Temporal Trends of brominated and chlorinated flame retardants in lake trout (*Salvelinus namaycush*) from Ontario (1979-2004)

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Introduction

Lake trout (*Salvelinus namaycush*) has been used as a sentinel bioindicator species for studies on temporal trends of halogenated organic pollutants in Canadian and US monitoring programs. The rationale is that lake trout are a high trophic level organism with a relatively long life span (approximately 25 years) and so changes in the inputs of chemicals into the environment would be reflected in changes in concentrations of chemical contaminants in this species over time. For new and emerging chemicals where there is a general paucity of information regarding emissions, constructing historical concentration profiles in lake trout is one means of assessing the chronology of contamination.

Our objective in this study was to construct a temporal trend dataset on archived lake trout for brominated and chlorinated flame retardants. In addition to the brominated diphenyl ethers (BDEs), hexabromo-cyclododecane (HBCD), 1,2,bis(tribromophenyoxy)ethane (BTBPE), pentabromoethylbenzene (PBEB) and the isomers of Dechlorane Plus (DP) were studied.

Materials and Methods

Samples. Lake trout (4 year olds) were collected from Lake Ontario as part of the Department of Fisheries and Oceans long term monitoring program in the Great Lakes. Individual whole fish were homogenized then stored frozen in glass jars at -80°C until analysis in the archive, maintained by the Great Lakes Laboratory for Fisheries and Aquatic Sciences.

Extraction and clean-up. The extraction and analysis protocols for brominated compounds have been described in Law *et al*. 2006. Briefly, thawed and weighed samples were mixed with Hydromatrix (600 ºC for 6 h) and placed into a 100 mL ASE cell. The void volume was filled with Ottawa sand (baked at 600 ºC for 6 h) then spiked with recovery internal standard (RIS); PBDEs-mix (BDE -71, -126, -197, -207), and ^{13}C-labeled α-, β-, and γ-HBCD 10 ng of each chemical. Extractions were carried with 50% hexane in dichloromethane (DCM). Extracts were reduced in volume and filtered with polytetrafluoroethylene syringe filter (1 μL Gelman). An aliquot of each extract was evaporated to dryness and lipid weights were determined gravimetrically. Lipid was removed from the remaining extract using gel-permeation chromatography and extracts were further cleaned up on a column of Florisil (1.2% deactivated, 8 g, 10.5 mm i.d. × 300 mm). Two fractions were collected using gradient elution with pure hexane (40 mL) to hexane-dichloromethane (85:15, 30 mL), as fraction 1. The column was further eluted with hexane-dichloromethane (85:15, 7 mL to (50:50, 50 mL) and collected fraction 2. PBDEs and DP were eluted in

F1 fraction while F2 fraction containing BTBPE and HBCD isomers. PBEB eluted in both F1 and F2 fractions.

Gas chromatography/mass spectrometry analysis. Detection of PBDEs, PBEB, BTBPE and isomers of DP was carried out using GC/MS methods. 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 280°C. 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 in 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 $[M-1]^-$.

Results and Discussion.

Figure 1 shows the mean concentrations (wet weight basis) of the compounds examined in this study in lake trout from Lake Ontario (1979-2004). Total BDEs (Σ BDE: sum of 28, 47, 99, 100, 154, 153) were by far the most dominant flame retardant examined in this study. The temporal profile shows a linear increase in concentration between 1979 to 1998 followed by what appears to be a decrease in 2004. These observations are consistent with the work of Zhu and Hites. These results are puzzling considering that it was only in 2004 that the BFR industry announced plans to discontinue production of the penta- and octa-BDE mixtures. An alternative explanation for our results would be that if emissions were assumed to have remained unchanged, then perhaps the feeding ecology of trout might have changed resulting in an apparent decline in Σ BDE-concentrations. The $\delta^{15}N$-data (Figure 2) clearly suggest that in fact the diet of trout from Lake Ontario have changed and from 1998 to 2004 these animals have been feeding on organisms at a lower trophic level. By accounting for this trophic level shift, the concentration difference between the animals in 1998 and 2004 are less dissimilar. Despite the change in feeding ecology of the animals, the concentration profile of BDE-209 shows a linear increase ($p<0.05$) over the study period. This is thought to be consistent with the use of this compound; BDE-209 accounts for ~ 80% of global production of PBDEs and today remains unregulated.

Concentrations of HBCD were between 2.03 ng/g and 4.11 ng/g. ww and showed a declining trend during sampling years. BTBPE, which is now replacing the popular penta-mix, shows a strong linear increase from 1979 to 1998 followed by small decline in concentrations in 2004; concentration corrections using

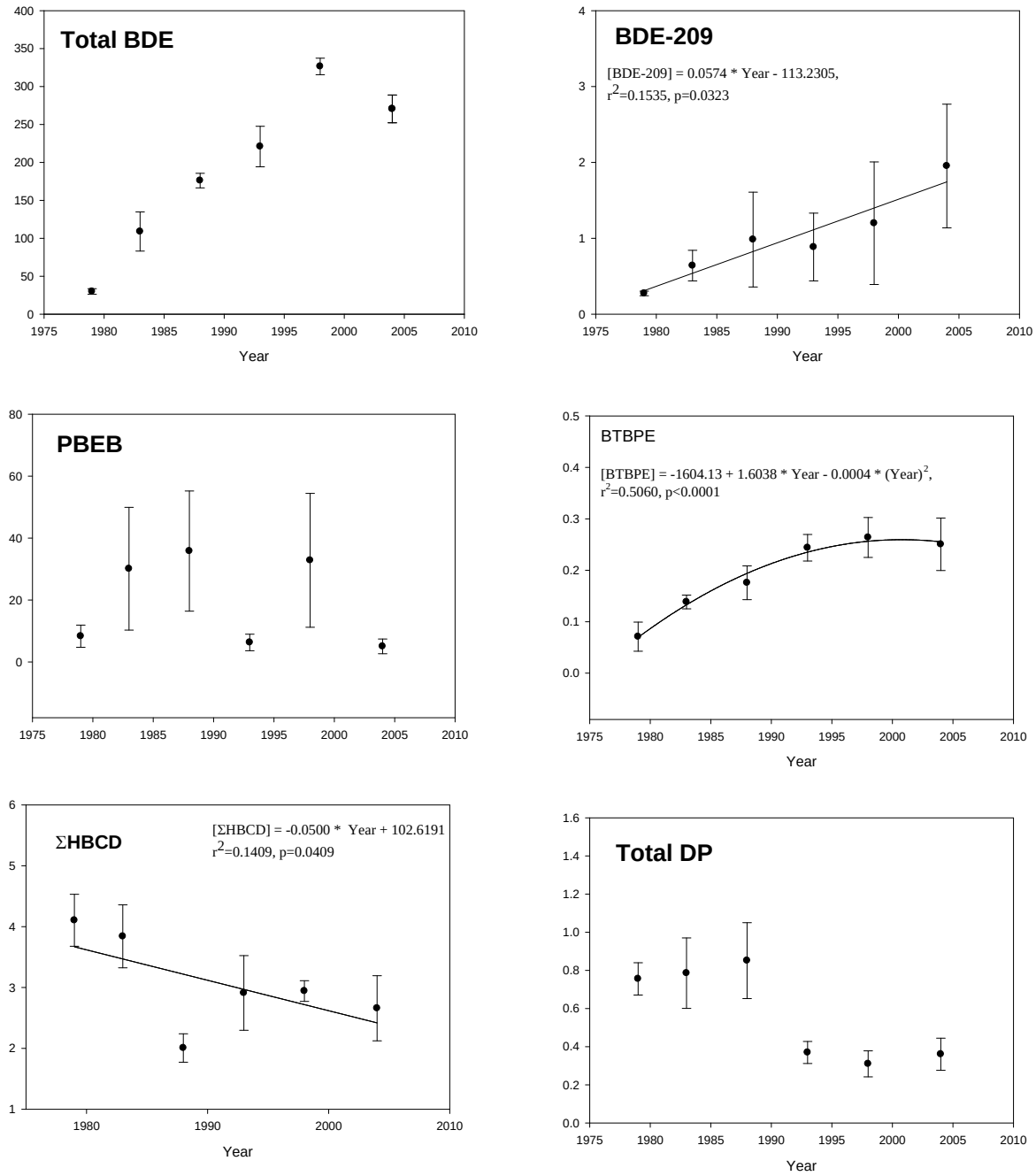

Figure 1. Temporal trends of total BDE (sum of 6 congeners, top left panel), BDE-209 (top right panel), PBEB (middle left panel), BTBPE (middle right panel) ΣHBCD (bottom left panel) and ΣDP (bottom, right panel).

the δ^{15}N-data showed higher concentrations in the 2004 animals compared to those in 1998 suggesting that BTBPE emissions are in fact continuing to increase. No clear temporal trend was observed for PBEB. The only chlorinated chemical DP showed an increase in concentration between 1979 to 1993 followed by a decline starting from 1993.

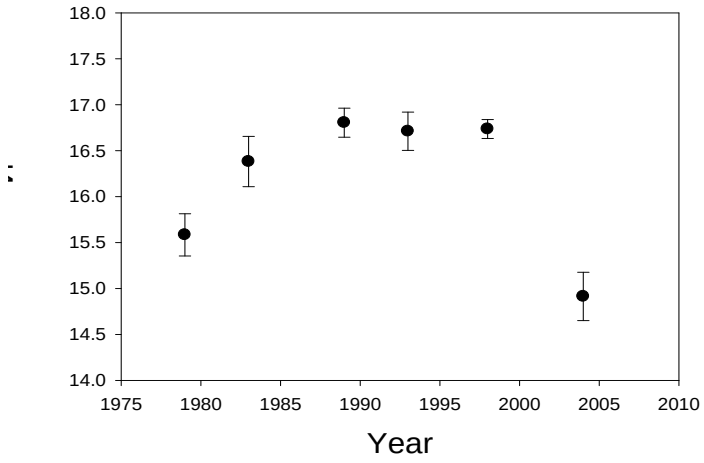

Figure 2. Mean δ^{15}N (±1 x standard error, *n=5*) of lake trout from Lake Ontario.

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