(Re)Emerging Halogenated Flame Retardants in Predator and Prey Fish From the Laurentian Great Lakes: Age-Dependent Accumulation and Trophic Transfer

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Introduction

Flame retardants (FRs) refer a diverse group of chemicals, which have been widely used over decades and added to various manufactured materials such as plastics, electronic product, textiles, surface finishes and coatings to delay the ignition of flame and to prevent the spread of fire [1,2]. Legacy FRs involved a few classes (i.e. commercial penta/octa bromodiphenyl ethers (penta/octa-BDEs), hexabromocyclododecane (HBCDD), hexabromobiphenyls (PBBs)), which in recent times were listed under Annex A of the persistent organic pollutant (POP) Stockholm Convention and are destined for global elimination [3]. Strict restrictions and ban on legacy FRs has led to the increasing demand for "novel" FRs as replacements, and are regarded as emerging FRs, i.e. dechlorane plus (DDC-COs), decabromodiphenyl ethane (DBDPE), 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE) and 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (EHTBB) [1,4]. Environmental monitoring studies demonstrated that both legacy and emerging FRs are pervasive in the environment samples (i.e. bird eggs, water, fish, atmospheric dust) of the Laurentian Great Lakes of North America [5,6]. Aquatic trophic transfer has been well-examined for the legacy PBDE FRs in some specific Great Lakes locations but not so much for emerging FRs [7]. In a mixed food web of native and non-native species in Lake Erie showed that non-native prey species such as rainbow smelt contributed significantly to the biomagnification of PBDEs [8]. Trophic transfer of DDC-COs was also investigated in a marine food web from Liaodong Bay, China, and significantly positive relationships were found between lipid equivalent concentrations of *anti*-DDC-CO and trophic levels with a trophic magnification factor (TMF) of 5.6 [9]. The objectives of this study were: 1) to investigate 48 FRs in Lake Ontario and Lake Erie aquatic food web; 2) to evaluate bioaccumulation of FRs in top predator fishes (i.e. Lake Trout and Walleye) with respect to sex and age; and 3) to examine if there are significant positive relationships between FR concentrations and trophic levels.

Materials and methods

The full list of the 48 target FRs (25 PBDEs and 23 other flame retardants (NPHFRs)), along with their full chemical names and chemical structures is provided in Figure 1. All aquatic samples were as part of fish contaminant monitoring and surveillance in the Great Lakes conducted by Environment and Climate Change Canada (ECCC). Detailed information on collection, sample preparation, and storage methods have been described elsewhere, and biological information on these fish can be found in our recent study [10]. In brief, a total of 65 aquatic biotic samples collected in 2010 from Lake Ontario (n=26) and Lake Erie (n=39) were analyzed for legacy and emerging FRs. Samples from Lake Ontario included Alewife (*Alosa pseudoharengus*; n=2), Deepwater Sculpin (*Myoxocephalus thompsonii*; n=2), Lake Trout (*Salvelinus namaycush*; n=15), Rainbow Smelt (*Osmerus mordax*; n=2), Round Goby (*Neogobius melanostomus*; n=2) and Slimy Sculpin (*Cottus cognatus*; n=2). Samples from Lake

Erie included Emerald Shiner (*Notropis atherinoides*; n=3), Freshwater Drum (Aplodinotus grunniens; n=3), Lake Trout (*Salvelinus namaycush*; n=7), Rainbow Smelt (Osmerus mordax; n=3), Round Goby (*Neogobius melanostomus*; n=3), Trout Perch (*Percopsis omiscomaycus*; n=3); Walleye (*Alosa pseudoharengus*; n=10), Whiter Perch (*Morone americana*; n=3) and Yellow Perch (*Perca flavescens*; n=3). After capture, fish are immediately frozen on dry ice and transported to the laboratory where they are partially thawed, weighed, measured, and sexed. Scales, fin rays, and/or otoliths are removed for aging.

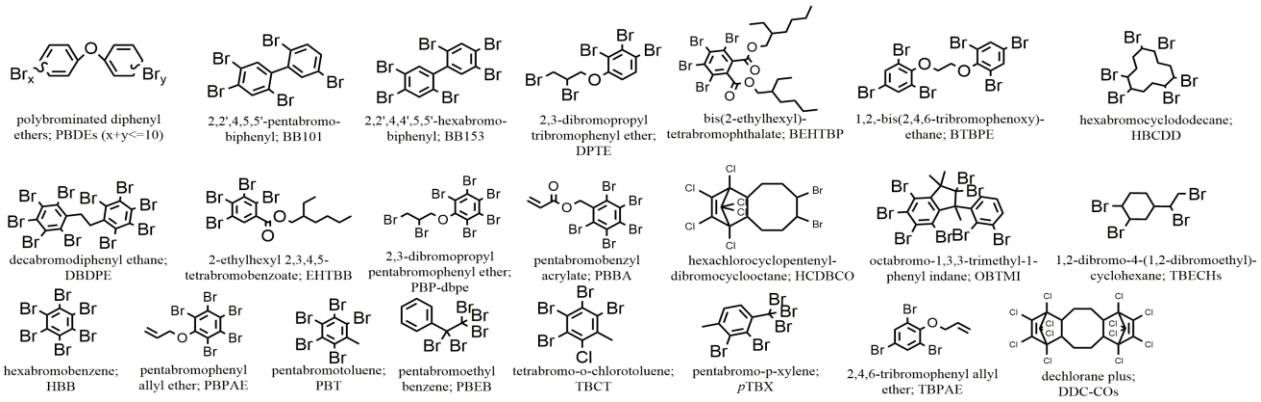

Figure 1. Chemical structures of the target legacy and emerging FRs in this study

Determination of these target 48 brominated FRs in biotic samples was carried out according to our previously published methods [11]. In brief, approximately 2.0 g of the biotic homogenate (wet weight, ww) was accurately weighed and homogenized with pre-cleaned diatomaceous earth (DE) for accelerated solvent extraction (ASE), and was spiked with a mixture of internal standards, i.e. BDE-30 and -156 and $^{13}C_{12}$-BDE-209. From the ASE sample extracts was taken a 10% volume for lipid determination. The remaining portion was concentrated under gentle nitrogen, and further cleaned up by high performance-gel permeation chromatography (HP-GPC) (Waters, Milford, MA), followed by further cleaned-up on a silica LC-Si SPE cartridge (500 mg X 6 mL; 6 gram; J.T. Baker, USA). The final sample fraction was analyzed by gas chromatography (6890 GC) -single quadrupole mass spectrometry (5973N MS) (GC-MS; (Agilent Technologies, Mississauga, ON, Canada)) operated in the electron capture negative ion (ECNI) mode. Brominated FR quantification was achieved via selected ion monitoring (SIM) for *m/z* $^{79}Br^-$ and $^{81}Br^-$, except for BDE-209 (*m/z* 487) and $^{13}C_{12}$-BDE-209 (*m/z* 495). The molecular ion (*m/z* 652) was used for quantifying *syn*- and *anti*-DDC-CO isomers.

The doubling time (T_2) was defined as the rate that FR concentrations doubled annually as a function of growth rate of the fish. T_2 was derived from the slope of the plots of natural log FR concentrations (wet or lipid weight) *versus* fish age in year as follows (Ln [FR] = A + S × [Age] and T_2 = Ln2/S) where S and A is the slope and intercept of fitted curves, respectively. [FR] and [Age] were FR concentrations (expressed as ng/g ww or ng/g lw) and fish ages, respectively. The $^{15/14}N$ and $^{13/12}C$ stable isotope ratios were determined by the Environmental Isotope Laboratory (EIL) at the University of Waterloo (Ontario, Canada). Stable $^{15/14}N$ and $^{13/12}C$ isotope analysis is described in a previous publication [12]. Trophic level (TL) was assigned relative to plankton using $TL_{sample} = 2 + (\delta^{15}N_{sample} - \delta^{15}N_{plankton}) \div \Delta N$. Here, ΔN is trophic enrichment factor that is estimated to be 3.4 ‰ [13]. The TMFs were based on the entire food web of Lake Ontario (n = 26) or Lake Erie (n = 39), and derived from the slope of the plots of natural

log concentrations (wet weight or lipid weight) *versus* TL as follows (Ln [FR] = A + S × [TL_{sample}] and TMF = e^S) where [FR] is FR concentrations (ng/g ww) determined in aquatic samples; S is the slope of the plots of natural log concentrations (wet weight) versus TL.

Results and discussion

Twenty-one PBDE congeners, including BDE-47, -100, -119, -154, -153, -99, -28, -49, -85/155, -209, -66, -183, -203, -207, -17, -15, -77, -205, -138, and -206, were quantifiable in at least one of the 65 fish. ΣPBDE concentrations varied dramatically with a range from 5.73 ng/g ww (Rainbow Smelt from Lake Erie) to 808 ng/g ww (Lake Trout from Lake Ontario), and BDE-47, -99, -100, -153 and -154 are the dominant congeners constituting > 80 % of the ΣPBDE concentrations. Lake Trout from Lake Ontario contained significantly greater PBDE concentrations than those from Lake Erie. Among 23 NPHFRs analyzed, BB-153 (100 %), BB-101 (83.1 %), *syn*-DDC-CO (87.7 %), *anti*-DDC-CO (89.2 %), HBCDD (83.1 %), pTBX (72.3 %), PBEB (56.9 %), TBP-AE (38.5 %), PBT (16.9 %), DBDPE (10.8 %), α/β-DBE-DBCH (7.7 %) and TBCT (6.2 %) were quantifiable in at least one sample, whereas DPTE, PBPAE, HBB, PBBA, EHTBB, HCDBCO, PBPA-DBPE, BTBPE, BEHTBP and OBTMI were not detectable in any samples. HBCDD was quantifiable in 100 % of the samples from Lake Ontario with concentrations ranging from 1.72 to 162 ng/g ww. Like PBDEs in Lake Ontario, the greatest mean HBCDD concentrations were in Lake Trout (86.4 ng/g ww), which was following by Slimy Sculpin (29.8 ng/g ww), Alewife (12.6 ng/g ww), Rainbow Smelt (12.2 ng/g ww), Deepwater Sculpin (7.94 ng/g ww) and Round Goby (2.29 ng/g ww), respectively. Lake Erie fish clearly contained less HBCDD than those from Lake Ontario, and greatest mean HBCDD concentrations were in Lake Trout (13.8 ng/g ww). *Syn*- and *anti*-DDC-CO were quantifiable in > 88% of all fish, but concentrations were consistently at sub-ppb level. Overall, TBPAE, pTBX, PBEB, PBT, TBCT were at low, sub-ppb levels or not detectable. DBDPE was detected in 7 of the 65 fish. α-/β-TBECH isomers were detected in 5 of the 65 fish at sub-ppb levels.

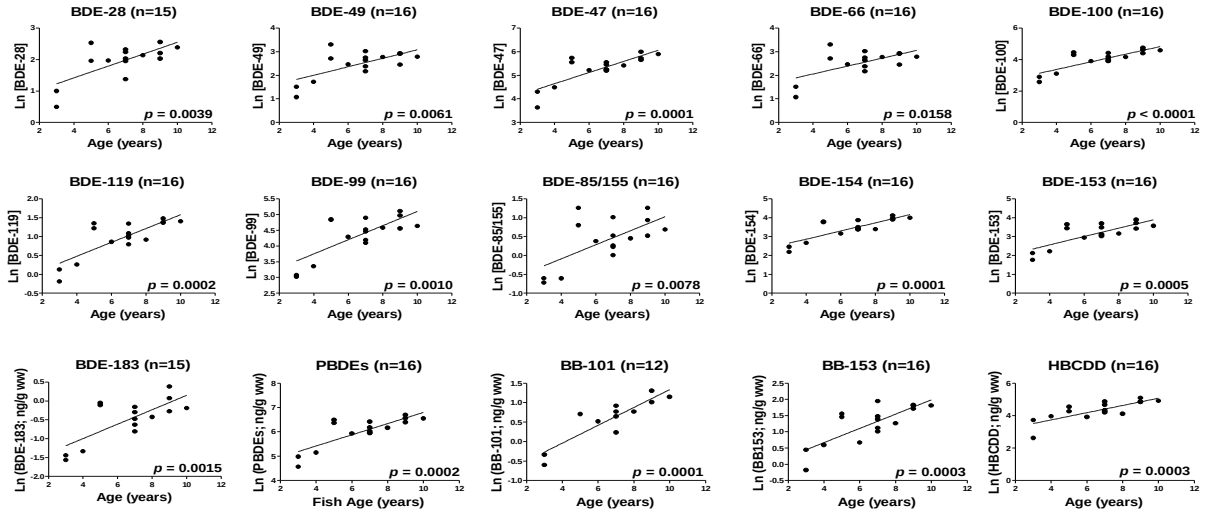

Figure 2. Chemical concentrations in Lake Trout of the Lake Ontario (ng/g wet weight; Log-transformed) versus fish ages (years) for selected FRs

Relationships between fish individual characteristics (i.e. sex, age, length and weight) and FR concentrations could be examined for the Lake Trout from Lake Ontario (n=15; 9 female and 6 male), and the Walleye from Lake Erie (n=10; 5 female and 5 male). For most of quantifiable FRs, significant differences were observed between male and female Walleye from Lake Erie. FR concentrations (wet weight) increased as a function of increasing fish age. Significant positive linear correlative relationships were observed for sixteen FRs (BDE-28, -49, -47, -66, -100, -119, -99, -95/155, -154, -153 and -183, $\sum_{20}$PBDEs, BB-101, BB-153 and HBCDD) in Lake Trout from Lake Ontario between Log-normalized concentration (wet weight) versus fish age (Figure 2), and T_2 values were derived from the slope of the fitted linear curves and ranged from 2.9 (BDE-47) to 3.9 (BDE-49) years. For FR concentrations in Walleye from Lake Erie, significant positive linear correlative relationships were observed BDE-66, -119, -99, -85/155, -154 and -153, pTBX, BB-153 and HBCDD, and T_2 ranged from 2.0 (HBCDD) to 5.5 (BB-153) years. Significant positive relationships ($p < 0.05$; slope > 0) were observed for most of the FRs from the plot of natural log wet weight concentrations versus TL (based on δ^{15}N-values) for both Lake Ontario and Lake Erie fishes. However, the significantly positive relationships disappeared whene the chemical concentrations were normalized with lipid weight. The exceptions were BDE-28, -47 and -119, BB-153 and HBCDD in Lake Ontario fish, with TMF values of 1.60, 2.11, 2.33, 2.25 and 2.23, respectively, indicatingbiomagnification potential in the Lake Ontario food web.

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