ORGANOPHOSPHATE TRIESTER FLAME RETARDANT AND PHOSPHORIC ACID DIESTER METABOLITE ANALYSIS AND SPATIAL AND TEMPORAL TRENDS IN HERRING GULLS FROM THE NORTH AMERICAN GREAT LAKES

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Introduction

Since as early as the 1960s organophosphate flame retardants (OPFRs) have been widely used in plastics, foams, textiles and furniture with high-production volume worldwide.[1] With the phase-out of brominated flame retardants such as polybrominated diphenyl ethers (PBDEs), the usage of certain triester OPFRs has increased (e.g., tris(methylphenyl) phosphate (TCrP), tris(1,3-dichloro-2-propyl) phosphate (TDCPP) and tris(1-chloro-2-propyl) phosphate (TCPP)). Although environmental contamination concerns date back to the 1970s, with the exception in the 1980s of some studies of possible bioaccumulation and uptake and clearance in fish, there have been limited OPFR studies in environmental compartments.[1-6] In the mid-2000s, OPFRs "re-emerged" as environmental contaminants with some reports of e.g., (TDCPP) in indoor dust, water, sediment, and sludge, and some Swedish studies in human blood and milk and fish.[2,3,7] However, to our knowledge there have been no scientific literature reports on OPFRs in wildlife.

Phosphate triesters can be metabolized to phosphoric acid diesters and monoesters, and these metabolites may be more logical for use as biomarker analytes rather than their parent triester OPFRs in biota samples. Some phosphoric acid diesters and monoesters, such as di(2-ethylhexyl) phosphate (DEHP) and mono(2-ethylhexyl) phosphate (MEHP), are also known to be industrially produced.[1] However, there are very few reports on diester and monoester OPFRs in complex environmental samples, which is largely due to a current lack of sufficiently sensitive analytical methods.

Using newly developed LC-MS based methodologies, we analyzed for and report on 15, non-halogenated, chlorinated or brominated triester OPFRs, as well as several phosphoric acid diester metabolites, and their occurrence, persistence, and spatial and temporal trends (1982-2010) in herring gulls (*Larus argentatus*) eggs for 15 colonies across the Laurentian Great Lakes of North America. The phosphoric acid diester methodology is via LC-MS using a post-LC dicationic complexation approach. We hypothesized that dicationic complexation of OPFR diesters and subsequent analysis by LC-ESI(+)-MS/MS would result in higher quantitative sensitivities relative to direct diester analysis via LC-ESI(-)-MS/MS.

Materials and Methods

Sub-samples of egg pool homogenates from 2009 or 2010 collection were analyzed from each of the 15 colony locations (all five Great Lakes). For selected colonies (Agawa Rocks, Gull Is., Chantry Is. Channel-Shelter Is., Fighting Is., Niagara River, and Toronto Harbour), pool homogenates were analyzed from the years 1982 to 2010. OPFR triesters under study included TCPP, tris(2-butoxyethyl) phosphate (TBEP), tributyl phosphate (TBP), triphenyl phosphate (TPP), tris(2-ethylhexyl) phosphate

(TEHP) and TDCPP, and extraction involved accelerated solvent extraction and solid phase extraction with aminopropyl silica, with analysis by LC-tandem quadrupole MS (electrospray(+)).

As for OPFR diesters, these included dibutyl phosphoric (DBP), diphenyl phosphoric (DPhP), DEHP and di(1,3-dichloro-2-propyl) phosphoric (DDCPP) acid. For post-LC complexation, several reagents were tested, overall, for DDCPP, DBP, DPP and DEHP the optimally reactive dicationic reagent was decamethonium hydroxide.[8] Based on ESI(+)-Q-ToF-MS full scan mass spectral results, quantitative analysis of the decamethonium dicationic ion complexes with the phosphoric acid diester anions was via LC-ESI(+)-QQQ-MS/MS.

Results and Discussion:

Of the 15, non-halogenated, chlorinated or brominated OPFRs analyzed for, several were quantifiable in gull egg pools. For example, for the chlorinated triesters TCDPP and TCEP, the concentrations ranged from 5.4 to 30.1 and 1.1 to 7.9, respectively (Figure 1a). For the non-halogenated triesters TBEP and TPP, the concentrations ranged from below detection to 39 ng/g and 2.1 to 8.2 ng/g lipid weight, respectively (Figure 1b).

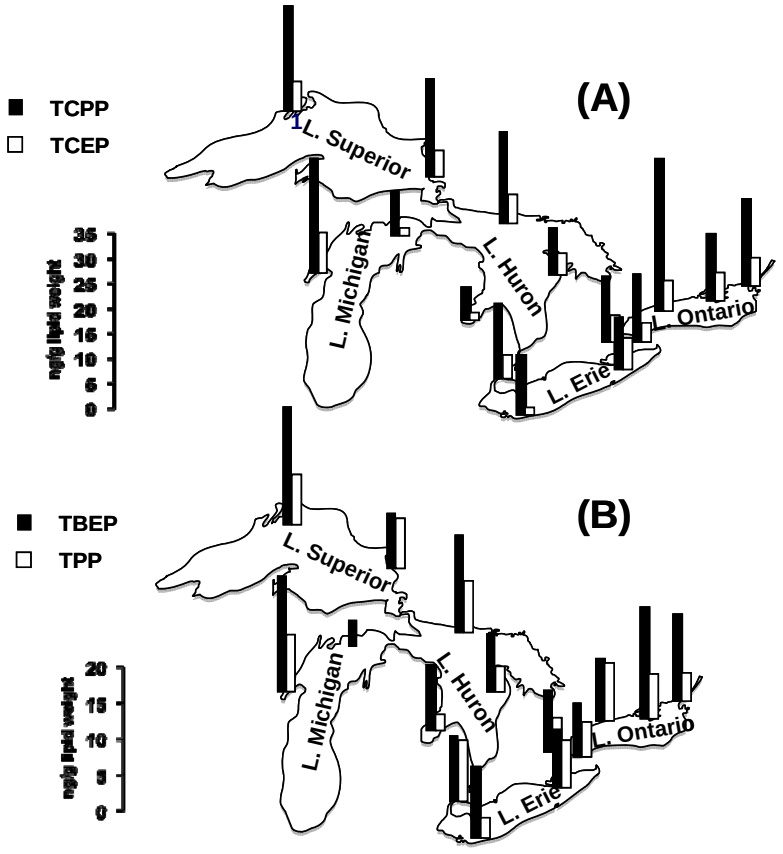

Figure 1. Mean concentration (ng/g lipid weight) of (A) and (B) in the egg pools (n=15 eggs per pool) of herring gulls from 15 colony sites across the Laurentian Great Lakes of North America. All eggs were collected during the period of May-June of 2009 and 2010.

Spatial trends were not obvious regarding the concentrations of triester OPFRs among colony sites. However, the residue levels of quantifiable triester OPFRs are likely governed in part by the capacity for metabolism/degradation by the maternal gulls and/or within their aquatic/terrestrial food web. In fact, we found that TDCPP is rapidly metabolized to DDCPP *in vitro* using rat liver micrsomes.[8] When considering the all quantifiable triester OPFRs, the sum OPFR concentrations were the highest among FRs (e.g. PBDEs, numerous non-PBDE FRs and chlorinated FRs *anti*- and *syn*-Dechlorane Plus) we have reported in eggs of herring gulls from across the Laurentian Great Lakes of North America.[9-12] Temporal trends (1982-2010) were observed and different among the quantifiable triester OPFRs.

Several trimester OPFRs that are widely used as flame retardants, i.e., TBP, TPP, TEHP and TDCPP, and can be metabolized in some organisms including humans to corresponding phosphoric acid diesters products DBP, DPP, DEHP and DDCPP, respectively.[8] In the absence of highly sensitive methods for the determination of these important phosphoric acid diester metabolites, especially for DDCPP, we developed a LC-MS based analysis method. Unique to this method is a post-column LC separation introduction of decamethonium hydroxide, a dicatonic reagent, to form complexes with the target phosphate acid diester anions, which were found to be highly sensitive determination by MS/MS with positive electrospray ionization (ESI+). For the phosphate acid diester complex, the mass spectrum (ESI(+)) showed that complex ion was the most abundant ion when single MS was used, and with tandem mass spectrometry (MS/MS) $[(CH_3)_2N(CH_2)_{10}N(CH_3)_3]^+$ (*m/z* 243.2795 amu) was the most abundant product ions of the complex ion, with lesser abundances of $[[M\text{-}H]^-[(CH_3)_3N^+(CH_2)_9CH_2]]^+$ and $[CH_2CH(CH_2)_8N(CH_3)_3]^+$ (*m/z* 198.2216 amu). For DDCPP, the fragment ions of $[[Cl]^-[(CH_3)_3N^+(CH_2)_{10}N^+(CH_3)_3]]^+$ (*m/z* 293.2718 amu) and $[[Cl]^-[(CH_3)_3N^+(CH_2)_9CH_2]]^+$ (*m/z* 234.1983 amu) could also be observed. The limits of quantitation by LC-ESI(+)-MS/MS (based on the complex ion to $[(CH_3)_2N(CH_2)_{10}N(CH_3)_3]^+$ fragment ion transition) were 0.14, 0.03, 0.14 and 0.02 ng/mL for DPP, DBP, DDCPP and DEHP, respectively. The response was highly linearly correlated ($r > 0.995$) with concentration over the range of 0 to 1000 ng/mL.

Based on the ESI fragmentation pattern of priority triester and diester OPFRs, we developed highly sensitive, quantitative methods for their determination in avian eggs. Using these novel methods, our results on triester and diester OPFRs demonstrate the increasing complexity of FRs and/or their persistent and bioaccumulative metabolites in the eggs of herring gulls from across the Laurentian Great Lakes. Clearly these substances are bioaccumulated in the gulls and subsequently transferred to their eggs during the breeding period. It is probable that these triester and diester OPFRs are biomagnified from the diet, which could include via the aquatic food web (fish) or via terrestrial feeding sources (human refuse, etc.) as a consequence of opportunistic feeding. Triester OPFR levels are likely influenced by their susceptibility to metabolism including to diester OPFRs.

Acknowledgements:
This study was financially supported by Chemicals Management Plan (CMP; Environment Canada), as well as by the Natural Science and Engineering Research Council (NSERC) of Canada (to R.J.L.). Dr Craig Hebert, Doug Crump, Kim Williams and the Environment Canada-National Wildlife Specimen Bank staff are thanked for their help in collecting and processing the herring gull egg samples.

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