

## SUPPORTING INFORMATION

### **Complementary Nontargeted and Targeted Mass Spectrometry Techniques to Determine Bioaccumulation of Halogenated Contaminants in Freshwater Species**

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## Chemicals

This study incorporated 16 PXDD/PXDF standards of which 8-bromo-2,3-dichlorodibenzofuran (8-Br-2,3-Cl<sub>2</sub>DF), 8-bromo-2,3,4-trichlorodibenzofuran (8-Br-2,3,4-Cl<sub>3</sub>DF), 4-bromo-2,3,7,8-tetrachlorodibenzofuran (4-Br-2,3,7,8-Cl<sub>4</sub>DF), 1,2-dibromo-7,8-dichlorodibenzofuran (1,2-Br<sub>2</sub>-7,8-Cl<sub>2</sub>DF), 2,3-dibromo-7,8-dichlorodibenzofuran (2,3-Br<sub>2</sub>-7,8-Cl<sub>2</sub>DF), 1,3-dibromo-2,7,8-trichlorodibenzofuran (1,3-Br<sub>2</sub>-2,7,8-Cl<sub>3</sub>DF), 7-bromo-2,3-dichlorodibenzo-p-dioxin (7-Br-2,3-Cl<sub>2</sub>DD), 2-bromo-3,7,8-trichlorodibenzo-p-dioxin (2-Br-3,7,8-Cl<sub>3</sub>DD), 2-bromo-1,3,7,8-tetrachlorodibenzo-p-dioxin (2-Br-1,3,7,8-Cl<sub>4</sub>DD), and 2,3-dibromo-7,8-dichlorodibenzo-p-dioxin (2,3-Br<sub>2</sub>-7,8-Cl<sub>2</sub>DD), and <sup>13</sup>C<sub>12</sub>-2,3-Br<sub>2</sub>-7,8-Cl<sub>2</sub>DD were obtained from Wellington Laboratories (Guelph, CAN), and 3-bromo-2,7,8-trichlorodibenzofuran (3-Br-2,7,8-Cl<sub>3</sub>DF), 1-bromo-2,3,7,8-tetrachlorodibenzo-p-dioxin (1-Br-2,3,7,8-Cl<sub>4</sub>DD), 2-bromo-3,6,7,8,9-pentachlorodibenzo-p-dioxin (2-Br-3,6,7,8,9-Cl<sub>5</sub>DD), 1-bromo-2,3,6,7,8,9-hexachlorodibenzo-p-dioxin (1-Br-2,3,6,7,8,9-Cl<sub>6</sub>DD), and 1-bromo-2,3,4,6,7,8,9-heptachlorodibenzo-p-dioxin (1-Br-2,3,4,6,7,8,9-Cl<sub>7</sub>DD) were obtained from Cambridge Isotope Laboratories (Andover, USA). EPA Method 1613 standard solutions containing PCDD/PCDFs were used as standards and internal standards (Wellington Laboratories, Guelph, CAN). PCN standard solutions from Cambridge Isotope Laboratories (Andover, USA) and Wellington Laboratories (Guelph, CAN), as well as a Cl-PAH standard solution obtained from Kurunthachalam Kannan (Wadsworth Center, New York State Department of Health), were used for analyte confirmation. All other materials were obtained from Caledon Laboratory Chemicals (Georgetown, CAN) unless otherwise indicated.

### Lipid and total organic carbon (TOC) analysis

Lipid content of test species was measured using gravimetric analysis. The TOC for the sediment mixture was calculated as the difference between total carbon and total inorganic carbon. Total carbon was determined by combustion using a LECO C-632 Carbon Determinator (LECO Corporation, St. Joseph, MI, USA), while inorganic carbon was determined using a coulometer to measure CO<sub>2</sub> produced from perchloric acid reacting with carbonate.<sup>1</sup>

### Sample extraction

Details of sediment extraction procedures may be found in the Ontario Ministry of the Environment Method 3418.<sup>2</sup> Briefly, approximately 5 g of homogenized dry sediment was spiked with 20 µL of a 100 pg/µL solution containing <sup>13</sup>C<sub>12-2,3,7,8-Cl<sub>4</sub>DF</sub>. Samples underwent Soxhlet extraction using 200 mL toluene for 12 hours. Toluene extracts were concentrated to dryness and reconstituted in 5 mL hexane. Chromatographic clean-up procedures consisted of acid-base silica, alumina, and carbon columns. The resulting dichloromethane extract was concentrated to dryness by rotary evaporation and reconstituted in 1 mL hexane. Extracts were transferred to glass GC vials and concentrated to dryness under nitrogen evaporation. Sample vials were stored at ≤ 8 °C until analysis.

Details of biota extraction procedures may be found in the Ontario Ministry of the Environment Method 3481.<sup>3</sup> Briefly, approximately 2.5 g of homogenized biota sample was spiked with 20 µL of a 100 pg/µL solution containing <sup>13</sup>C<sub>12-2,3,7,8-Cl<sub>4</sub>DF</sub> and mixed with diatomaceous earth. Sample extraction was performed in a Pressurized Liquid

Extractor (Fluid Management System (FMS) Inc., Watertown, MA, USA) with 10:90 (v/v) dichloromethane (DCM)/hexane solution for two hours. Sample extracts were concentrated to 2 mL by rotary evaporation. If visual oil residue was observed, the extract was loaded on an acidified silica column, eluted with hexane and DCM, and concentrated to 2 mL. Sample clean-up was performed in an Automated Clean-up System Power Prep using pre-packed acid-base silica, alumina, and carbon columns (Fluid Management System (FMS) Inc., Watertown, MA, USA). Resulting toluene extracts were concentrated to 1 mL by rotary evaporation, transferred to glass GC vials and concentrated to dryness under nitrogen evaporation. Sample vials were stored at 2-8 °C until analysis.

#### FTICR-MS analysis

A Varian 920 FTICR-MS, positioned in a Varian 9.4 Tesla superconducting magnet, was used to obtain ultrahigh-resolution mass spectra using electron ionization (EI). The *Hexagenia* spp. sample extracts were injected (1 µL) on a Varian CP-3800 GC with a Varian CP-8400 autosampler coupled to a Varian J320 triple quadrupole mass spectrometer. Analytes were separated on a DB-5HT GC column (15 m x 0.25 mm ID x 0.10 µm, J&W Scientific, USA). The GC oven program held 120 °C for 1 min, increased to 245 °C at 20 °C/min, then to 280 °C at 5 °C/min, and finally to 320 °C at 40 °C/min and held for 2 min. The injector temperature was 250 °C and the transfer line and source temperatures were both 280 °C. The FTICR-MS was operated at a resolution of 40 000 at  $m/z$  400 full width at half maximum (FWHM). Using arbitrary waveform excitation, mass spectra were obtained for a mass range of  $m/z$  150-650. The acquisition and cycle times were 275 ms and 1.5 s, respectively. Perfluorotributylamine (PFTBA) was used for

external mass calibration and background siloxane and phthalate ions were used for internal calibration of mass spectra. Elemental compositions from accurate mass measurements were determined using Varian Elemental Composition Calculator.

#### Description of mass defect filtering on an H/Cl mass scale

Complex mass spectra were produced from FTICR-MS analysis of the *Hexagenia* spp. sample extracts and were interpreted using mass defect filtering on an H/Cl mass scale. In this approach, mass spectral data were converted from the International Union of Pure and Applied Chemistry (IUPAC) mass scale (C = 12.0000 Da) to an H/Cl mass scale (-H/+Cl = 34.0000 Da), using Equation 1.

$$(1) \quad \text{H/Cl mass} = \left[ \text{IUPAC mass} \times \left( \frac{34}{33.96102} \right) \right]$$

From the converted mass spectral data set, mass defect values were calculated by subtracting the nominal mass (rounded down) from the accurate mass (H/Cl mass scale), using Equation 2.

$$(2) \quad \text{mass defect (H/Cl scale)} \\ = \text{accurate mass (H/Cl scale)} - \text{nominal mass (rounded down, H/Cl scale)}$$

The resulting mass defect values based on the H/Cl mass scale (y-axis) were plotted against the IUPAC mass scale  $m/z$  values (x-axis) to generate a mass defect plot.

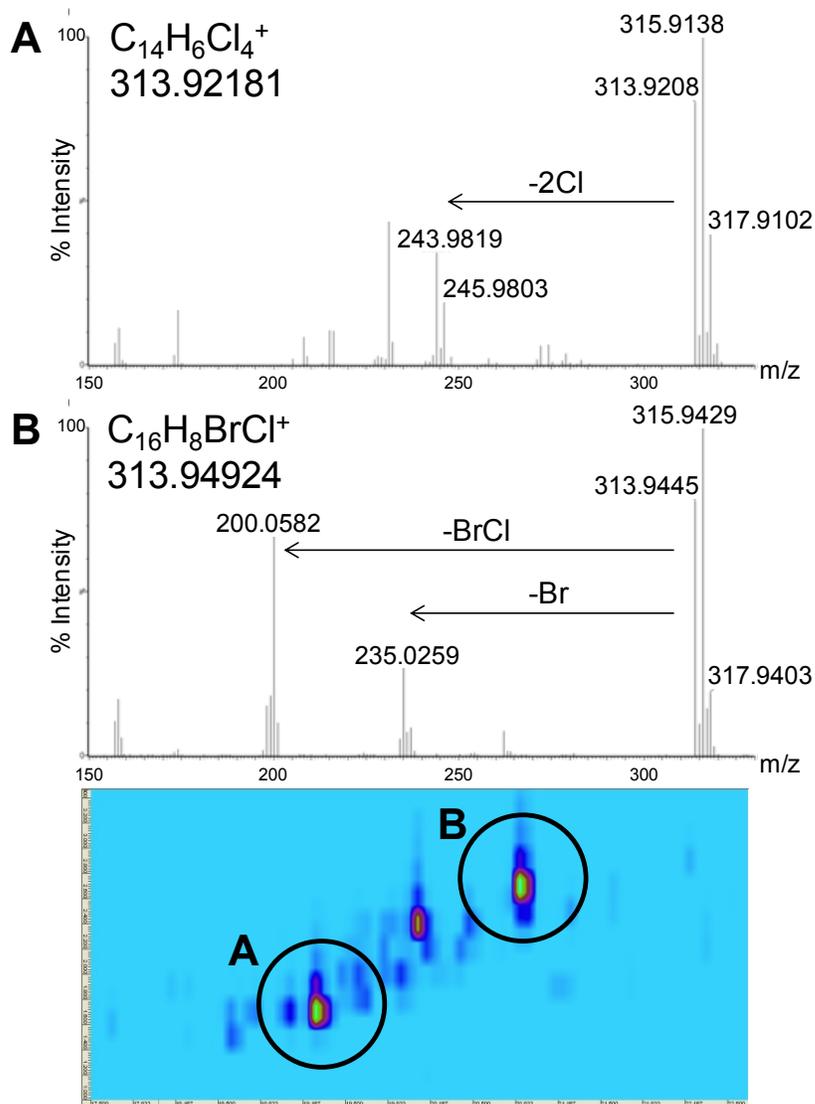
Congener classes related by chlorine substitution have the same mass defect value based on the H/Cl mass scale and are therefore aligned on the plot horizontally.

### GCxGC-HRToF analysis

PCN, Cl-PAH, and X-PAH analyses were performed on an Agilent HP 6890 gas chromatograph (Agilent Technologies, Mississauga, CAN) coupled to a Waters GCT Premier time of flight mass spectrometer with EI (Waters, Milford, USA). The GCxGC system consisted of a single jet loop modulator (Zoex Corporation, Houston, USA), an Rtx-5MS first dimension column (30 m x 0.25 mm ID x 0.25  $\mu$ m) and an Rtx-50 second dimension column (2.3 m x 0.18 mm ID x 0.2  $\mu$ m) (Restek Corporation, Bellefonte, USA). The injection volume was 1  $\mu$ L and injector temperature was 270  $^{\circ}$ C. The GC oven program held 140  $^{\circ}$ C for 1 min, increased to 170  $^{\circ}$ C at 20  $^{\circ}$ C/min, then to 330  $^{\circ}$ C at 5  $^{\circ}$ C/min, and held for 5 min. The modulation time was 4 seconds. A mass range of  $m/z$  150 to 800 was scanned at an acquisition rate of 10 Hz. The system was operated at a source temperature of 250  $^{\circ}$ C, electron energy of 70 eV, and trap current of 100  $\mu$ A. The mass resolution was approximately 7000 FWHM. Mass spectra were internally calibrated on background PFTBA masses. MassLynx software (Waters, Milford, USA) data files were converted for data processing of two-dimensional chromatograms in GC Image (GC Image, LLC, Lincoln, USA).

**Table S1.** APGC-MS/MS MRM method parameters

Compound	Mass to charge ratio (m/z)		Reaction	Collision energy (V)	Standard peak area ratio	Transitions used to quantify
	Parent Ion	Product Ion				
<b>PXDFs</b>						
C <sub>12</sub> OH <sub>5</sub> BrCl <sub>2</sub>	313.8	206.8	M-COBr	40	1	*
	313.8	171.7	M-COBrCl	50	0.25	*
C <sub>12</sub> OH <sub>4</sub> BrCl <sub>3</sub>	347.7	240.8	M-COBr	35	1	*
	347.7	170.9	M-COBrCl <sub>2</sub>	55	0.55	*
C <sub>12</sub> OH <sub>3</sub> BrCl <sub>4</sub>	381.8	274.5	M-COBr	35	1	*
	381.8	204.9	M-COBrCl <sub>2</sub>	55	0.77	
C <sub>12</sub> OH <sub>4</sub> Br <sub>2</sub> Cl <sub>2</sub>	393.6	286.9	M+2-COBr	30	1	*
	391.6	284.8	M-COBr	30	0.41	
C <sub>12</sub> OH <sub>3</sub> Br <sub>2</sub> Cl <sub>3</sub>	427.6	320.9	M+2-COBr	35	1	*
	425.6	318.7	M-COBr	40	0.59	
<b>PXDDs</b>						
C <sub>12</sub> O <sub>2</sub> H <sub>3</sub> BrCl <sub>2</sub>	329.6	222.7	M-COBr	30	0.46	*
	329.6	159.8	M-(CO) <sub>2</sub> BrCl	50	1	*
C <sub>12</sub> O <sub>2</sub> H <sub>4</sub> BrCl <sub>3</sub>	363.7	256.7	M-COBr	35	0.35	*
	363.7	193.8	M-(CO) <sub>2</sub> BrCl	50	1	
C <sub>12</sub> O <sub>2</sub> H <sub>3</sub> BrCl <sub>4</sub>	397.6	334.8	M-COCl	30	0.62	n/a
	397.6	290.5	M-COBr	35	0.45	n/a
	397.6	227.5	M-(CO) <sub>2</sub> BrCl	45	1	n/a
C <sub>12</sub> O <sub>2</sub> H <sub>4</sub> Br <sub>2</sub> Cl <sub>2</sub>	407.6	193.8	M-(COBr) <sub>2</sub>	50	0.89	n/a
	407.6	300.8	M-COBr	35	0.60	n/a
	409.6	302.6	M+2-COBr	35	1	n/a
C <sub>12</sub> O <sub>2</sub> H <sub>2</sub> BrCl <sub>5</sub>	433.6	326.7	M-COBr	35	0.71	n/a
	433.6	263.6	M-(CO) <sub>2</sub> BrCl	50	1	n/a
C <sub>12</sub> O <sub>2</sub> HBrCl <sub>6</sub>	467.6	360.4	M+2-COBr	35	0.42	n/a
	467.6	297.8	M+2-(CO) <sub>2</sub> BrCl	50	1	n/a
	465.6	295.6	M-(CO) <sub>2</sub> BrCl	50	0.56	n/a
C <sub>12</sub> O <sub>2</sub> BrCl <sub>7</sub>	499.6	329.7	M-(CO) <sub>2</sub> BrCl	50	0.48	n/a
	501.6	331.7	M+2-(CO) <sub>2</sub> BrCl	50	1	n/a
	501.6	394.4	M+2-COBr	40	0.24	n/a
<b>Internal Standards</b>						
<sup>13</sup> C <sub>12</sub> -2,3-Br <sub>2</sub> -7,8-Cl <sub>2</sub> DD	421.6	313.7	M+2- <sup>13</sup> COBr	35	1	*
	419.6	311.6	M- <sup>13</sup> COBr	30	0.60	*
	419.6	203.5	M-( <sup>13</sup> COBr) <sub>2</sub>	50	0.75	*
<sup>13</sup> C <sub>12</sub> -2,3,7,8-Cl <sub>4</sub> DF	315.7	251.8	M- <sup>13</sup> COCl	40	1	*
	315.7	216.9	M- <sup>13</sup> COCl <sub>2</sub>	50	0.31	*



**Figure S1.** Two-dimensional selected ion chromatogram for mass range 313.9000-313.9700 highlighting two chromatographically separated peaks corresponding to  $C_{14}H_6Cl_4$  and  $C_{16}H_8BrCl$  in an *L. variegatus* extract analyzed by GCxGC-HRToF. Corresponding mass spectra identify elemental compositions through accurate mass, isotopic ratios and fragments.

## References

- (1) *The Determination of Total Carbonate-Carbon in Soil and Sediments by Coulometry*, Method 3012; Ontario Ministry of the Environment: Toronto, Ontario, Canada, 2010.
- (2) *The Determination of Polychlorinated Dibenzo-p-dioxins, Polychlorinated Dibenzofurans and Dioxin-like Polychlorinated Biphenyls (DLPCBs) in Environmental Matrices by Gas Chromatography-High Resolution Mass Spectrometry (GC-HRMS)*, Method 3418; Ontario Ministry of the Environment: Toronto, Ontario, Canada, 2010.
- (3) *The Determination of Polychlorinated Dibenzo-p-dioxins, Polychlorinated Dibenzofurans, Dioxin-like Polychlorinated Biphenyls (DLPCBs), Polychlorinated Naphthalenes (PCNs) and Polybrominated Diphenyl Ethers (PBDEs) in Biota Samples by Automated Sample Extraction/Cleanup and Gas Chromatography-High Resolution Mass Spectrometry (GC-HRMS)*, Method 3481; Ontario Ministry of the Environment: Toronto, Ontario, Canada, 2012.