NOVEL METHOXYLATED POLYBROMINATED DIPHENOXYBENZENE CONGENERS AND POSSIBLE SOURCES IN HERRING GULLS FROM THE LAURENTIAN GREAT LAKES OF NORTH AMERICA

Chen D[1,2], Letcher RJ[1,2], Gauthier LT[1], Chu SG[1], McCrindle R[2], Potter D[3]

[1]Ecotoxicology and Wildlife Health Division, Science and Technology Branch, Environment Canada, National Wildlife Research Centre, Carleton University, Ottawa, ON, K1A 0H3, Canada; [2]Department of Chemistry, Carleton University, Ottawa, Ontario, K1S 5B6, Canada; [3]Chemistry Department, University of Guelph, Guelph, Ontario, Canada; [4]Wellington Laboratories, Research Division, Guelph, Ontario, Canada

Introduction

Recent restrictions and regulation of usage of polybrominated diphenyl ether formulations have resulted in the increased use of alternative flame retardant chemicals to meet flammability standards for commercial products. While some alternative BFRs have been characterized in environmental samples including bioaccumulation in wildlife, many others may be currently manufactured and in commercial use but have yet to be identified in environmental compartments. The array of possible yet environmentally unknown flame retardants and other brominated substances raises new challenges and concerns for scientists to investigate their presence, sources, bioavailability, ecosystem behavior and effects in the environment.

The herring gull (*Larus argentatus*) has been considered as a key bio-indicator wildlife species in the Laurentian Great Lakes of North America, and recently we have characterized several novel flame retardants in the Great Lakes herring gull eggs.[1,2] However, in the BFR-containing fractions isolated from these herring gull eggs, numerous (and some rather intense) bromide anion responsive peaks observed in electron capture negative ionization (ECNI) mass spectra remain to be identified.[2] The current study reports on the characterization and identification of several highly novel brominated contaminants, as well as their quantification and assessment of recent spatial distribution, and the potential sources in herring gull eggs from fourteen colony sites spanning the Laurentian Great Lakes.

Materials and methods

Identification of Unknown Analytes

For qualitative identification of target unknown analytes, approximately 40 grams of pooled egg homogenate from Channel-Shelter Island (Lake Huron) (1999-2001) were extracted in ten replicates. Each replicate (4 g) was ground with diatomaceous earth and then subjected to accelerated solvent extraction (ASE). The extract was purified by gel permeation chromatography (GPC) followed by cleanup on a 500 mg Bakerbond SPE^{TM} SiOH SPE cartridge. The fraction containing target analytes was obtained by eluting the cartridge with 8 mL 20:80 DCM:HEX, and was further cleaned and separated on a column packed with 7 g silica gel. The first fraction containing PBDEs was eluted with 100 mL HEX, followed by 30 mL 20:80 DCM:HEX. The second fraction eluted with 16 mL 80:20 DCM:HEX contained the target analytes and were concentrated for GC-low resolution MS analysis in both ECNI (scan range 30 – 800 amu) and EI (scan range 30 – 800 amu) ionization modes, and also for GC-high resolution MS analysis in EI mode (50 – 1000 amu). The oven was temperature programmed as follows: 100 °C for 2 min, 25 °C/min to 250 °C, 1.5 °C/min to 260 °C, 25°C/min to 325 °C, Hold at 325 °C for 5 min. The transfer lines and source were also at 280 °C. The GC-LRMS(ECNI) mass chromatogram of a partial time range of 14 to 18 min for the Channel-Shelter herring gull egg fraction reveals three major (U1, U2 and U3) and three minor (U4, U5 and U6) unknown analytes.

Quantitative Analysis in Herring Gull Eggs

The target analytes (U1 – U6) are identified as methoxylated polybrominated diphenoxybenzene (MeO-PBDPB) congeners (see Results and Discussion section). To semi-quantify the six MeO-PBDPBs, approximately 1 g egg pool homogenate from each of fourteen gull colony sites spanning the Great Lakes (2009 collection) were spiked with 20 ng of methoxylated BDE-137 (MeO-BDE-137) as an internal standard and extracted with the previously mentioned method. The generated fraction containing MeO-PBDPBs was concentrated and analyzed on the GC-LRMS using ECNI mode. Semi-quantification of target analytes U1, U2, U5 and U6 was achieved via selected ion monitoring (SIM) for 79Br- and 81Br-, and based on the calibration curve for BDE-194. This could be accomplished due to the comparable molecular structures and equitable cleanup and isolation from egg homogenate, as well as

their close retention times on GC. For the same reason, U3 and U4 were semi-quantified based on the calibration curves for BDE-206 and BDE-170, respectively.

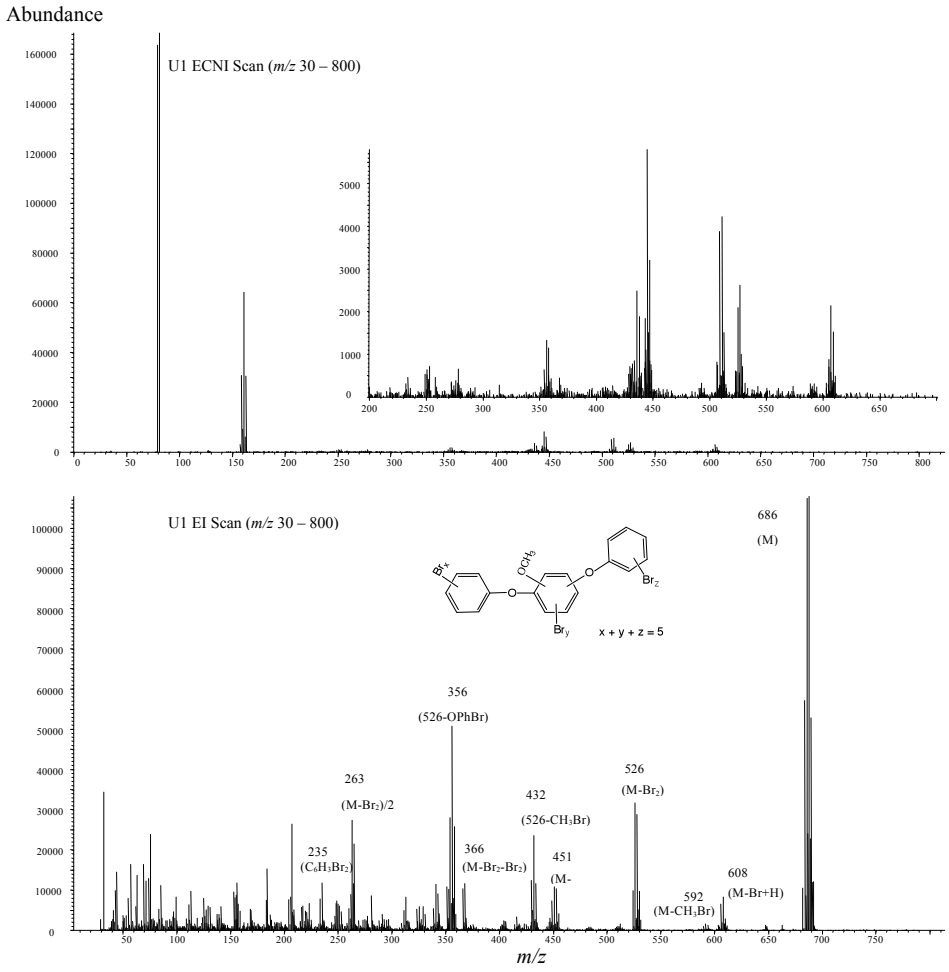

Figure 1. GC-LRMS ECNI and EI mass spectra of the MeO-PBDPB congener U1 in the extract of Channel-Shelter Island herring gull eggs. The base structure of this congener is also shown (hydrogen atoms are omitted for clarity).

Results and discussion

Isolation and Mass Spectral Characterization of MeO-PBDPBs in Herring Gull Eggs

Thorough cleanup and separation of MeO-PBDPBs from the egg matrix, particularly the application of 7-g silica gel SPE, greatly reduced the interferences in the GC-MS analyses by removal of lipids and closely eluting compounds, particularly PBDEs. The optimally isolated MeO-PBDPB-containing fraction facilitated the acquisition of high quality ECNI and EI mass spectra for the target analytes. The LRMS(ECNI) mass spectrum of U1 reveals the dominance of the $[Br]^-$ fragment ion isotopes at *m/z* 79 and 81 amu, and to a lesser extent by *m/z* 159, 161 and 163 amu (Figure 1). However, the ECNI spectrum of U1 provides very limited structural information.

Additional LRMS(EI) analysis of the egg fraction shows that the ion cluster centered at *m/z* 686 appears to be the molecular ion, as essentially no ions are observed above *m/z* 686 to a maximum of *m/z* 1000 amu by GC-HRMS(EI) analysis. The GC-HRMS(EI) analysis reveals that the elemental composition of U1 is $C_{19}H_{11}O_3Br_5$ since the exact mass of $[M]^+$ ($^{12}C_{19}{}^1H_{11}{}^{16}O_3{}^{79}Br_5$) is *m/z* 681.6005 amu, 0.002 amu from the calculated mass. The EI mass spectrum for U1 (Figures 1) exhibits an abundant $[M\text{-}Br_2]^+$ fragment, which resembles the spectra of certain PBDE congeners (e.g., BDE-47) that have bromine atoms *ortho* to the ether linkage.[3] This $[M\text{-}Br_2]^+$ fragment is formed by the elimination of two *ortho* bromines, upon electron impact, and involves the formation of a dibenzofuran-like ion.[3] The ion at *m/z* 356 amu is the second most abundant in the U1 mass spectrum, and the HRMS(EI) full scan analysis

indicated its exact mass to be *m/z* 353.8898 amu, which corresponds to an elemental composition of $C_{13}H_8O_2Br_2$ with an error of 0.0007 amu from the calculated exact mass. The mass difference between *m/z* 356 and *m/z* 526 ($[M-Br_2]^+$) may correspond to the loss of a phenoxy group with a bromine substituent (C_6H_4OBr). Therefore, the transition from M^+ to $[M-Br_2]^+$ and then to the ion at *m/z* 356 amu may represent the loss of two *ortho* bromine substituents to form a dibenzofuran-like structure, followed by loss of a brominated phenoxy group. A similar fragmentation pattern is consistently observed in the EI mass spectra of polychlorinated diphenoxybenzenes, which may suggest a diphenoxybenzene-like structure for U1.[4]

A loss of CH_3Br from $[M]^+$ is observed in the LRMS(EI) spectrum of U1. For MeO-PBDE congeners with a methoxy group and a bromine atom *ortho* to the aromatic ether linkage, e.g., 6-MeO-BDE-47, EI has been shown to produce a stable brominated dibenzo-*p*-dioxin ion due to the loss of CH_3Br from the molecular ion.[3] The $[M-CH_3Br]^+$ fragment ion is present in the U1 spectrum at *m/z* 592 amu in low abundance. In contrast, loss of CH_3Br from $[M-Br_2]^+$ produces an abundant ion $[M-Br_2-CH_3Br]^+$ at *m/z* 432 amu. Thus, a very likely EI fragmentation pathway for U1 is formation of a dibenzofuran ion due to loss of Br_2 from $[M]^+$, followed by loss of CH_3Br to form a dibenzo-*p*-dioxin structure. Formation of such a fragment ion (*m/z* 432 amu) that contains both dibenzo-*p*-dioxin and dibenzofuran moieties appears to require a molecular ion with a diphenoxybenzene base structure, as well as a methoxy group *ortho* to one of the phenyl ether atoms. This proposed backbone structure, i.e., diphenoxybenzene, is further supported by the presence of the ion at *m/z* 366 amu in the EI spectrum (Figure 1). This fragment ion cluster may be produced by loss of Br_2 from the dibenzofuran ion $[M-Br_2]^+$ and consequently contains two dibenzofuran moieties.

The EI and ECNI spectra of U2, U5 and U6 were essentially the same as for U1, and thus according to the mass spectral rationale for U1, they appear to have structures very similar to that for U1, i.e., a diphenoxybenzene backbone bearing a methoxy group and five bromine atoms. U3 and U4 have a molecular ion at *m/z* 766 amu and *m/z* 608 amu, respectively. They also exhibit similar mass spectra as those for U1. Therefore, considering the evidence of LRMS(EI), HRMS(EI) and GCMS(ECNI) spectra combined, it is clear that the base structures of U1, U2, U5 and U6 are MeO-pentabromoDPBs, U3 a MeO-hexabromoDPB and U4 a MeO-tetrabromoDPB (Figure 1).

Spatial Distribution and Possible Sources of MeO-PBDPBs in Herring Gulls

The spatial distribution of MeO-PBDPB contamination in the Great Lakes basin was examined by a preliminary assessment of recent egg pool homogenates of herring gulls from fourteen colony sites spanning the Great Lakes. Highest concentrations were observed in the pool from the Channel-Shelter Island (Lake Huron), where ∑MeO-PBDPB (including U1 – U6) concentration was 39 ng/g ww. MeO-PBDPB congeners were also detected in egg homogenates from most of the other 13 colony sites, although comparatively to Channel-Shelter Island the concentrations were at least one order of magnitude lower, i.e., ∑MeO-PBDPBs ranging <0.2 – 9 ng/g ww.

To our knowledge, there are no published literature reports on the environmental presence of MeO-PBDPBs prior to the current study. Absolutely nothing is known about their sources and environmental behavior. Enlightened by the relationship between PBDEs and MeO-PBDEs, we hypothesize that the MeO-PBDPB congeners are metabolites/degradation products of PBDPBs such as tetradecabromodiphenoxybenzene (commercially known as SAYTEX 120) or polybromo 3P2E flame retardants. SAYTEX 120 is a currently used BFR that finds primary applications in high performance polyamide and linear polyester engineering resins and alloys.[5] We hypothesize that MeO-PBDPBs are produced via transformations of PBDPBs that may be impurities in technical SAYTEX 120 or produced by degradation of SAYTEX 120. In addition to SAYTEX 120, polybromo 3P2E may be another potential source for MeO-PBDPBs. Polybromo 3P2E belongs to a general class of halogenated polyphenyl ethers that were incorporated as flame retardants into a number of polymer and foam rubber products.[6] It's a mixture of congeners with a general structure of polybrominated dephenoxybenzene. For example, one of the isomers introduced in the United States Patent 3760003 was 2,5-dibromo-*p*-bis(2,4-dibromophenoxy)benzene, which may be a potential precursor for MeO-PBDPB-Br_5. In our opinion, there is the possibility of exposure and accumulation via terrestrial diet consumption of herring gulls and subsequent *in ovo* transfer to their eggs. There is also a more remote possibility that the MeO-PBDPBs may be of natural origin, rather than being anthropogenic, and accumulated in the herring gull food web.[7] Nonetheless, the discovery of MeO-PBDPBs in Great Lakes herring gull eggs clearly indicates their substantial bioaccumulation potential, which, as well as the spatial distribution pattern across the Great Lakes, raises concerns about wildlife and human health risks.

Acknowledgements

We thank Chip Weseloh, Craig Hebert, Kim Williams, Doug Crump, Guy Savard, and other field personnel involved in the herring gull egg collection and archiving. Special thanks go to Brock Chittim and Wellington Laboratories. Funding support for this study was provided by Environment Canada's Chemicals Management Plan (to R.J.L.). Postdoctoral support for D. Chen is in part from the Natural Science and Engineering Research Council (NSERC) of Canada (to R.J.L.).

References:

1. Letcher RJ, Chu S. (2010); *Environ Sci Technol.* 44: 8615-21.
2. Gauthier LT, Hebert CE, Weseloh DVC, Letcher RJ. (2007); *Environ. Sci. Technol*. 41: 4561-7.
3. Hites RA. (2008); *Environ Sci Technol.* 42: 2243-52.
4. Timmons L, Cannon M, Grese D, Brown R, Haile C, Murrill E. (1984); *Anal. Lett*. 17: 277-96.
5. Albemarle® Corporation. (1999). http://www.albemarle.com/TDS/Flame_retardants/bc1003f.pdf.
6. Asadorian AA, Langer HG, Funsher JA. (1973); http://www.freepatentsonline.com/3760003.pdf.
7. Gribble GW. (1998); *Acc. Chem*. 31: 141-52.