Temporal and spatial trends of brominated and chlorinated flame retardants in beluga (*Delphinapterus leucas*) from the Canadian Arctic

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Introduction

The Canadian arctic has become a sentinel geographical location for studies on temporal trends of halogenated organic pollutants. Any changes in the inputs of chemicals into the environment are reflected in changes in concentrations of chemical contaminants in wildlife over time (OSPAR Commission). For new and emerging chemicals, there is a general paucity of information regarding emissions and as such, constructing historical concentration profiles is the only means of assessing the chronology of contamination.

Because of its vast area, the Canadian arctic is often used to highlight geographical differences in chemical contamination. Regional chemical 'hot-spots' is an important piece of information that can sometimes be gleaned from a spatial trend analysis study.

In this study, concentration profiles of brominated and chlorinated flame retardants were examined in beluga from the Canadian arctic. These included the PBDEs, HBCD, BTBPE and isomers of Dechlorane Plus®. Our objectives were twofold: examine the temporal variation (1993, 1995, 2001 and 2005) in concentrations of these compounds in beluga from Hendrickson Island (HI, western Canadian arctic) and to determine if they are geographical differences in the concentration of these contaminants in Canadian arctic beluga.

Materials and Methods.

Samples and geographic locations. Beluga samples were obtained through the Department of Fisheries and Oceans (DFO) ongoing collection programs as part of subsistence hunts of Northern communities. Collected samples are stored in our archived repository at -30°C. The animals selected for study were from our archived databank (see Figure 1 for locations). Beluga from Hendrickson Island (HI: 1993, 1995, 2001, 2005) was the basis of our time-trend study. Our geographic trend study was based on beluga from Arviat (2003), Sanikiluaq (2003), Kimmirut (2003) and Pangnirtung (2002) and animals from HI collected in 2005. To mitigate the effects of age and sex on contaminant levels, male animals (*n=10*) from each collection year and ranging from 5 to 15 years were selected for study. Unfortunately, blubber from 1984 was unavailable.

Extraction and Analysis. Blubber was analyzed for the brominated and chlorinated compounds. Blubber (0.1 g) was spiked with BDE-71, 126, 197, 207, ^{13}C-α, β and γ recovery internal standards and extracted by ball-mill using a mixture of hexane:DCM:acetone (50 mL, 45:45:10 w/w). Extracts were reduced in volume to 8 mL and a 1 mL aliquot was removed for gravimetric lipid determination. Sample extracts were further reduced in volume (1 mL) and partitioned on a column (300 mm x 10.5 mm i.d.) of reagent-grade Florisil (1.2% deactivated (w/w), 8 g, 60-100 mesh size, Fisher Scientific). PBDE, BTBPE and isomers of DPs were eluted using 40 mL of hexane followed by 30 mL of hexane:DCM (85:15, F1); HBCDs were eluted using 7 mL of hexane:DCM (85:15) followed by 50 mL of hexane:DCM (50:50, F2).

F2 was reconstituted in isopropanol and reduced in volume 200 µL; F1 was reduced to 100 µL. F1 was then spiked with BDE-156 (5 ng), and F2 was spiked with d_{18} α-, β-, and γ-HBCD (20 ng) labelled instrument performance matrix internal standard.

Gas chromatography/mass spectrometry analysis. Detection of BDEs, BTBPE and isomers of DP was carried out using GC/MS methods (Law *et al*. 2006). In brief, a DB-5 capillary column (10m; 0.25 mm i.d; film thickness 0.25 µm) with helium as a carrier gas at a flow rate of 2mL/min was used in our separation. The initial oven temperature was set on 90 ºC with no hold time, ramped at a rate of 20 ºC/min to 310 ºC and held for 5 min. The injector temperature was set at 280ºC. For the Br-containing compounds the MS was operated under ECNI conditions using methane as buffer gas and monitoring the *m/z* 79 and 81 ions. For DP, the *syn* and *anti* isomers were detected using the most abundant ion in the molecular ion cluster i.e., *m/z* 651.8 and *m/z* 653.8. Quantitation was done using the external standard method.

LC-MS/MS analysis. Details of HBCD analysis is described elsewhere (Tomy *et al*. 2005). Briefly, separations were performed on a Genesis C_{18} analytical column (particle size 4 µm silica 2.1 i.d. × 100mm), using a linear gradient elution of water-methanol (30:70) to pure methanol for 3 min, held for 3.5 min and returned to initial condition in 3 min at a flow rate of 300 µL/min. All samples were prepared in isoprpanol (HPLC grade). The samples were analyzed on a Sciex API 2000 (MDS Sciex, ON, Canada) with electrospray ionization in negative ion mode. The detection was achieved by multiple reaction monitoring (MRM) conditions; monitoring of $[M-H]^-$ (*m/z* 640.6) › Br^- (*m/z* 79) ion transition (both isotopes). MS/MS detection of the two surrogates: d_{18}-HBCD (used as instrument performance internal standard: IPIS) and $^{13}C_{12}$-HBCD (employed as recovery internal standard: RIS) was based on the analogues $[M-H(D)]^- \rightarrow Br^-$ reaction monitored for the native HBCD [d_{18}-HBCD *m/z* 657.6 $(M-2)]^-$ and $^{13}C_{12}$ *m/z* 652.4 $[M-1]^-$.

QA/QC. Two types of blanks were employed in this study. Instrument blanks for LC/MS/MS were injections of methanol run after every five samples and were used to monitor contamination from the LC/MS/MS instrument. Extraction blanks were used to monitor the potential for contamination to occur during extraction and work-up of the sample. The SRM material (SRM 1941) which has certified concentrations of BDE congeners was run in duplicate and we obtained very good agreement between our values and the certified ones. Average recoveries of BDE-71, 126, 197 and 207 were 82% while ^{13}C-α-, β- and γ- recoveries were greater than 90%. No recovery correction was applied to the data. Some BDE congeners [range: <detection limits to 1.1 ng (BDE-181)] and HBCD (range: <detection limits for α and β-isomers to γ: 22.6 pg) were observed in the method blanks (*n=5*) and small differences were observed in the signals of instrument performance internal standards (BDE-156 and the d_{18} labelled HBCD isomers) in the sample to that in the external standard solution. The mean corrected integrated ion signals of target congeners in the blanks (correction based on the instrument performance internal standard ratio) were subtracted from the analogous corrected ion signal in the samples. This corrected area was then normalized to sample weight to generate a concentration value.

Results and Discussion.

Time trends. Both HBCD and BDE time-trend profiles fitted that of a non-linear parametric relationship (Figure 1). There was however no discerning temporal trend for either Σ BDE or Σ HBCD (one-way

ANOVA, p=0.3 and p=0.07, respectively) in HI beluga. BDE concentrations (sum of 17 congeners) remained steady between 1993 and 2005 (average of ~ 20 ng/g, lipid weight (lw)) and were ~ 15x greater than that of Σ HBCD (average ~ 1.5 ng/g, lw). For both compounds, there was a peak in concentration in 2001 at 2.2 ± 0.4 ng/g (lw, geomean ± standard error (SE)) for Σ HBCD and 23.7 ± 2.6 ng/g (lw) for Σ BDEs.

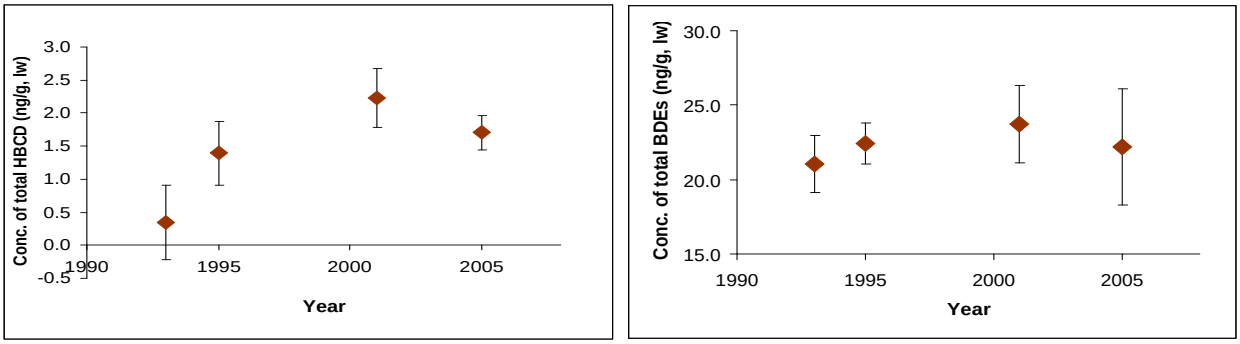

Figure 1. Change in Σ HBCD (left panel), Σ BDE (right panel) concentrations (ng/g, lipid weight) in Hendrickson Lsland beluga blubber. Each data point represents the geometric mean (*n=10*) ± 1 standard error.

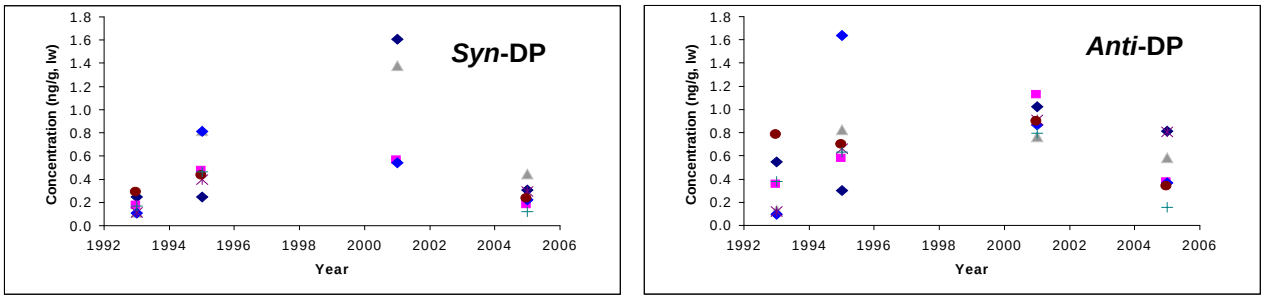

Figure 2. Temporal trends in concentrations of syn (left panel) and anti-DP (right panel, ng/g, lw) in Hendrickson Island beluga blubber. Concentrations found in the individual animals are shown on each plot.

There were no clear discerning trends in the concentrations of the DP-isomers in the HI beluga (Figure 2): concentrations were small (<2 ng/g) and have remained fairly constant over our study time period. To our knowledge, this is the first report of the DP in aquatic animals from remote locations. BTBPE was also detected in a few animals from HI (ranging from 0.1 to 2.5 ng/g, lw) but in too few cases to generate any meaningful graphical plots. Stable isotope determination on the animals showed little change in the $\delta^{15}N$ values suggesting that the diet of these animals have changed little over our study time period.

Spatial trends. There were no clear trend geographic trends for either compound: Σ HBCD concentrations were greatest in animals from Sanikiluaq (3.9 ± 0.4 ng/g, lw) and smallest in animals from Pangnirtung

(0.05 ± 0.02 ng/g, lw). Rank order of Σ BDE concentrations were Sanikiluaq (35.3 ± 5.2 ng/g lw) > HI > Arviat > Kimmirut > Pangnirtung (7.0 ± 2.3 ng/g, lw). There were statistically significant differences for both compound classes and region (one-way ANOVA, $p<0.001$ in both cases). HBCD concentrations in Pangnirtung beluga were different (Dunn's test: $p<0.05$) than those from Sanikiluaq, Kimmirut and Arviat while Sanikiluaq and HI beluga also had statistically different concentrations of HBCD. For BDEs, there were concentration differences (Tukey's test: $p<0.001$) between Saniqiluaq animals with those from Pangnirtung, Kimmirut and Arviat while differences between HI and Pangnirtung animals were also evident. BTBPE was also detected in animals from Arviat and concentrations were similar to that of HBCD.

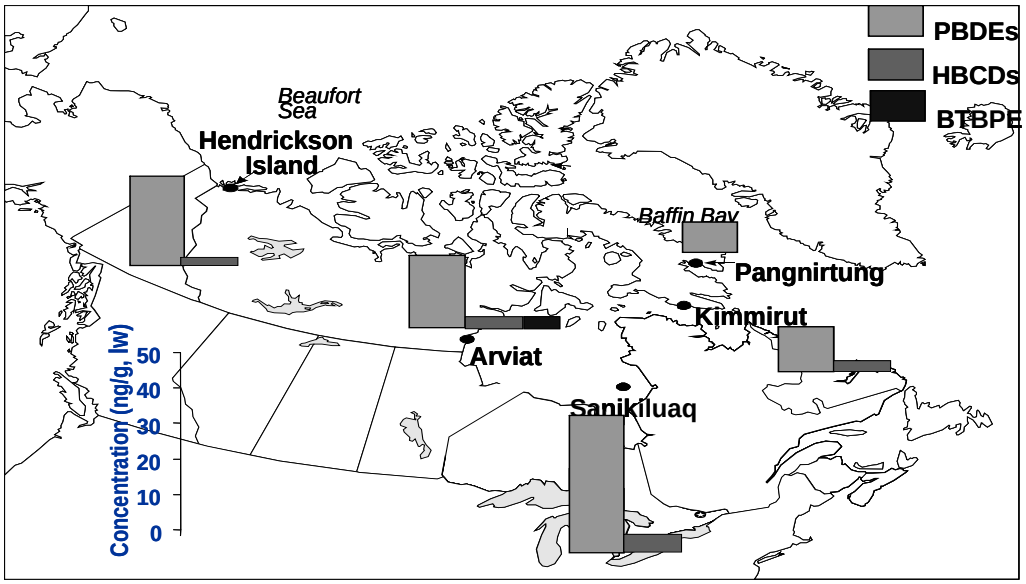

Figure 2. Spatial trends in concentrations of some BFRs in beluga from the Canadian Arctic.

References.

OSPAR Commission. JAMP Guidelines for monitoring contaminants in biota. 1999-2, 1-49. 1999.

Law K, Halldorson T, Danell RW, Stern GA, Gerwutz S, Alaee M, Marvin C, Whittle DM, Tomy GT. 2006a. Environ Toxicol Chem 25, 2177.

Tomy GT, Halldorson THJ, Danell RW, Law K, Arsenault G, Alaee M, MacInnis G, Marvin, C. H. 2005. Rapid Commun.Mass Spectro. *19*, 2819.