Disposition of phenolic and sulfated metabolites after inhalation exposure to 4-chlorobiphenyl (PCB3) in female rats

Kiran Dhakal[§,†], Eric Uwimana[§,†], Andrea Adamcakova-Dodd[†], Peter S. Thorne[§,†], Hans Joachim-Lehmler[§,†], and Larry W. Robertson[§,†,]*

[§]Interdisciplinary Graduate Program in Human Toxicology, The University of Iowa, Iowa City, IA, United States.

[†]Department of Occupational and Environmental Health, The University of Iowa, Iowa City, IA, United States.

*Corresponding author. Phone: 319-335-4346. Fax: 319-335-4290.
E-mail: larry-robertson@uiowa.edu

Supporting information

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Table S1. Serum chemistry and free thyroxine (T4) levels in rats exposed to PCB3 and laboratory air for 2 h. Values are mean±SD, control (n=6), and each exposure time point (n=3)

		*Time after inhalation exposure to PCB3 (hour)**				
	Control	0	1	2	4	24
Renal Function						
Creatinine (mg/dL)	0.3±0.1	0.3±0.05	0.4±0.1	0.3±0.1	0.4±0.1	0.3±0.1
Blood Urea Nitrogen (mg/dL)	11±5	15±4	17±2	15±5	18±4	11±0.05
Serum Protein						
Total protein (g/dL)	5±2	5±1	6±0.2	5±1	6±0.5	4±0.3
Albumin (g/dL)	3±0.8	3±0.6	3±0.2	3±0.7	3±0.3	3±0.2
Electrolytes balance						
Calcium (mg/dL)	9±2	10±2	11±2	8±2	11±1	10±0.6
Phosphorous (mg/dL)	8±2	11±3	12±2	10±4	8±1	8±0.5
Sodium (mmol/L)	107±24	111±22	129±7	106±30	127±12	115±5
Potassium (mmol/L)	5±2	6±1	8±2	7±3	5±1	4±0.3
Chloride (mmol/L)	73±16	77±14	90±4	76±22	86±10	77±3
Pancreas Function						
Glucose (mg/dL)	235±47	271±71	304±115	165±26	246±44	264±12
Liver function						
Alkaline phosphatase (U/L)	157±73	152±46	179±8	122±28	183±58	130±33
Alanine Aminotransferase (U/L)	45±13	40±5	94±44	95±65	66±23	41±4
Gamma-glutamyltransferase (U/L)	0±0	0±0	0.3±0.6	1.3±1.5	0.3±0.6	0.0±0.0
Lipid metabolism						
Cholesterol	80±22	89±27	97±5	76±29	90±9	76±11
Triglycerides (mg/dL)	67±41	11±48	41±4	51±21	59±40	48±11
Respiratory/metabolic disorder						
CO_2 mmol/L	21±6	19±5	23±2	17±4	24±2	25±1
Thyroid hormone						
Free thyroxine (T4) (pmol/L)	39±13	31±14	38±4	38±16	49±11	35±4

* The estimated dose of inhalation in the rats killed at 0, 1, 2, and 4 hour after exposure was 23 µg/rat. The rats killed at 24 h have an estimated dose of 35µg/rat.

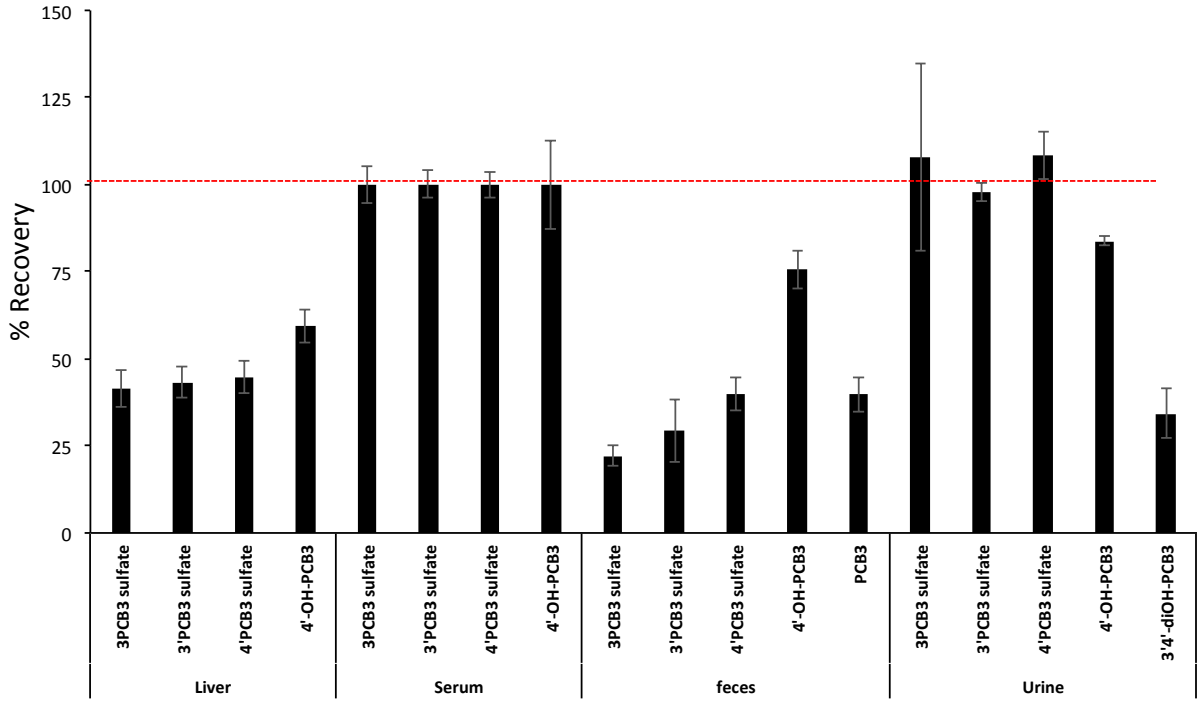

Figure S1. Recovery of authentic standards in spiked samples. Liver (1 g), serum (1 mL), feces (1 g), and urine (1 mL) samples derived from rats exposed to clean air were spiked with 0.5 µg of each authentic standard, extracted in acetonitrile, cleaned in WAX solid phase extraction column, concentrated to nearly dryness, reconstituted in 35% acetonitrile in water (200 µL), internal standard (50 µL, 3-F, 4'PCB3 @ 0.5 µg/mL in 35% acetonitrile in water) was added, and analyzed for sulfates by LC/MS. To the same vial, after analysis of sulfates, internal standard (50 µL, 3-F, 4'OH-PCB3 @ 0.5 µL/mL in 100% methanol) was added and analyzed for phenols by LC/MS. For the analysis of PCB3, final acetonitrile extract was concentrated to exactly 500 µL and 2 µL was injected into GC/MS.

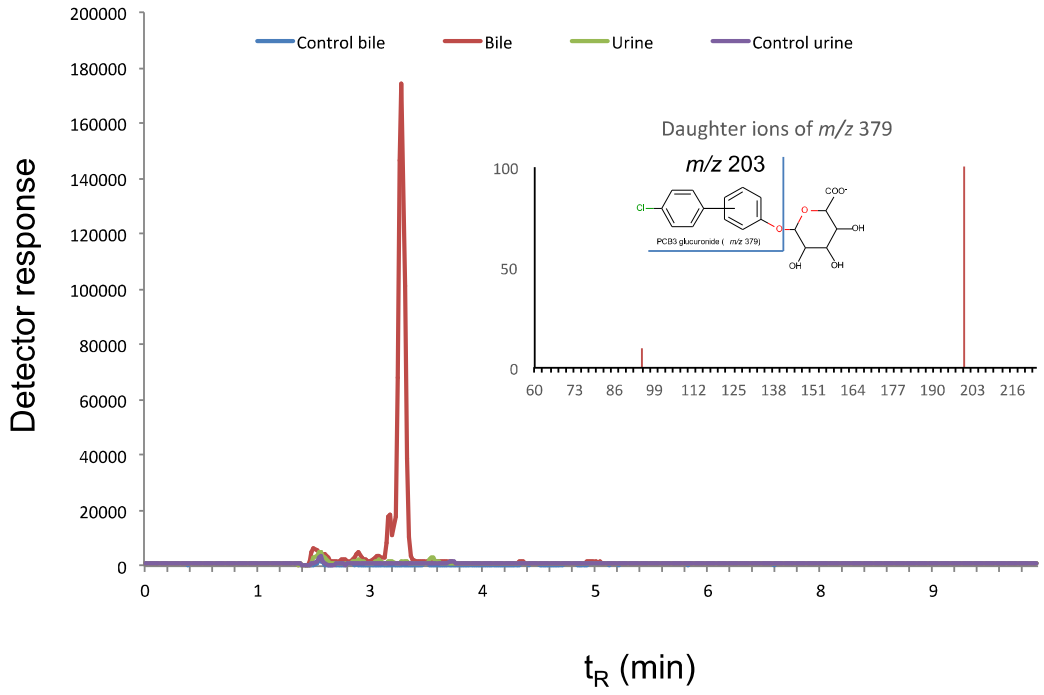

Figure S2. Identification of a glucuronide conjugate of PCB3 in bile. SIM chromatogram showing a putative glucuronide (*m/z* 379) in bile. Urine or bile (1 mL) was extracted in acetonitrile, cleaned in WAX column, concentrated by solvent evaporation, reconstituted in 35% acetonitrile in water (200 µL), and analyzed by LC/MS. A peak for putative glucuronide of *m/z* 379 was observed at 3.2 min. Fragmentation of *m/z* 379 resulted in a major daughter ion of *m/z* 203, confirming that this is a glucuronide conjugate of monohydroxylated PCB3. The mass spectra is shown in the inset. This glucuronide metabolite was present only in bile.

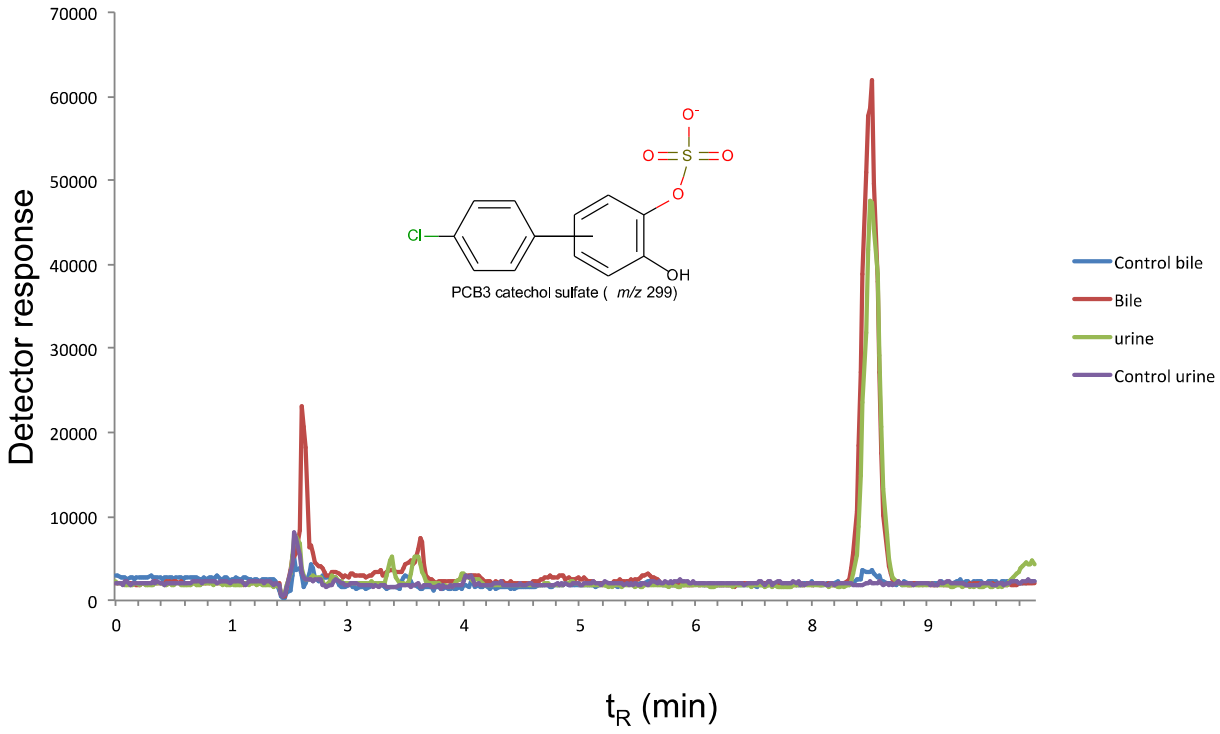

Figure S3. Identification of putative PCB3 catechol sulfate. SIM chromatogram showing a putative PCB3 catechol sulfate in urine and bile. Urine or bile (1 mL) was extracted in acetonitrile, cleaned in WAX column, concentrated by solvent evaporation, reconstituted in 35% acetonitrile in water (200 µL), and analyzed by LC/MS. A peak for putative PCB3 catechol sulfate of *m/z* 299 was observed at 8.5 min. This metabolite was confirmed by its fragmentation to a major daughter ion of *m/z* 219, and also appearance of a peak corresponding to 3'4'-diOH-PCB3 (*m/z* 219) after incubation of the sample with sulfatase (refer text for detail).