

SUPPORTING INFORMATION

Polybrominated Diphenyl Ethers, Polychlorinated Biphenyls and Organochlorine Pesticides in Human Milk from Massachusetts, USA

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Supplementary Information

1. Analysis of human breast milk samples and QA/QC

1.1 Extraction and clean-up. PBDEs and OCPs were analyzed following the method described elsewhere (Johnson-Restrepo et al. 2005; Kannan et al., 1992).

Approximately 10 g of the each breast milk sample were homogenized with anhydrous sodium sulfate and extracted in a Soxhlet apparatus for 16 h using dichloromethane and hexane (3:1; 400 mL). PCB congeners CB-30 and CB-204 were spiked as surrogate standards. The extract was rotary-evaporated at 40°C to 10 mL, and 1 mL aliquot of the extract was used for the determination of fat content by gravimetry. The remaining extract was spiked with ¹³C-labelled PBDE congeners #3, 15, 28, 47, 99, 100, 118, 153 and 183 as internal standards. Samples were then purified by passage through a series of layers of silica gel (Davisil, 100-200 mesh size, Aldrich, Milwaukee, WI) in the following order: 1 g of silica gel, 2 g of 40% sulfuric acid-silica gel, 1 g of silica gel, 2 g of 40% sulfuric acid-silica gel, and 1 g of

silica gel at the bottom. The packed silica was cleaned with 50 mL of hexane prior to the transfer of the sample extracts. Samples were then eluted with 15% dichloromethane in hexane (150 mL), which was then rotary-evaporated to 10 mL. The extracts were treated with sulfuric acid (5 mL) and then concentrated to 100 μ L under a gentle stream of nitrogen for the analysis of PBDEs and OCPs.

1.2 Quantification. The measurement of tri- to hexa-BDE congeners was accomplished by use of a Thermo Finnigan Trace GC Ultra gas chromatograph / MAT95XP high resolution mass spectrometer (HRGC-HRMS). Measurements were carried out at a resolution of $R > 9,000$ - $10,000$. The ion source temperature was held at $280\text{ }^{\circ}\text{C}$. A DB-5MSITD capillary column coated with 5 % phenyl methylpolysiloxane (30 m length, 0.25 mm i.d., 0.25- μm film thickness; J&W Scientific, Folsom, CA, USA) was used for the separation of congeners. Helium was employed as the carrier gas, at a flow rate of 1 mL/min. The injection port temperature was held at $260\text{ }^{\circ}\text{C}$. The column oven was programmed from $140\text{ }^{\circ}\text{C}$ (1.5 min) to $205\text{ }^{\circ}\text{C}$ at $15\text{ }^{\circ}\text{C}/\text{min}$, and then to $325\text{ }^{\circ}\text{C}$ (5 min) at $6\text{ }^{\circ}\text{C}/\text{min}$. The following native PBDE congeners (BDE-LMS; AccuStandard Inc., New Haven, CT, USA) were used to prepare an external calibration standard: 2,4,4'-triBDE (BDE-28); 2,2',4,4'-tetraBDE (BDE-47); 2,3',4,4'-tetraBDE (BDE-66); 2,2',3,4,4'-pentaBDE (BDE-85); 2,2',4,4',5-pentaBDE (BDE-99); 2,2',4,4',6-pentaBDE (BDE-100); 2,2',3,4,4',5'-hexaBDE (BDE-138); 2,2',4,4',5,5'-hexaBDE (BDE-153); 2,2',4,4',5,6'-hexaBDE (BDE-154). PBDE congeners were monitored using the two most abundant masses of the ion clusters $[\text{M}]^+$ (m/z 405.8026 and 407.8006) for tri-BDE, $[\text{M}]^+$, (m/z 283.6955 and 285.6935) for tetra-BDE, $[\text{M}-\text{Br}_2]^+$ (m/z 403.7800 and 405.7800) for penta-BDE,

and $[M-Br_2]^+$ (m/z 481.6975 and 483.6955) for hexa-BDE. A six-point calibration curve (0.2 - 100 ng/mL) was run every time when samples were measured.

The analysis of decabromodiphenyl ether (BDE-209), 2,2',3,4,4',5,6-heptabromodiphenyl ether (BDE-183), and 2,2',3,4,4',5,5',6-octabromodiphenyl ether (BDE-203) was performed using an Agilent Technologies 6890N gas chromatograph-electron capture detector (GC-ECD) and a ZB-5 capillary column coated with 5 % phenylmethylpolysiloxane (5 m length, 0.25 mm i.d., 0.25- μ m film thickness; Phenomenex, Torrance, CA, USA). Helium was used as the carrier gas, and nitrogen as the make-up gas. The flow rate of helium was 3 mL/min. A splitless injection was used, and the purge flow to split vent was 0.5 mL/min for 0.75 min. The oven temperature was programmed from 80 °C (2 min) to 180 °C at 20 °C/min, then to 210 °C at 2°C/min, then to 280°C at 8°C/min, and then held for 5 min. The injector and detector temperatures were 250 and 280 °C, respectively. The commercial octa-BDE (Great Lakes DE79) and deca-BDE (Great Lakes DE83) products (Great Lakes Chemical Corporation, West Lafayette, IL, USA) were used as qualitative standards for the identification of octa- and deca-BDE congeners, respectively. Composition and retention times of commercial octa- and deca-BDE specific congeners were confirmed by GC-MS. A calibration curve was run for each congener, to quantify the concentrations in samples. The term total PBDE (Σ PBDEs) denotes the sum of all of the identified tri- to deca-BDE congeners.

Organochlorine pesticides were analyzed on an Agilent Technologies 6890N GC-ECD. A capillary column coated with RTX-5MS (30 m length x 0.25 mm i.d. x 0.25 μ m film thickness; Restek Corp, Bellefonte, PA) was used for the separation of pesticides. An external six-point standard calibration curve was used to quantify the sample concentrations from the peak areas for each OCP. The term DDTs refers to

the sum of *o,p'*-DDE, *p,p'*-DDE, *p,p'*-DDT, and *p,p'*-DDD; The term CHLs refers to the sum of *trans*-chlordane, *cis*-chlordane, *cis*-nonachlor, *trans*-nonachlor, and oxychlordane; The term HCHs refers to the sum of α -, β -, γ -, and δ -HCH isomers. The identities of the detected OCPs were confirmed by GC-MS. Concentrations of PBDEs, PCBs, and OCPs are reported on a lipid-weight basis. PCBs and PBDEs are identified according to the IUPAC numbering system.

1.3 Quality Assurance and Quality Control. Carryover and cross-contamination were monitored by injection of solvents between two samples in the HRGC-HRMS. Each compound measured by HRGC-HRMS and GC-MS had to meet the following criteria: (1) Ratios of ions selected for native or ^{13}C -labelled compounds must not be different from their theoretical values more than 20%. (2) If the values calculated were below of limit of detection (calculated as 3 times the standard deviation of blank measurements), values were reported as zero. Retention time, ion chromatogram, and abundance of the monitored ions were used for the identification. The cleanup and fractionation procedures used were evaluated by measuring the absolute recovery of the substances analyzed. The entire analytical procedure, including extraction, cleanup, and fractionation steps was evaluated by spiking of ^{13}C -labelled internal and native surrogate standards. Mean (\pm SD) recoveries of ^{13}C -labelled BDE 28, 47, 99 and 154 spiked after the extractions were $78.1 \pm 15\%$, $80 \pm 17\%$, $77 \pm 18\%$, and $78 \pm 18\%$, respectively. Overall recoveries of PBDEs ranged from 61 to 127%. Overall recoveries (mean \pm SD) of PCB congeners, spiked before extractions were $89.2 \pm 16\%$ (range: 58 - 111%) for CB-30 and $82.4 \pm 16\%$ (range: 61 to 111%) for CB-204. Overall mean (\pm SD) recoveries of pesticides were $71 \pm 15\%$ for HCB, $72.7 \pm 20\%$ for α -HCH, $100 \pm 7.2\%$ for β -HCH, $86.4 \pm 9.9\%$ for γ -HCH, $93.5 \pm 3.2\%$ for oxy-CHL, $101 \pm 3\%$ for *trans*-CHL, $99.3 \pm 5\%$ for *cis*-CHL,

93.8 ± 3% for *trans*-nonachlor, 96.2 ± 6.8% for *p,p'*-DDE, 94.4 ± 3.6% for *cis*-nonachlor, 91.1 ± 4% for *p,p'*-DDD, 100 ± 5.4% for *p,p'*-DDT. Overall recoveries of pesticides ranged from 56.4 to 107%. The limit of detection for PBDE homologues (tri- to deca-BDE) ranged from 0.02 to 204 ng/g, lipid weight; The limits of detection for pesticides were 0.8 ng/g for HCB, 0.5 ng/g for α -HCH, 2.3 ng/g for β -HCH, 1.3 ng/g for γ -HCH, 6.5 ng/g for δ -HCH, 15.4 ng/g for oxy-chlordane, 0.01 ng/g for *trans*-chlordane, 0.01 ng/g for *cis*-chlordane, 4.2 ng/g for *trans*-nonachlor, 4.1 ng/g for *p,p'*-DDE, 2.1 ng/g for *cis*-nonachlor, 2.0 ng/g for *p,p'*-DDD, and 5.2 ng/g for *p,p'*-DDT. Procedural blanks were analyzed for every set of 10 samples, to check for interferences and contamination. The reported concentrations were not corrected for recoveries.

Literature Cited in the Supplementary Information

Johnson-Restrepo, B.; Kannan, K.; Rapaport, D.P.; Rodan, B.D. Polybrominated diphenyl ethers and polychlorinated biphenyls in human adipose tissue from New York. *Environ. Sci. Technol.* **2005**, *39*, 5177-82.

Kannan, K.; Falandysz, J.; Yamashita, N.; Tanabe, S.; Tatsukawa, R. Temporal trends of organochlorine concentrations in cod-liver oil from the southern Baltic proper, 1971-1989. *Mar. Pollut. Bull.* **1992**, *24*, 358-63.

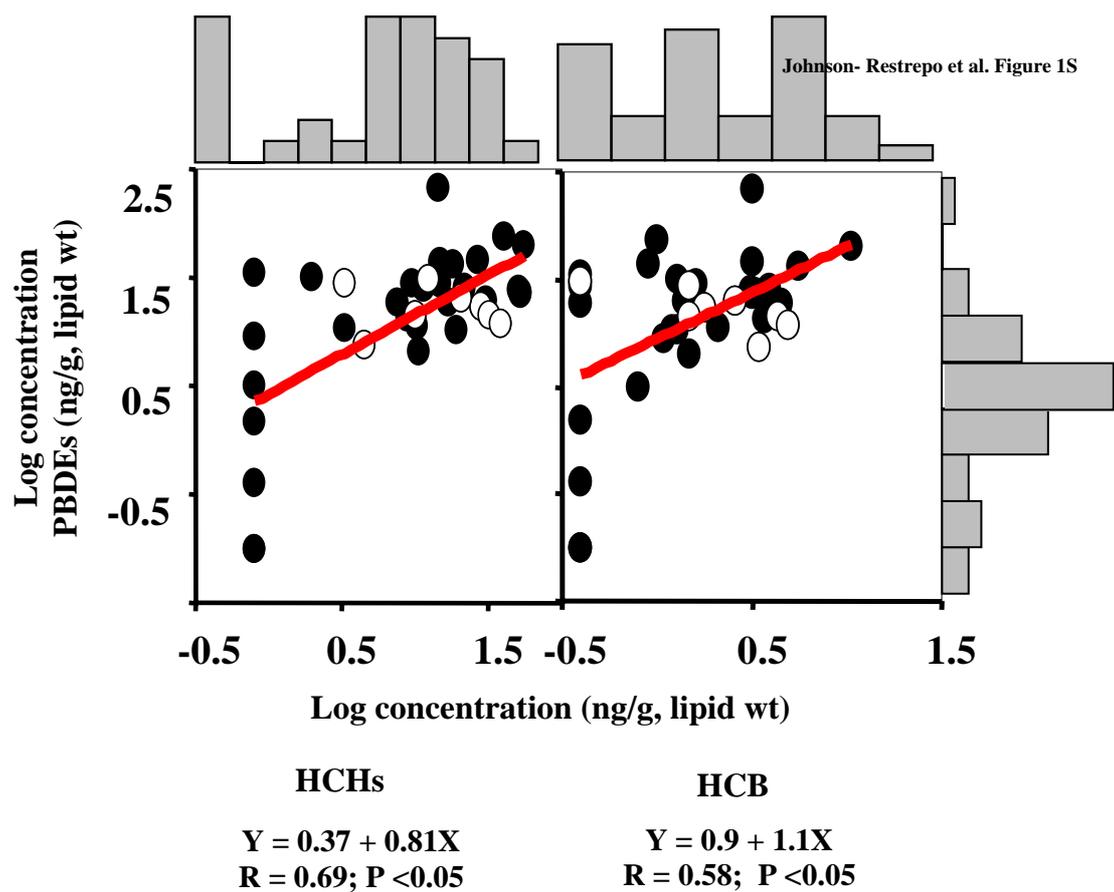


Figure 1S.