KCl	potassium chloride

LC-PCB	lower-chlorinated PCB
MRM	multiple reaction monitoring
MTBE	methyl- <i>tert</i> -butyl ether
OH-PCB	hydroxylated PCB
PCB	polychlorinated biphenyl
SULT	cytosolic sulfotransferase
T4	3,3',5,5'-tetraiodo-L-thyronine/L-thyroxine
TTR	transthyretin

References

- Ampleman MD, Martinez A, DeWall J, Rawn DF, Hornbuckle KC, Thorne PS. Inhalation and dietary exposure to PCBs in urban and rural cohorts via congener-specific measurements. *Environ Sci Technol*. 2015; 49:1156–1164. [PubMed: 25510359]
- Barron MG, Yurk JJ, Crothers DB. Assessment of Potential Cancer Risk from Consumption of PCBs Bioaccumulated in Fish and Shellfish. *Environ Health Perspect*. 1994; 102:562–567. [PubMed: 9679116]
- Bergman A, Klasson-Wehler E, Kuroki H. Selective retention of hydroxylated PCB metabolites in blood. *Environ Health Perspect*. 1994; 102:464–469. [PubMed: 8593850]
- Burse VW, Head SL, Korver MP, McClure PC, Donahue JF, Needham LL. Determination of selected organochlorine pesticides and polychlorinated biphenyls in human serum. *J Anal Toxicol*. 1990; 14:137–142. [PubMed: 1695698]
- Carpenter DO. Exposure to and health effects of volatile PCBs. *Rev Environ Health*. 2015; 30:81–92. [PubMed: 25822318]
- 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]
- 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]
- 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]
- Dirinck E, Dirtu AC, Malarvannan G, Covaci A, Jorens PG, Van Gaal LF. A Preliminary Link between Hydroxylated Metabolites of Polychlorinated Biphenyls and Free Thyroxine in Humans. *Int J Environ Res Public Health*. 2016; 13:421. [PubMed: 27089353]
- Dirtu AC, Covaci A. Estimation of daily intake of organohalogenated contaminants from food consumption and indoor dust ingestion in Romania. *Environ Sci Technol*. 2010; 44:6297–6304. [PubMed: 20704229]
- Dirtu AC, Jaspers VL, Cernat R, Neels H, Covaci A. Distribution of PCBs, their hydroxylated metabolites, and other phenolic contaminants in human serum from two European countries. *Environ Sci Technol*. 2010; 44:2876–2883. [PubMed: 20384379]
- Dirtu AC, Dirinck E, Malarvannan G, Neels H, Van Gaal L, Jorens PG, et al. Dynamics of organohalogenated contaminants in human serum from obese individuals during one year of weight loss treatment. *Environ Sci Technol*. 2013; 47:12441–12449. [PubMed: 24074050]
- 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]
- Erickson MD, Kaley RG 2nd. Applications of polychlorinated biphenyls. *Environ Sci Pollut Res Int*. 2011; 18:135–151. [PubMed: 20848233]
- Fangstrom B, Hovander L, Bignert A, Athanassiadis I, Linderholm L, Grandjean P, et al. Concentrations of polybrominated diphenyl ethers, polychlorinated biphenyls, and polychlorobiphenyls in serum from pregnant Faroese women and their children 7 years later. *Environ Sci Technol*. 2005a; 39:9457–9463. [PubMed: 16475322]
- Fangstrom B, Strid A, Grandjean P, Weihe P, Bergman A. A retrospective study of PBDEs and PCBs in human milk from the Faroe Islands. *Environ Health*. 2005b; 4:12. [PubMed: 16014177]
- Ferguson RN, Edelhofer H, Saroff HA, Robbins J, Cahnmann HJ. Negative cooperativity in the binding of thyroxine to human serum prealbumin. Preparation of tritium-labeled 8-anilino-1-naphthalenesulfonic acid. *Biochemistry*. 1975; 14:282–289. [PubMed: 1120103]
- Frenich AG, Vidal JL, Frias MM, Olea-Serrano F, Olea N. Quantitative determination of endocrine-disrupting polychlorinated biphenyls and organochlorinated pesticides in human serum using gas chromatography with electron-capture detection and tandem mass spectrometry. *J Mass Spectrom*. 2000; 35:967–975. [PubMed: 10972996]
- 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]
- Grimm FA, He X, Teesch LM, Lehmler HJ, Robertson LW, Duffel MW. Tissue Distribution, Metabolism, and Excretion of 3,3'-Dichloro-4'-sulfooxy-biphenyl in the Rat. *Environ Sci Technol*. 2015a; 49:8087–8095. [PubMed: 26046945]
- Grimm FA, Hu D, Kania-Korwel I, Lehmler HJ, Ludewig G, Hornbuckle KC, et al. Metabolism and metabolites of polychlorinated biphenyls. *Crit Rev Toxicol*. 2015b; 45:245–272. [PubMed: 25629923]
- 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*. 2015c; 228:1–8. [PubMed: 25595224]
- Grossman E. Nonlegacy PCBs: pigment manufacturing by-products get a second look. *Environ Health Perspect*. 2013; 121:A86–93. [PubMed: 23454657]
- Herrick RF, McClean MD, Meeker JD, Baxter LK, Weymouth GA. An unrecognized source of PCB contamination in schools and other buildings. *Environ Health Perspect*. 2004; 112:1051–1053. [PubMed: 15238275]
- Hovander L, Athanasiadou M, Asplund L, Jensen S, Wehler EK. Extraction and cleanup methods for analysis of phenolic and neutral organohalogenes in plasma. *J Anal Toxicol*. 2000; 24:696–703. [PubMed: 11110024]
- Hovander L, Linderholm L, Athanasiadou M, Athanassiadis I, Bignert A, Fangstrom B, et al. Levels of PCBs and their metabolites in the serum of residents of a highly contaminated area in eastern Slovakia. *Environ Sci Technol*. 2006; 40:3696–3703. [PubMed: 16830529]
- Hu D, Martinez A, Hornbuckle KC. Discovery of non-aro-chlor PCB (3,3'-dichlorobiphenyl) in Chicago air. *Environ Sci Technol*. 2008; 42:7873–7877. [PubMed: 19031874]
- Hu D, Hornbuckle KC. Inadvertent polychlorinated biphenyls in commercial paint pigments. *Environ Sci Technol*. 2010; 44:2822–2827. [PubMed: 19957996]
- Hu D, Lehmler HJ, Martinez A, Wang K, Hornbuckle KC. Atmospheric PCB congeners across Chicago. *Atmos Environ*. 2010; 44:1550–1557.
- James, MO. Polychlorinated Biphenyls: Metabolism and Metabolites. In: Robertson, LW.; Hansen, LG., editors. *PCBs: Recent advances in environmental toxicology and health effects*. Lexington, KY: University of Kentucky Press; 2001. p. 35–46.
- Kaiser J, Enserink M. Environmental toxicology. Treaty takes a POP at the dirty dozen. *Science*. 2000; 290:2053. [PubMed: 11187821]
- Kania-Korwel I, Zhao H, Norstrom K, Li X, Hornbuckle KC, Lehmler HJ. Simultaneous extraction and clean-up of polychlorinated biphenyls and their metabolites from small tissue samples using pressurized liquid extraction. *J Chromatogr A*. 2008; 1214:37–46. [PubMed: 19019378]

- Kester MH, Bulduk S, Tibboel D, Meinel W, Glatt H, Falany CN, et al. Potent inhibition of estrogen sulfotransferase by hydroxylated PCB metabolites: a novel pathway explaining the estrogenic activity of PCBs. *Endocrinology*. 2000; 141:1897–1900. [PubMed: 10803601]
- Koh WX, Hornbuckle KC, Thorne PS. Human Serum from Urban and Rural Adolescents and Their Mothers Shows Exposure to Polychlorinated Biphenyls Not Found in Commercial Mixtures. *Environ Sci Technol*. 2015; 49:8105–8112. [PubMed: 26053216]
- Koh WX, Hornbuckle KC, Marek RF, Wang K, Thorne PS. Hydroxylated polychlorinated biphenyls in human sera from adolescents and their mothers living in two U.S. Midwestern communities. *Chemosphere*. 2016a; 147:389–395. [PubMed: 26774304]
- Koh WX, Hornbuckle KC, Wang K, Thorne PS. Serum polychlorinated biphenyls and their hydroxylated metabolites are associated with demographic and behavioral factors in children and mothers. *Environ Int*. 2016b; 94:538–545. [PubMed: 27352881]
- Lehmann GM, Christensen K, Maddaloni M, Phillips LJ. Evaluating health risks from inhaled polychlorinated biphenyls: research needs for addressing uncertainty. *Environ Health Perspect*. 2015; 123:109–113. [PubMed: 25302536]
- Lehmle HJ, Robertson LW. Synthesis of hydroxylated PCB metabolites with the Suzuki-coupling. *Chemosphere*. 2001a; 45:1119–1127. [PubMed: 11695625]
- Lehmle HJ, Robertson LW. Synthesis of polychlorinated biphenyls (PCBs) using the Suzuki-coupling. *Chemosphere*. 2001b; 45:137–143. [PubMed: 11572605]
- Li X, Parkin S, Duffel MW, Robertson LW, Lehmle HJ. An efficient approach to sulfate metabolites of polychlorinated biphenyls. *Environ Int*. 2010; 36:843–848. [PubMed: 19345419]
- Liu Y, Apak TI, Lehmle 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]
- Liu Y, Lehmle HJ, Robertson LW, Duffel MW. Physicochemical properties of hydroxylated polychlorinated biphenyls aid in predicting their interactions with rat sulfotransferase 1A1 (rSULT1A1). *Chem Biol Interact*. 2011; 189:153–160. [PubMed: 21130751]
- 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]
- Marek RF, Thorne PS, DeWall J, Hornbuckle KC. Variability in PCB and OH-PCB serum levels in children and their mothers in urban and rural U.S. communities. *Environ Sci Technol*. 2014; 48:13459–13467. [PubMed: 25300024]
- Norstrom K, Czub G, McLachlan MS, Hu D, Thorne PS, Hornbuckle KC. External exposure and bioaccumulation of PCBs in humans living in a contaminated urban environment. *Environ Int*. 2010; 36:855–861. [PubMed: 19394084]
- Pauwels A, Wells DA, Covaci A, Schepens PJ. Improved sample preparation method for selected persistent organochlorine pollutants in human serum using solid-phase disk extraction with gas chromatographic analysis. *J Chromatogr B Biomed Sci Appl*. 1999; 723:117–125. [PubMed: 10080639]
- Porterfield SP. Thyroidal dysfunction and environmental chemicals—potential impact on brain development. *Environ Health Perspect*. 2000; 108(Suppl 3):433–438. [PubMed: 10852841]
- Purkey HE, Palaninathan SK, Kent KC, Smith C, Safe SH, Sacchettini JC, et al. Hydroxylated polychlorinated biphenyls selectively bind transthyretin in blood and inhibit amyloidogenesis: rationalizing rodent PCB toxicity. *Chem Biol*. 2004; 11:1719–1728. [PubMed: 15610856]
- Robertson LW, Ludewig G. Polychlorinated Biphenyl (PCB) carcinogenicity with special emphasis on airborne PCBs. *Gefahrst Reinhalt Luft*. 2011; 71:25–32. [PubMed: 21686028]
- Rodriguez EA, Li X, Lehmle HJ, Robertson LW, Duffel MW. Sulfation of Lower Chlorinated Polychlorinated Biphenyls Increases Their Affinity for the Major Drug-Binding Sites of Human Serum Albumin. *Environ Sci Technol*. 2016; 50:5320–5327. [PubMed: 27116425]
- Sacco JC, Lehmle HJ, Robertson LW, Li W, James MO. Glucuronidation of polychlorinated biphenyls and UDP-glucuronic acid concentrations in channel catfish liver and intestine. *Drug Metab Dispos*. 2008; 36:623–630. [PubMed: 18180271]

- Safe S. Polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs): biochemistry, toxicology, and mechanism of action. *Crit Rev Toxicol.* 1984; 13:319–395. [PubMed: 6091997]
- 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]
- 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]
- Troisi GM, Haraguchi K, Kaydoo DS, Nyman M, Aguilar A, Borrell A, et al. Bioaccumulation of polychlorinated biphenyls (PCBs) and dichlorodiphenylethane (DDE) methyl sulfones in tissues of seal and dolphin morbillivirus epizootic victims. *J Toxicol Environ Health A.* 2001; 62:1–8. [PubMed: 11205532]
- Wang LQ, Lehmler HJ, Robertson LW, Falany CN, James MO. In vitro inhibition of human hepatic and cDNA-expressed sulfotransferase activity with 3-hydroxybenzo[a]pyrene by polychlorobiphenyls. *Environ Health Perspect.* 2005; 113:680–687. [PubMed: 15929889]
- Wang LQ, Lehmler HJ, Robertson LW, James MO. Polychlorobiphenyls are selective inhibitors of human phenol sulfotransferase 1A1 with 4-nitrophenol as a substrate. *Chem Biol Interact.* 2006; 159:235–246. [PubMed: 16413005]
- Wethington DM 3rd, Hornbuckle KC. Milwaukee, WI, as a source of atmospheric PCBs to Lake Michigan. *Environ Sci Technol.* 2005; 39:57–63. [PubMed: 15667075]

Highlights

- Sulfated metabolites of PCB 11 were detected in serum of PCB-exposed individuals
- Conjugated PCB metabolites are not co-extracted in conventional analytical procedures
- Overall PCB exposure levels in exposed populations may have been underestimated

Figure 1.

Identification of dichlorinated PCB metabolites in human serum. UPLC/Q-TOF ion chromatograms and schematic depictions of fragmentation patterns indicate the presence of 4-OH-PCB 11 ($m/z = 236.98$), 4-PCB 11 sulfate ($m/z = 316.94$) and 4-PCB 11 glucuronide ($m/z = 413.02$) in human serum sample #45. Data were acquired using a Waters Q-TOF Premier high resolution (HR) mass spectrometer in negative ion mode.

Figure 2.

Identification of dichlorinated PCB metabolites in human serum (sample #45). LC-MS spectra, recorded on a Waters Q-TOF Premier high resolution (HR) mass spectrometer in negative ion mode indicated the presence and chlorination patterns of 4-PCB 11 glucuronide ($m/z=413.02$) and its fragment ion at $t_r=6.57$ min (**A**), 4-PCB 11 sulfate ($m/z=316.94$) and its fragment ion at $t_r=8.13$ min (**B**) and 4-OH-PCB 11 ($m/z=236.98$) at $t_r=10.40$ min (**C**). Dihydroxylated PCBs and mono-OH-PCB sulfates were not detectable.

Figure 3.

Inter-individual variability in the metabolic PCB 11 profile. A comparison between human serum samples with clearly identifiable PCB 11 sulfate indicated the presence of the glucuronic acid conjugate and the OH-PCB in some individuals (#11, #45), whereas these were absent in others (#27, #29). The numbers refer to subject identification numbers noted in Table 1.

Table 1Identification of dichlorinated PCB metabolites in human serum^a

Sample Information		UPLC/TQD detection of 4-PCB 11 Sulfate			UPLC/Q-TOF Confirmation		
ID	Description	Origin	detected	nM	ng/mg lipid	PCB sulfate	OH-PCB
1	Mother #1,	East Chicago	-	-	-	-	-
2	Daughter #1	East Chicago	-	-	-	-	-
3	Mother #2	East Chicago	Yes	<LOQ	<LOQ	Yes	-
4	Daughter #2	East Chicago	-	-	-	-	-
5	Mother #3	East Chicago	-	-	-	-	-
6	Daughter #3	East Chicago	-	-	-	-	-
7	Mother #4	East Chicago	-	-	-	-	-
8	Daughter #4	East Chicago	-	-	-	-	-
9	Mother #5	East Chicago	-	-	-	-	-
10	Daughter #5	East Chicago	-	-	-	-	-
11	Mother #6	East Chicago	Yes	19.7	1.19	Yes	Yes
12	Daughter #6	East Chicago	-	-	-	-	-
13	Daughter #7	East Chicago	-	-	-	-	-
14	Mother #7	East Chicago	-	-	-	-	-
15	Mother #8	East Chicago	-	-	-	-	-
16	Daughter #8	East Chicago	-	-	-	-	-
17	Mother #9	East Chicago	Yes	<LOQ	<LOQ	Yes	Yes
18	Daughter #9	East Chicago	-	-	-	-	-
19	Mother #10	East Chicago	-	-	-	-	-
20	Daughter #10	East Chicago	-	-	-	-	-
21	Mother #11	East Chicago	-	-	-	-	-
22	Daughter #11	East Chicago	Yes	9.7	0.61	Yes	Yes

Sample Information		UPLC/TQD detection of 4-PCB 11 Sulfate			UPLC/Q-TOF Confirmation		
ID	Description	Origin	detected	nM	ng/mg lipid	PCB sulfate	PCB glucuronide
23	Mother #12	East Chicago	-	-	-	-	-
24	Daughter #12	East Chicago	-	-	-	-	-
25	Daughter #13	Columbus Junction	-	-	-	-	-
26	Mother #13	Columbus Junction	-	-	-	-	-
27	Daughter #14	Columbus Junction	Yes	61	4.47	Yes	-
28	Mother #14	Columbus Junction	Yes	<LOQ	<LOQ	Yes	Yes
29	Daughter #15	Columbus Junction	Yes	<LOQ	<LOQ	Yes	-
30	Mother #15	Columbus Junction	-	-	-	-	-
31	Daughter #16	Columbus Junction	-	-	-	-	-
32	Mother #16	Columbus Junction	-	-	-	-	-
33	Daughter #17	Columbus Junction	Yes	6.6	0.44	Yes	Yes
34	Mother #17	Columbus Junction	-	-	-	-	-
35	Daughter #18	Columbus Junction	-	-	-	-	-
36	Mother #18	Columbus Junction	-	-	-	-	-
37	Mother #19	Columbus Junction	-	-	-	-	-
38	Daughter #19	Columbus Junction	-	-	-	-	-
39	Daughter #20	Columbus Junction	-	-	-	-	-
40	Mother #20	Columbus Junction	-	-	-	-	-
41	Daughter #21	Columbus Junction	-	-	-	-	-
42	Mother #21	Columbus Junction	Yes	<LOQ	<LOQ	Yes	-
43	Daughter #22	Columbus Junction	-	-	-	-	-
44	Mother #22	Columbus Junction	-	-	-	-	-
45	Mother #23	Columbus Junction	Yes	14.9	1.22	Yes	Yes
46	Daughter #23	Columbus Junction	-	-	-	-	-

^aFor more detailed demographic information, see Marek et al. 2013 and Marek et al. 2014.