LEVELS AND BIOACCUMULATION OF HALOGENATED FLAME RETARDANTS IN THE LAKE ERIE AQUATIC FOOD WEB

Mackintosh SA[1*], Pérez-Fuentetaja A[2], Zimmerman LR[1], Pacepavicius G[3], Clapsadl M[2], Alaee M[3], Aga DS[1]

[1]Department of Chemistry, University at Buffalo, State University of New York, Buffalo NY 14260, USA; [2]Department of Biology and Great Lakes Center, The State University of New York College at Buffalo, Buffalo, NY 14222, USA; [3]Aquatic Ecosystem Protection Research Division, Water Science and Technology Directorate, Environment Canada, Burlington, Ontario L7R 4A6, Canada

Introduction

A flame retardant is a substance added or applied to a material in order to increase the flame resistance of that material and reduce fire risk. Halogenated flame retardants (HFRs) comprise about 25% by volume of the global production of flame retardants (FRs)[1]. Structurally diverse, HFRs are commonly chlorinated or brominated and added or reacted with polymers, textiles, and electronics. The most commonly applied HFRs are brominated flame retardants (BFRs), with over 75 different compounds that have been commercially produced[2].

Polybrominated diphenyl ethers (PBDEs) are among the most frequently used BFRs and are of particular concern due to their persistence and bioaccumulative nature. In animals, these compounds have been shown to have toxic effects on the endocrine system, thyroid function, and early neurodevelopment[3,4,5]. PBDEs in Great Lakes sediment is a well documented issue[6,7] and more recent studies show that levels in fish have been increasing in the last decades[8,9]. Lake Erie endures large impacts from urbanization, agriculture, and industry[10] with PBDE-loading estimated to be around 3,400 kg/yr from contaminated water inflows and atmospheric deposition[6]. Studies on levels, trophic transfer, and bioaccumulation of PBDEs in Eastern Lake Erie are still limited.

Production of Penta-BDE and Octa-BDE technical formulations has been banned in North America as a result of their toxicity and ubiquitous occurrence in the environment. However, products containing these compounds are still in use, hence continued monitoring of environmental levels is warranted. In addition, new HFRs have emerged as replacements for the banned formulations, for which environmental occurrence and fate needs to be understood.

The aim of this study was to investigate the current status of PBDEs and replacement HFRs in the Eastern Lake Erie aquatic food web. PBDEs and selected chlorinated and brominated flame retardants were detected and quantified in biota. Trophic positions and interactions were determined through stable isotope and fish diet analyses. By relating contaminant loads and trophic analyses insights on bioaccumulation, and trophic transfer were obtained. This study is of importance as it provides baseline data for HFRs in Eastern Lake Erie. In addition, emerging HFRs potential accumulation and trophic transfer in aquatic food webs is investigated.

Materials and methods

Study Area and Samples. Fish were collected in 2009 in Eastern Lake Erie species include: Lake Trout (n=5), Walleye (n=5), Steelhead Trout (n=5), Small Mouth Bass (n=5), Yellow Perch (n=5), Rainbow Smelt (individuals n=4, group composite n=5), Round Gobies (individuals n=5, group composite n=5), Emerald Shiners (group composite n=10). Group composite sample sets were determined based on fish with similar total lengths (mm). Invertebrates were collected during 2009-2010 species include: amphipods (n=2), dreissenid mussels (n=2), and zooplankton (n=6). Sediment (n=6, 2009) and water (2011) samples were also collected.

Biological measurements. Total length (mm), weight, and sex of fish samples were determined. Stomach contents were removed to determine fish diet, and to avoid their influence on the stable isotope analysis and

contaminant levels. Samples were sent to Colorado Plateau Stable Isotope Laboratory in Northern Arizona for ^{13}C and ^{15}N stable isotopes determination.

Sample Prep for Chemical Analysis. Before extraction, whole fish samples or group composites were homogenized. Four grams, wet weight (ww), of each sample was extracted using accelerated solvent extraction (ASE™) with dichloromethane:hexane (1:1 v/v). Samples were spiked with a ^{13}C-surrogate mixture prior to extraction. Crude extracts were then concentrated and underwent purification by Gel Permeation Chromatography (GPC) and further clean-up with an acidified silica gel column prior to instrumental analysis.

Instrumental Analysis. PBDEs (BDE-1, -2, -3, -7, -10, -15, -17, -28, -30, -47, -49, -66, -71, -77, -85, -99, -100, -119, -126, -138, -139, -140, -153, -154, -156, -169, -171, -180, -183, -184, -191, -196, -197, -201, -203, -204, -205, -206, -207, -208, -209), and selected emerging HFRs (Table 1.) were analyzed by gas-chromotaography-mass spectrmetery (GC-MS). PBDEs were analyzed by GC-high resolution-MS (GC-HR-MS) using a Micromass Ultima magnetic sector mass spectrometer coupled with an HP 6890 GC (Waters Micromass, Manchester, U.K.) operated in selected ion monitoring (SIM) positive electron ionization (EI) mode. HFRs were analyzed by GC-triple quadrupole tandem- MS (GC-QQQ MS/MS) operated in selected reaction monitoring (SRM) positive EI mode. This analysis was carried out using a Trace GC Ultra coupled to a TSQ Quantum triple quadrupole mass spectrometer (Thermo Fisher Scientific, San Jose, CA USA).

Table 1. Emerging HFRs, precursor ions for SRM QQQ- MS/MS analysis, collision energies, and product ions.

HFR	Precursor ion (*m/z*) SRM	Collision energy (eV)	Product Ions (*m/z*) (Q/C/C)[a]
Allyl 2,4,6-tribromophenyl ether (ATE)	372	8	212/ 291
2,3,5,6-Tetrabromo-p-xylene (pTBX)	422	22	343/ 262
2-Bromoallyl 2,4,6-tribromophenyl ether (BATE)	450	8	329/ 290
Pentabromotoluene (PBT)	488	18	409/ 407/ 328
Pentabromoethylbenzene (PBEB)	502	15	487/ 421
2,3-Dibromopropyl 2,4,6-tribromopehnyl ether (DPTE)	533	3	334/ 330
Hexabromobenzene (HBB)	552	45	313/ 393
Hexachlorocyclopentenyl-dibromocyclooctane (HCDBCO)	542	6	300/ 377
2-ethylhexyl-2,3,4,5-Tetrabromobenzoate (EHTBB)	421	20	393/ 312
1,2-Bis(2,4,6-tribromophenoxy)ethane (BTBPE)	693	10	359/ 356
Bis(2-ethyl-1-hexyl)tetrabromophthalate (BEHTBP)	465	35	381/ 305/ 340
Octabromotrimethylphenylindane (OBIND)	854	8	773/ 774/ 405
Decabromodiphenylethane (DBDPE)	973	12	487/ 815
Syn-Dechlorane Plus® (sDP)	654	8	571/ 301
Anti-Dechlorane Plus® (aDP)	654	6	315/ 571/ 274

[a] Q: quantifying ion C: confirming ion

Results and discussion

Stomach content analysis (Figure 1.) revealed that, at the time of capture, the fish prey species fed primarily on chironomid larva and pupae and some larval fish and plankton, except for round gobies that had an exclusive diet

of dreissenid mussels. For the predator species, lake trout and walleye fed heavily on rainbow smelt, while steelhead trout fed mostly on emerald shiners and the small mouth bass' diet was comprised of round gobies. In terms of total PBDE loads in fish, all prey fishes (yellow perch, rainbow smelt, emerald shiners and round gobies) were significantly different ($p<0.05$) from the top predator fishes (lake trout, walleye, steelhead trout, small mouth bass). In addition, total PBDE content in fish was correlated with their $\delta^{15}N$ ($r=0.772$, $p<0.0001$), thus the trophic level of the fish is a good indicator of their PBDE loads. In other words, the higher the $\delta^{15}N$ content of a fish, the higher the likelihood that their PBDE content is also high due to bioaccumulation of the contaminant.

All biota samples analyzed contained PBDE congeners. Mean concentrations (arithmetic mean ± standard error) of ∑PBDEs (sum of congeners) ranged from 0.30 ± 0.1 ng/g ww in dreissenid mussels to 31.44 ± 5 ng/g ww in walleye (Figure 2.). Comparison of the BDE congener profiles detected in the species analyzed shows a similarity in concentrations of various congeners in those species that occupy the same trophic level. These profiles are effective tracers of food web connections and trophic levels.

Biomagnification factors (BMF) were calculated as the ratio of wet weight concentrations in predator/ wet weight concentrations in prey for individual species (BMF= [predator]/[prey]). Predator/Prey feeding relationships were established based on stomach content diet analysis. BMF values for ∑PBDE congeners were determined for all feeding relationships and are outlined in table 2. BMF values for BDE-47, BDE-99, BDE-100, BDE 153, BDE 154, and BDE 209 were determined for the top predator feeding relationships (lake trout, walleye, steelhead trout, and small mouth bass) and are outlined in table 3. BMF values larger than 1 suggest biomagnifications between trophic levels is taking place. For ∑PBDE congeners all feeding relationships except for perch/amphipods and zooplankton and shiners/zooplankton BMFs were greater than 1. BMF values for BDE-47, BDE-99, BDE-100, BDE 153, BDE 154, and BDE 209 in the top predator species were all greater than one except small mouth bass / round gobies for BDE-209. BMFs will also be determined for lipid corrected concentrations and compared to those obtained from other studies. Finally BMF values for emerging HFRs will be obtained and compared with values for PBDEs.

This study provides vital baseline data on the current PBDE levels in the biota, sediment and water from eastern Lake Erie as well as insights on trophic interactions and bioaccumulation. An instrumental method for analysis of emerging HFRs has been developed (Table 1.) and this work will include quantifying levels of these compounds in biota from this study. Conclusions will be drawn about the behavior of emerging HFRs in an aquatic food web and the potential for bioaccumulation and biomagnification.

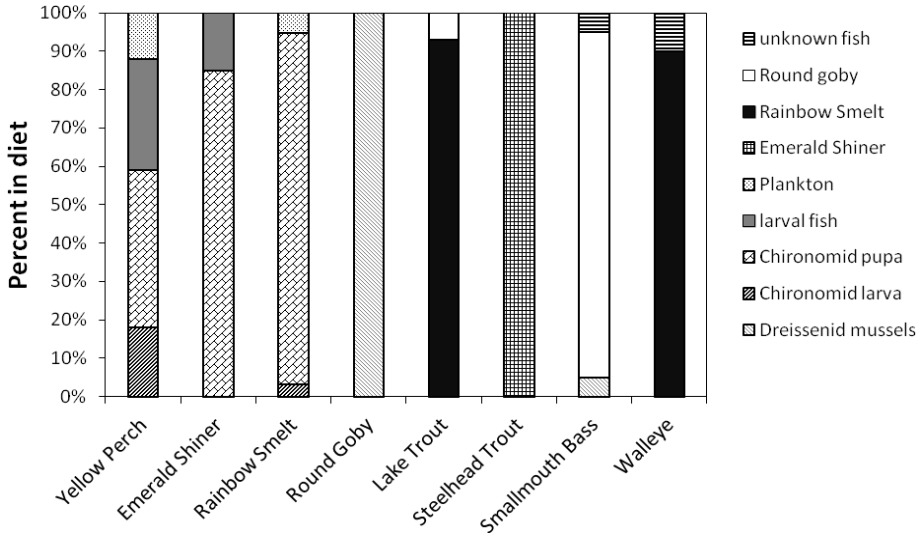

Figure 1. Percent diet composition based on stomach content analysis for prey and predator fish species collected in eastern L. Erie.

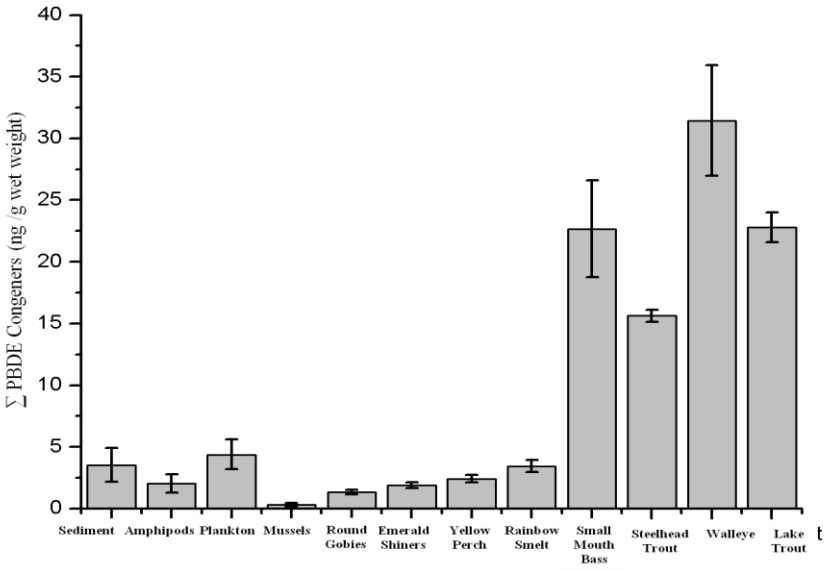

Figure 2. PBDE congeners (ng/g ww) (mean ± SE), species are arranged in order by their mean $\delta^{15}N$ content.

Table 2. BMF values for ∑PBDE in Lake Erie predator/prey feeding relationships

Predator/ Prey	BMF ∑PBDE
Yellow Perch/ Amphipods and Zooplankton	0.75
Emerald Shiner Groups/ Zooplankton	0.92
Rainbow Smelt / Zooplankton	1.68
Round Goby/ Dressinaid Mussels	4.53
Lake Trout / Rainbow Smelt	6.65
Steelhead Trout / Emerald Shiners	8.31
Walleye / Rainbow Smelt	9.17
Small Mouth Bass / Round Gobies	16.96

Table 3. BMF for individual BDE congeners for Lake Erie predator/prey feeding relationships

	BMF					
Predator/ Prey	**BDE 47**	**BDE 99**	**BDE 100**	**BDE 153**	**BDE 154**	**BDE 209**
Lake Trout / Rainbow Smelt	6.07	5.13	7.33	7.31	5.54	n/a
Walleye / Rainbow Smelt	7.23	9.16	10.34	11.14	7.62	1.26
Steelhead Trout / Emerald Shiners	7.67	n/a	6.40	6.54	5.54	n/a
Small Mouth Bass / Round Gobies	10.73	6.65	17.01	16.11	15.52	0.77

Acknowledgements
We are thankful to the following people and organizations for helping to obtain samples and perform analyses. Jessica Wuerstle and Beryl Ankrah, The Dunkirk Fisheries Unit - Dept. of Environmental Conservation, NY; Funding was provided by the Great Lakes Protection Fund Large Grants Program to A. Perez-Fuentetaja. ERIE IGERT National Science Foundation Grant (NSF Grant No. 0654305).

References:

1. Covaci A, Harrad S, Abdallah M, Ali N, Law R, Herzke D, de Wit C. (2011) *Environ Int.* 37: 532-556
2. Alaee M, Arias P, Sijodin A, Bergmen A. (2003) *Environ Int.*29: 683-689
3. McDonald T. (2002) *Chemosphere* 46: 745-755
4. Meerts I, Letcher R, Hoving S, Marsh G, Bergman A, Lemmen J, van der Burg B, Brouwer A. (2001) *Environ. Health Perspect.*109: 399-407
5. Siddiqi M, Laessig R, Reed K. (2003) *Clin.Med. Res.* 1: 281-290
6. Song W, Ford J, Li A, Sturchio N, Rockne K, Buckley D, Mills W. (2005) *Environ. Sci. Technol*. 39 (15): 5600-5605
7. Samara F, Tsai C, Aga D. (2006) *Environ. Pollut*. 139 (3): 489-497
8. Carlson D, De Vault D, Swackhamer D. (2010) *Environ. Sci. Technol*. 44 (6): 2004-2010
9. Montory M, Habit E, Fernandez P, Grimalt J, Barra R. (2010) *Chemosphere* 78: 1193-1199
10. Pérez-Fuentetaja A, Lupton S, Clapsadl M, Samara F, Gatto L, Biniakewitz R, Aga D. (2010) *Chemosphere* 81: 541-547