COMPARATIVE PROFILING OF FLAME RETARDANTS AND OTHER BROMINATED CONTAMINANTS IN BODY COMPARTMENTS AND IN OVO TRANSFER IN NORTH AMERICAN GREAT LAKES HERRING GULLS

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Introduction

Flame retardant (FR) chemicals are a class of chemicals used in polymeric materials in commercial products to adhere to fire safety regulations. As a consequence of substantial, long-term use, many FRs have been reported in humans, wildlife, air, water, soil, sediment, and even in remote areas such as the Arctic.[1] Halogenated FRs have been widely used and to date, in the order of 75 different brominated FRs have been commercially produced, and with other unknown FRs likely being part of newer FR technical formulations. The three major brominated FRs that have been produced in the highest volumes include polybrominated diphenyl ethers (PBDEs), hexabromocyclododecane (HBCDD), and tetrabromobisphenol A (TBBPA).[2] These groups of BFRs are persistent and bioaccumulative and have been found in wildlife and human tissues.[2,3] PBDEs have been produced since the 1970's with three main Penta, Octa, and DecaBDE formulations. PentaBDE is mainly composed of BDE-47, -99, and -100, OctaBDE consists mostly of BDE-183 and some octa-BDE congeners, and DecaBDE contains more than 97% BDE-209.[4] The recent worldwide restriction of the use of the PentaBDE and OctaBDE formulations has led to an increasing use of alternative FRs, many of which are not regulated.[5]

Environment Canada's Great Lakes Herring Gull Monitoring Program (GLHGMP) monitors the distribution and trends of persistent organic pollutants (POPs) throughout the Great Lakes by analyzing herring gulls eggs on an annual basis.[6] We recently examined 19 alternative brominated FRs in egg pools of herring gulls based on eggs collected from 1982-2006 from seven colony sites across the Laurentian Great Lakes of North America.[7] Over these years and at these sites, several of these FRs were detectable, including hexabromobenzene (HBB), 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE), decabromodiphenyl ethane (DBDPE), and the α-, β-, γ-, and δ-isomers of 1,2-dibromo-4-(1,2-dibromoethyl)cyclohexane (TBECH). Spatial trends were observed between the colony sites, but temporal trends were not. In 2008, the same pools were used to determine the temporal and spatial trends of PBDEs in Great Lakes herring gulls.[4] BDE-209 showed increasing concentration levels starting in 1997, whereas BDE-47, -99, and -100 congener levels showed rapid increases until 2000, but with no further increasing trends until 2006. Further monitoring up until 2012 of PentaBDE and OctaBDE derived congeners is showing continual concentration decreases.

Despite numerous reports on spatial and temporal trends of emerging POPs in wildlife, few studies have investigated the levels of POPs, specifically FRs, in the body and egg compartments of avian wildlife, including Great Lakes herring gulls.[6] For example, Gebbink and Letcher (2012) examined perfluorinated sulfonates (PFSAs) and carboxylates (PFCAs) in several herring gull body tissues and the yolk and albumen egg fractions with results exhibiting tissue-specific accumulation.[8] These same gull samples have very recently been used to examine organophosphate flame retardants (OPFRs) and their metabolites.[9] In the present study, a comprehensive suite of PBDEs and FR compounds were examined in the body and egg compartments of eight female herring gulls from a Great Lakes' colony site in order to investigate tissue distribution and *in ovo* transfer.

Materials and methods

Female herring gulls (*Larus argentatus*) (n = 8) and their corresponding eggs (n = 17) were collected from Chantry Island (Lake Huron) in 2010 for the analysis of a group of legacy and emerging compounds. The maternal herring gull tissues (liver, adipose, muscle), red blood cells (RBCs) and whole egg clutches (yolk and albumen) were monitored for 45 PBDEs and 22 non-PBDE FRs.

Approximately 1 g of sample tissue was ground with diatomaceous earth and subjected to accelerated solvent extraction (ASE) with 1:1 dichloromethane:hexane. Extraction cells were spiked with 20 ng of internal standards, which included BDE-30, -156, $^{13}C_{12}$-209, $^{13}C_{10}$-*syn*-DDC-CO, $^{13}C_{10}$-*anti*-DDC-CO, and 6-MeO-BDE-137. A portion of the extracts were removed for lipid analysis, and the remaining extracts were purified by gel permeation chromatography. Final cleanup was performed with a 6 mL, 0.5 g silica (SiOH) adsorbent Bakerbond disposable SPE catridge. The SPE cartridge was conditioned with 10% methanol:dichloromethane and 5% dichloromethane:hexane, and analytes were extracted with 8 mL of 5% dichloromethane:hexane.

The final extracts were concentrated to 250 uL in isooctane and analyzed on a gas chromatograph mass spectrometer working in electron capture negative ionization mode. Samples were injected in pulsed-splitless mode and analytes were separated on a 15 m x 0.25 mm x 0.10 um DB-5 HT fused-silica analytical column. Quantification was based on selected ion monitoring of the most abundant ions for each compound, mainly the isotopic bromine anions. [5]

Results and discussion

Of the 45 PBDE congeners monitored, BDE-47, -99, -100, -153, -154, and -209 accounted for approximately 90% of the total PBDE concentrations in all body compartment and egg samples with slight variations between the tissues (see Figure 1A). Overall, the most dominant congeners were BDE-99 (25-32%) and BDE-47 (11-31%), followed by BDE-100 (10-17%), -153 (9-17%), -154 (3-10%), and -209 (3-9%). The adipose and yolk samples constituted a smaller proportion of BDE-47 and a larger proportion of BDE-153 compared to the other tissues. As the adipose and yolk tissues contain a much larger lipid fraction, this suggests selective congener uptake for higher brominated congeners in lipid rich tissues.

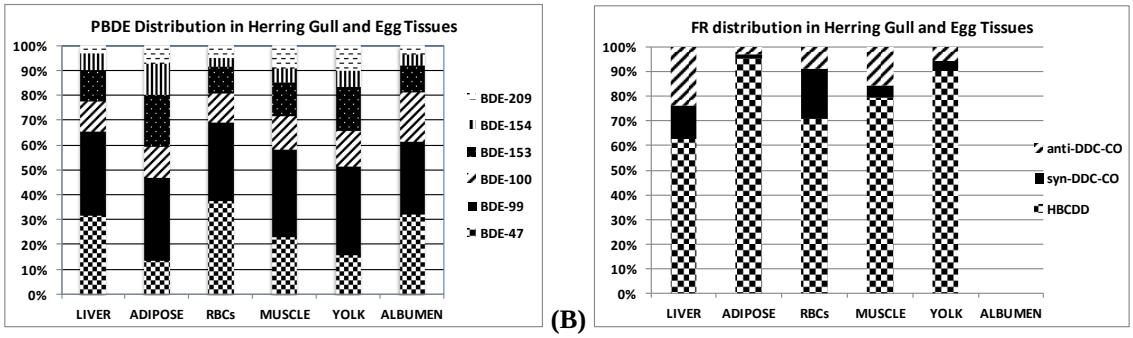
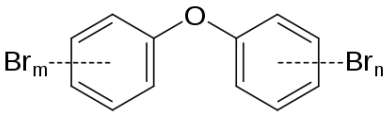
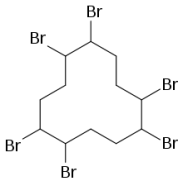


Figure 1. PBDE (A) and non-PBDE FRs (B) distributions in herring gull body compartments and egg tissues.

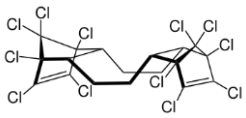
Of the 22 non-PBDE FRs monitored, HBCDD, *syn*-DDC-CO and *anti*-DDC-CO (see Figure 2 for molecular structures) were the only detected FRs in the majority of samples, with HBCDD representing between 60-90% of the total ∑FRs. Similarly, HBCDD represented a larger proportion of the sum of FRs in lipid rich tissues, such as adipose and yolk. In addition, BTBPE was measured in most of the adipose and half of the yolk samples, with greater concentrations in the lipid rich adipose.



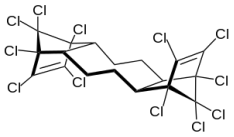
(A) polybrominated diphenyl ethers (PBDEs)



(B) hexabromocyclododecane (HBCDD)



(C) *syn*-Dodecachlorodimethano-dibenzocyclooctane (*syn*-DDC-CO)



(D) *anti*-Dodecachlorodimethano-dibenzocyclooctane (*anti*-DDC-CO)

Figure 1. Dominant FRs compounds observed in herring gull compartments and egg tissues.

Table 1 lists the median concentrations for the BDE congeners and non-PBDE FRs as well as the lipid content of the studied tissues and egg yolk and albumen. On a wet weight (ww) basis, the concentrations are greatest in the adipose tissue followed by the yolk, muscle, liver, RBCs, and albumen. The median $\sum_6$PBDE concentrations were 6375, 415, 331, 88, 21, and 6 ng/g ww for adipose, yolk, muscle, liver, RBCs, and albumen, respectively. The median $\sum_4$FR concentrations were 366, 22, 19, 3, and 1 ng/g ww in adipose, yolk, muscle, liver, and RBCs, respectively, while no FRs were detectable in the albumen of the egg. The $\sum_4$FR concentration represents approximately 5% of the $\sum_6$BDE concentrations. Higher concentrations are found in tissues with a higher percentage of lipid, such as adipose, yolk, and muscle, which suggests the tissue or egg accumulation of these compounds may be associated with lipid content. Work is currently in progress to examine wet weight concentration and lipid content to investigate whether any correlations exist.

Table 1. Concentrations (ng/g ww) of the FR compounds measured in herring gull and egg tissues.

	MATERNAL TISSUES				EGG	
	Liver	**Adipose**	**RBCs**	**Muscle**	**Yolk**	**Albumen**
	ng/g ww				**ng/g ww**	
Median $\sum_6$PBDEs*	88	6,375	21	331	415	6.1
Range $\sum_6$PBDEs	44 - 241	4,344 - 17,101	12 - 65	212 - 695	299 - 1,035	4.5 - 15
	SUM = 6,815				**SUM = 421**	
Median $\sum_4$BFRs**	2.8	366	0.81	19	22	N.D.
Range $\sum_4$BFRs	1.5 - 7.0	183 - 1,174	0.70 - 1.3	8.7 - 27	14 - 56	N.D.
	SUM = 389				**SUM = 22**	
Median HBCDD	1.5	345	0.55	12	20	N.D.
Median *syn* -DDC-CO	0.30	9.1	0.16	1.1	0.78	N.D.
Median *anti* -DDC-CO	0.59	9.6	0.08	2.1	2.2	N.D.
Median BTBPE	N.D	12	N.D.	N.D.	0.46	N.D.
% Lipid	2.6	66	0.26	5.3	30	0.05

*** Sum concentrations of the 6 major PBDE congeners were based on BDE-47, -99, -100, -153, -154, -209.**
**** Sum concentrations of the 4 major non-PBDE FRs were based on HBCDD, *syn*-DDC-CO, *anti*-DDC-CO, and BTBPE.**

There was a wide range of BDE congener and FR concentrations from one herring gull to another, although the general compound distribution and tissue patterns remained the similar. Furthermore, individual herring gulls with high levels of PBDEs also had correspondingly higher levels of non-PBDE FR compounds, suggesting similar exposure and accummulation pathways via the diet.

The median Σ_6PBDE concentrations in the maternal tissues combined was 6815 ng/g ww and the total concentrations in the whole egg fractions was 421 ng/g ww. For the non-PBDE FRs, the maternal compartments represented 389 ng/g ww and the yolk and albumen represented 22 ng/g ww. The PBDE and FR patterns were comparable in the maternal tissues compared to the combined egg fractions, with approximately 5% of the total

maternal levels transferred to the herring gull egg. This supports the use of the egg a reflective monitoring compartment of FRs in Great Lakes herring gulls.

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