Published in final edited form as:

Chem Res Toxicol. 2013 June 17; 26(6): 853–855. doi:10.1021/tx4001539.

Sulfate conjugates are urinary markers of inhalation exposure to 4-chlorobiphenyl (PCB3)

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Abstract

PCBs are contaminants in the air of older buildings and cities, which raises the concern of inhalation exposure. No reliable biomarker of such exposure is available. We exposed rats to air containing 2 mg/m³ PCB3 via nose-only inhalation for 2 h, collected urine, and analyzed it by LC/MS. Each rat inhaled an estimated dose of 35 µg PCB3, and excreted 27±2 % of it as sulfates within 24 h. Peak excretion occurred within 6 h. PCB sulfates were stable in urine for at least three days at room temperature without chemical preservatives. These data support the use of PCB sulfate conjugates as suitable urinary biomarkers of PCB3 and other airborne PCBs.

Keywords

PCB; biomarker; sulfate conjugate

Polychlorinated biphenyls (PCBs) are ubiquitous persistent organic pollutants. PCBs have been identified in high levels inside buildings constructed or remodeled before 1977. The US EPA reported PCBs in several older public school buildings in New York City in 2012.¹ The primary sources of PCBs in the US schools were electric ballasts and caulks. However, surfaces within the buildings and surroundings also absorbed and emitted PCBs as secondary sources. Elevated air concentrations have also been reported in cities like Chicago.² One of the sources of air contamination across Chicago is the transfer of PCBs from the sediments of Lake Michigan to water and then to air.³ Some lower chlorinated PCBs, such as PCB11, may be inadvertently generated during the manufacturing of commercial paints and pigments.⁴ Vaporization of PCBs may arise from obsolete electrical transformers, aged electronics, and appliances. Airborne PCBs may also be a problem in places where E-waste recycling is carried out.⁵

Inhalation is an efficient route of PCBs uptake. Hu et al. conducted inhalation exposures to Aroclor 1242, a representative Chicago air mixture, and PCB11.⁶⁻⁸ These studies demonstrated that airborne PCBs are quickly absorbed and distributed to tissues from the lung. PCBs are eliminated within minutes to hours after exposure from rat liver, lung, brain and serum. Their rates of elimination differ by the nature of target organ as well as the

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Supporting Information **Available.** Stability of lower chlorinated PCB sulfates in human urine. This information available free of charge via the Internet at <http://pubs.acs.org/>.

These studies form a portion of the dissertation research of K.D. The opinions expressed are solely those of the authors and do not reflect an official policy of the granting agency. The authors declare no competing financial interest.

substitution pattern of congeners. The US EPA has estimated that inhalation may contribute over seventy percent of exposures in the children attending contaminated schools.¹

A reliable biological marker of effect of PCB exposure is not available because toxicities occur through various mechanisms associated with complex adverse effects, such as initiation of carcinogenesis,⁹ and endocrine disruption.^{10,11} The International Agency for Research on Cancer (IARC) has upgraded PCBs to Group 1 carcinogens.¹² Therefore, accurate assessment of recent exposure of the occupants of contaminated buildings is a pressing public health issue.

Biomonitoring of PCB exposure is presently based on serum analysis of PCBs and, in research settings, OH-PCBs.¹³ There are limitations to this approach especially given the short half-lives of lower chlorinated PCBs. Only higher chlorinated OH-PCBs are selectively retained in the serum.¹⁴ Hydroxylated metabolites are not the final biotransformation products. We previously reported the oxidation and conjugation products of PCB3 in urine following a single i.p. administration in rats, and showed that sulfation is a major metabolism pathway for PCB3.¹⁵ In this study, we investigated if sulfate conjugates can serve as urinary biomarkers of inhalation exposure to air-borne PCB3 in rats. We chose PCB3 because it has a higher capacity of volatilization, is bioactivated to genotoxic metabolites, and is a major component in indoor air.¹⁶

The nose-only inhalation exposure was carried out as previously described.⁶ Three female Sprague Dawley rats (190-225 g) were exposed to PCB3 vapor for 2 h and transferred immediately thereafter to metabolism cages. Urine was collected every 6 h for up to 24 h, and stored at -20°C until analysis. Three control animals were exposed to laboratory air in a second nose-only system in an adjacent room. The vapor of PCB3 was generated from a round bottom glass flask containing 300 g glass beads, 4.75 mm diameter, coated with 2 g of the study material held in a 25°C water bath. A cartridge filled with 10 g polymeric resin (XAD, Supelco) was attached to the outlet of the exposure system to capture PCB3. Concentration of PCB3 in air was determined by $C_{\text{air}} = \text{PCB3 in XAD} / (\text{Flow rate} * \text{Time})$.⁶ We generated an atmosphere containing 2.0 mg/m³ of vapor phase PCB3 at an average flow rate of 10.8 L/min. In our trial experiments without animals, we achieved an air concentration of 3.3±1.7 mg/m³ PCB3 (n=7). The inhalation exposure dose was estimated by $\text{Dose/Rat} = f * V * C_{\text{air}} * \alpha * T$.⁶ Assuming breathing frequency (f) in female rats of 94 breaths/min, vital volume (V) of 1.5 mL/breath, C_{air} of 2 mg/m³, and uptake through inhalation (α) of 100 %, each animal was estimated to have inhaled 35 µg PCB3. This is approximately one thousand times lower than i.p. administration dose in our previous study.¹⁵ For a quick reference, PCB levels in contaminated school buildings in New York are 500±154 ng/m³ and in Chicago air 75-5500 pg/m³.^{1,2} The Occupational Safety and Health Administration stipulates that workers not be exposed to 1 mg/m³ by inhalation over a period of 40 working hour week.

We have previously described a simultaneous extraction method for hydroxylated and conjugated metabolites of PCB3 from urine and serum.¹⁵ Here we modified the method for selective extraction of PCB sulfate, and briefly describe the changes. Urine (1 mL) was acidified with 40 µL glacial acetic acid, and processed as previously described for serum. The acetonitrile (ACN) extract was reconstituted in 1 mL sodium acetate (SA) buffer (25 mM, pH 4.0), and applied to 1 cc capacity Oasis® WAX SPE cartridge, pre-conditioned with 1 mL methanol (MeOH) and 1 mL water. The column was sequentially washed with 1 mL SA buffer, 1 mL 100% MeOH, and 1 mL of 2% NH₄OH (8M) in H₂O:MeOH (50:50, v/v). Elution of the sulfate conjugates was accomplished with 1 mL of 2% NH₄OH in MeOH:ACN (20:80, v/v). Finally, the solvent was evaporated, and the extract was reconstituted in 200 µL MeOH:H₂O (20:80, v/v). The ultraclean sample (5 µL) was injected into LC/MS

(Acquity TQD, Waters). Chromatography was performed on a BEH C18 column (2.1 × 10 mm, 1.7 μm) at 35°C. Mobile phases were 5 mM ammonium acetate (A), and 100% acetonitrile (B). Isocratic elution was achieved with 30% B at flow rate of 300 μL/min. MS conditions were same as before,¹⁵ except the data were acquired in multiple reaction monitoring (MRM) of m/z 283 to 203, and 285 to 205. Quality control samples consisting of urine and solvent blanks, and spiked urine were simultaneously analyzed. The LOQ, as determined from the calibration standards,¹⁵ were 5, 2, and 8 ng/mL for 3PCB3, 3'PCB3, and 4' PCB3 sulfates. The concentrations in all cases were above the LOQ. The recoveries in the spiked samples were above 90% (Supporting figure S1).

Four different sulfate conjugates of PCB3 were identified. Three conjugates derived from mono-OH-PCB3 were confirmed by standards (Figure 1A). 3PCB3 sulfate and 2'PCB3 sulfate standards co-eluted as the first peak. We assigned this peak as 3PCB3 sulfate based on literature evidence.¹⁷ In single reaction monitoring (SRM) of m/z 299, we detected an intense signal for a sulfate conjugate of PCB3 derived from di-OH-PCB3 (Figure 1B), and this was confirmed by the pattern of fragmented daughter ions in MS/MS analysis (Figure 1C). We also detected metabolites that could be attributed to PCB3 mercapturate (m/z 348) as a good signal, and PCB3-glucuronide (m/z 379) as minor metabolites (data not shown). Figure 2 shows the cumulative excretion of different forms of sulfates over 24 h. The rate of excretion (the difference of cumulative excretion over time, as indicated by the slope of line between two time points), indicates that sulfates were formed rapidly until 12 h and decreased afterwards. Higher slope for 2-6 h indicates that peak excretion of sulfates occurred within this time. A strong signal for sulfates was already seen in the pooled droplets of urine droplets collected from nose-only inhalation system after 2 h. This early excretion suggests rapid absorption and metabolism of PCB3 after inhalation. Approximately 27±2% of the dose was excreted as 3PCB3 sulfate, 3'PCB3 sulfate, and 4' PCB3 sulfate within 24 h.

These results are consistent with our previous study in male rats after i.p. exposure, except in this study we found a of higher fraction of the dose excreted as sulfates.¹⁵ Although sulfotransferases (SULTs) and UDP glucuronosyl-transferases (UGTs) both utilize phenols as substrates, there are complex relationships that govern the rates of sulfation and glucuronidation with various individual molecules. It is generally considered that glucuronidation predominates sulfation at higher substrate concentrations, but we did not find a great deal of PCB3 glucuronides, even when the exposure dose was one thousand times higher than this study.¹⁵ OH-PCBs with chlorine atom substitutions in the meta- and para-positions have been shown to be less favorable as substrates for glucuronidation.¹⁸ Quantitative structure activity relationships for glucuronidation of OH-PCBs indicate that there are multiple physicochemical parameters involved in the interaction of these molecules with UGTs, and both hydrophobic and electronic characteristics were identified as major contributors.¹⁹ Several studies have described an inhibitory effect of OH-PCBs on SULTs, however, some of the most potent inhibitors of the sulfation reaction studied were actually alternative substrates for the enzyme.^{10,20-22} Rat SULT1A1 is a major isoform that efficiently catalyzes many lower chlorinated OH-PCBs.²¹ OH-PCB3s and OH-PCB11 are good substrates for human SULT1A1.¹¹ This suggests that PCB sulfates may be similarly formed in humans.

Along with sensitivity and specificity, stability is an essential characteristic of a useful biomarker. We determined the stability of 11 different sulfate conjugates of typical airborne PCBs in human urine at different storage conditions for six weeks. Approximately 90% of all tested sulfates were recovered from storage at room temperature without aid of chemical preservatives after three days (Supporting information).

In conclusion, we showed that a significant amount of PCB3 was excreted as sulfate conjugates in urine after inhalation exposure. We described a straightforward procedure for storage, processing, and determination of PCB sulfates in urine. This study may be directly applied to biomonitoring of PCB3 exposure in the occupants of contaminated buildings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Drs. Xueshu Li, and Xianran He for the synthesis of authentic standards used in these studies, and members of our laboratory for their help with the animal studies.

Funding Sources

This study was supported by funding from NIH (ES013661 and ES005605). K.D. gratefully acknowledges support from the Iowa Superfund Research Program Training Core.

ABBREVIATIONS

OH-PCBs	hydroxylated polychlorinated biphenyls
SULT	sulfotransferase
UGT	UDP glucuronosyltransferase
EPA	Environmental Protection Agency
LOQ	limit of quantification
SPE	solid phase extraction
SA	sodium acetate
MeOH	methanol
ACN	acetonitrile

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Figure 1. LC/MS chromatograms of urine after 6 h exposure showing the presence of several PCB3 sulfate conjugates

(A) MRM chromatogram of m/z 383 to 203 showing conjugates of mono-OH-PCB3. (B) SIR chromatogram of m/z 299 and 283 showing relative peak height of di-OH-PCB3. (C) Mass spectrum of MS/MS analysis of m/z 299, showing relative abundances of daughter ions.

Figure 2. Time-course excretion of sulfates in urine

Sulfate conjugates were rapidly excreted in urine within 12 h. Approximately 27 ± 2 % dose was recovered as sulfate conjugates from 13 ± 2 mL urine within 24 h. Values are mean of cumulative excretion at each time point $\pm$ SD, n=3.