Supporting information for

Polybrominated Diphenyl Ethers and Their Hydroxylated Analogues in Ringed Seals (Phoca hispida) from Svalbard and the Baltic Sea

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Method for analysis of PBDEs in liver

The method of extraction and clean-up for analysis of PBDEs in liver sample, which is similar to that for analysis of PCBs, has been previously described (*1*). Briefly, seal liver (1-10 grams) was homogenised with Na_2SO_4, packed into Suprex standard extraction vessels (10 mL) and spiked with an internal standards (IS) consisting of a mixture of $^{13}C_{12}$-labeled BDE77 (375 pg) and BDE139 (750 pg). The samples were extracted by Suprex Autoprep/Accutrap Supercritical Fluid Extraction (Suprex, Pittsburgh, USA) using CO_2 as the supercritical fluid (40°C, 280 bar, 1.5 ml/min for 25 min). All the analytes were trapped on a C_{18} solid sorbent (ODS, Octadecylsilica) and the trap was rinsed with n-hexane (HEX)/dichloromethane (DCM) (1:1). After concentration, the samples were applied on combination mini silica columns consisting of NaOH silica and H_2SO_4 silica using HEX as elution solvent. The concentrated eluant was spiked the recovery standard (RS) containing $^{13}C_{12}$-labeled PCB178 (3.1 ng), changed the solvent to tetradecane and reduced the volume to 25 ul for gas chromatography - mass spectrometry (GC-MS) analysis. Lipid determination was determined gravimetrically by applying liver homogenate on a small column and quantitatively extracting with HEX/DCM (1:1). For liver samples, PBDEs were determined by gas chromatography (GC) (Agilent 6890, Agilent, Waldorff, Germany) coupled with low resolution mass spectrometry (MS) (Agilent 5973) with electron capture negative impact ionization (ECNI) mode monitoring m/z 79/81.

In accordance with routine QA/QC measures, a standard reference material (SRM, human adipose tissue) and a laboratory blank sample was run with each batch of seven to ten samples. All PBDE levels of the SRM were within 2 × the RSD which was less than 20% for all individual congeners. The variance of individual BDE congeners of the SRM (human adipose tissue) was less than 20%. Laboratory blank samples did not contain the reported target compounds at levels > 15% of the levels found in the seal samples reported in Table 1. The reported seal sample size was reduced for BDE28, -85, -99, -153 and -154 due to contamination in blank samples. In one blank for the Baltic seal liver samples, BDE99 was detected at concentrations > 15% of the levels found in five samples of the batch.

The same blank contained also traces of BDE85, -153 and -154 detected at concentrations > 15 % the levels detected in one sample of the batch. Another blank run together with the Svalbard samples contained traces of BDE153 at concentrations >15% of the levels detected in seven samples. Concentrations of BDE28 and 183 were not reported for the seals sampled in 2007, because these congeners were constantly detected in blank samples run together with 2007 seal samples at concentrations > 15%. No subtraction of blank levels was performed for any of the samples.

No replicates of seal samples were included. The glassware and supercritical fluid extraction vessels were cleaned with ethanol, dichloromethane and hexane. The minimum level of quantification (MLOQ) was defined as a signal-to-noise ratio of 3. All calculations of the MLOQ used for PBDE concentrations in liver were based on the procedural blanks run with each set of samples. These values are significantly higher than the 'instrumental' level of detection because of the detection of some of BDE congeners in the procedural blanks. IS recoveries were 73% [95% CI: 63%, 84%] for $^{13}C_{12}$-BDE77 and 74% for [95% CI: 64%, 84%] $^{13}C_{12}$-BDE139. Using the isotope dilution /internal standards, all data was automatically adjusted for recovery. The laboratory participates regularly part in international interlaboratory comparison studies with good results (z-scores < 2) and is currently a reference laboratory for POP analysis for the UN within the UNEP program.

Method for analysis of OH-PBDEs and PBDEs in plasma

Extraction and clean-up method for analysis of OH-PBDEs in plasma sample, similar to that of OH-PCBs, has been described elsewhere (*1*). Plasma (~ 3 g) was spiked 2'-OH-BDE28 (5 ng) for OH-PBDEs and BDE30 for PBDEs (10 ng) as IS. First, the plasma was denatured by 6 M HCl and 2-propanol, and the phenols and other pollutants were extracted from plasma with organic solvent of methyl tert-butyl ether (MtBE)/HEX (50:50). After the organic phase was partitioned from aqueous phase, the phenols were extracted with 1 M KOH in ethanol/water (50:50) from organic phase, and then back extracted from the acidified solution with MtBE /HEX (50:50). The concentrated phenolic fraction was dried by anhydrous Na_2SO_4 and reduced the volume to ~ 1 mL. The phenolic compounds were then

derivatized to their methyl ester with diazomethane and cleaned up by H_2SO_4 silica (22% H_2SO_4). The sample was ready for GC-MS analysis after solvent was evaporated and the sample was reinstituted in 100 μL isooctane. The neutral fraction, which contained PBDEs, was obtained after the extraction of phenols. This fraction was cleaned up with LC-Si solid phase extraction (SPE) cartridge (500 mg, 6 mL, J.T.Baker, USA). After the sample was loaded on the cartridge PBDEs were eluted with DCM/HEX (5:95). The collected fractions were then concentrated and translated the solvent to isooctane for GC-MS analysis.

The methoxy-derivatized phenolic, containing OH-PBDEs, and the neutral, containing PBDEs, fractions from samples were analyzed by GC-MS (Agilent 6890 and 5973; Agilent Technologies, CA) with ECNI source in the SIM mode. Two columns with different polarities were used. First, GC separation was performed using a silica DB-5 capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) (J&W Scientific, Folsom, CA) with the GC conditions described elsewhere (*2*). The oven temperature was programmed as follows: 80 °C, hold for 2 min, then at 10 °C/min to 290°C, hold for 20 min. The injector temperature was 300 °C and the purge time was 1 min after injection. Second, the OH-PBDEs were separated on a polar SP-2331 column (30 m × 0.25 mm i.d., 0.2 μm film thickness) (Supelco, Bellefonte, USA) using the same GC conditions as described previously (*3*).

The replicate determination (n = 4) of a polar bear plasma pool, used at NWRC as an in-house plasma reference material, showed 32% variation in the Σ-PBDE concentrations. No OH-PBDE residues were detected in method blank samples (n = 5), which were run with every second batch of five samples. Traces of the BDE47, -99 and -100 were observed at levels < 10 % of the levels found in the reported samples. Results for BDE99 for three seals and those of BDE100 for two seals were eliminated, because the blank samples run with these seal samples contained these congeners > 10% of the levels detected in these seal samples. The replicate determination was run with every second batch of five samples. No replicates of seal samples were included. All the glassware was cleaned with ethanol, dichloromethane and hexane. The minimum level of quantification (MLOQ) was defined as a signal-to-noise ratio of 10.

The method detection limit, which was tested for recovery efficiency and MLOQs by replicate spiking of chicken egg and bovine plasma, is listed in Table 1.

References

1. Routti, H.; Letcher, R.J.; Arukwe, A.; van Bavel, B.; Yoccoz, N.G.; Chu, S.G.; Gabrielsen, G.W. Biotransformation of PCBs in relation to phase I and II xenobiotic-metabolizing enzyme activities in ringed seals (*Phoca hispida*) from Svalbard and the Baltic Sea. Environ. Sci. Technol. **2008,** 42, 8952-8958.

2. Gebbink, W.A.; Sonne, C.; Dietz, R.; Kirkegaard, M.; Riget, F.F.; Born, E.; Muir, D.C.G.; Letcher, R. Tissue-specific congener composition of organohalogen and metabolite contaminants in east Greenland polar bears (*Ursus maritimus*). Environ. Pollut. **2008,** 152, 621-629.

3. Marsh, G.; Athanasiadou, M.; Bergman, A.; Asplund, L. Identification of hydroxylated and methoxylated polybrominated diphenyl ethers in Baltic Sea salmon (*Salmo salar*) blood. Environ. Sci. Technol. **2004,** 38, 10-18.

Figure S1. Mass chromatograms of OH-PBDEs on DB-5 colum (30 m × 0.25 mm) (A) and SP-2331 column (30 m × 0.25 mm) (B) in plasma sample from Svalbard after derivatization. IS: internal standard of 2'-MeO-BDE28; X: interference compound. The GC/MS operation parameters are described in text.