FLAME RETARDANT VERSUS FLUORINATED CONTAMINANTS OF EMERGING CONCERN IN HERRING GULL AND CASPIAN TERN EGGS FROM UNITED STATES COLONY SITES IN THE GREAT LAKES OF NORTH AMERICA

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Introduction:

Manufactured materials such as plastics, foams, textiles, furniture, paper coatings and many others materials include added chemicals (flame retardants (FRs), and per- and poly-fluoroalkyl substances (PFASs)) to meet specified use requirements and safety standards for commercial and industrial applications.[1,2,3]. In order to retard fires, organic and inorganic categorized FR chemicals are added to materials.[1,3] Organic FRs include organophosphate esters (OPEs) and other organohalogen substances, and have received increasing environmental attention based on their substantial share of the FR chemical market (e.g., more than 45 % of total volume in 2011).[1,3]

Environmental concern, restoration and protection of the Laurentian Great Lakes of North America is shared by both United States (U.S.) and Canadian governments.[4] The Canada-U.S. International Joint Commission (IJC) renewed the U.S.-Canada Great Lakes Water Quality Agreement (GLWQA) in September, 2012[4] and highlighted the importance to restore and protect the chemical, physical and biological integrity as well as the quality of waters of the Laurentian Great Lakes, and the identification and monitoring of chemicals of emerging concern (CECs) in various environmental compartments including biota. Retrospectively over the last three decades up to as recently as 2010, eggs and mainly egg pools from several herring gull (*Larus argentatus*) colonies have been monitored annually for an increasing number of CECs, i.e. PBDEs, a wide range of non-PBDE FR replacements such as numerous brominated FRs and OPEs, and PFASs and in particular PFAAs.[5,6,7] Other than the herring gull (which is not a totally aquatic fish consumer), there is presently limited information on CECs in other bird species such as the purely fish-eating Caspian tern (*Hydroprogne caspia*), which is currently a state-threatened species in the State of Michigan.

The objectives of the present study are to 1) To contribute to the GLWQA-2012 binational coordination by monitoring CECs in recently collected bird eggs (2013-2014) from four Great Lakes colonies within U.S. (Michigan), 2) to screen for, identify and/or quantify (flame retardant) CECs for the first time in the Great Lakes Caspian tern eggs, and 3) to compare of BFR and PFAS CEC concentrations between Caspian terns (an obligate piscivore listed as threatened in Michigan) and herring gulls (primarily aquatic but also a terrestrial feeder).

Materials and Methods:

The collection of the herring gull and Caspian tern eggs at the U.S. colony sites was carried out by Keith A. Grasman of Calvin College and David Best (retired) of USFWS, from three sites in the Laurentian Great Lakes area within the borders of the United States. For each of the three sites on Charity Reef and the Confined Disposal Facility (Saginaw Bay, Lake Huron; location 3 and 4 in Figure 1) and Two Tree Island (St. Mary's River; location

1 in Figure 1), n=10 eggs of Caspian terns were collected for an overall total of n=30 tern eggs. For comparison, n=10 herring gull eggs were also collected from the Pipe Island Twins colony at the St. Mary's River (Lake Huron/Lake Superior connecting channel; location 2 in Figure 1).

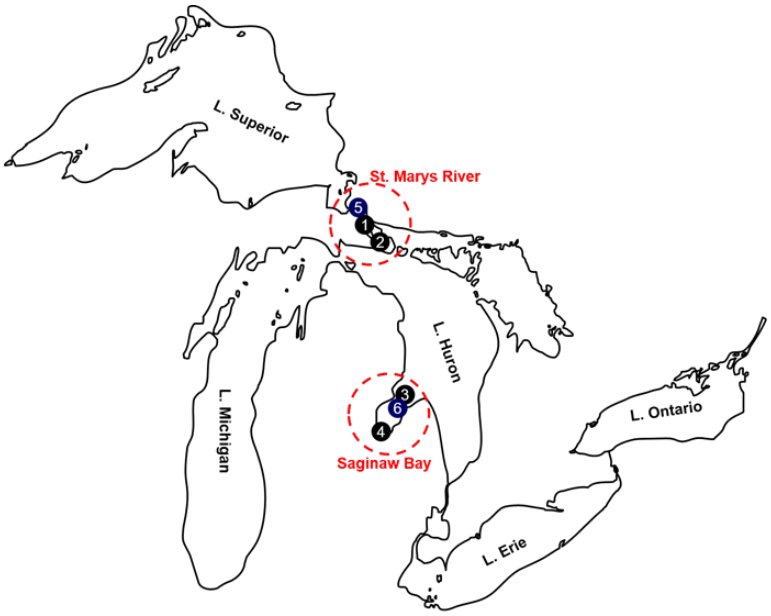

Figure 1. Sampling locations of herring gull or Caspian tern eggs in the North American Laurentian Great Lakes. In the present study, n=10 Caspian tern eggs were collected from colonies on Two Tree Island (location: 1), Charity Reef (3) and Confined Disposal Facility (4), respectively, and n=10 herring gull eggs were collected from the colony on Pipe Island Twin (2). The locations of 5 and 6 represented colonies on Five Mile Island and Little Charity Island, where CEC concentrations have been reported in our previous publications, and are used for comparison to results in this study.

A total of 87 target CEC-FRs (including 26 PBDEs, 23 other non-PBDE halogenated flame retardants (NPHFRs), 16 OPE-FRs and 22 per-/poly-fluoroalkyl substances (PFASs)) were screened for in the present Caspian tern and herring gull egg samples. Details descriptions of the methods used for FR and PFAS analysis can be found elsewhere.[5,6,7] In brief, all PBDEs and NPHFRs were determined in egg fractions using an Agilent 6890 gas chromatography (Agilent Tech., Palo Alto, CA, USA) coupled to a single quadrupole mass analyzer (Agilent 5973 MS) in electron capture negative ionization (ECNI) mode. Brominated FR quantification was achieved via selected ion monitoring (SIM) for $^{79}Br^-$ 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. Detailed information on the method development of determination of 16 OPEs in biotic samples was reported previously.[5] In brief, egg fractions were analyzed for OPEs using a ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). Detailed descriptions of PFAS sample extraction and instrumental analysis (UPLC-MS/MS) can be found elsewhere.[7]

Statistical analyses were performed by use of GraphPad Prism 5, and only conducted on CECs with a detection frequency greater than 80 %. One-way ANOVA with a Tukey's HSD test was carried out to assess the CEC concentration differences among 3 or more egg groups (i.e. egg from different colonies), and a nonparametric Mann-Whitney test was performed to assess CEC concentration differences between two egg groups (i.e. tern *vs.* gull; St. Mary's River *vs.* Saginaw Bay). For all statistical analysis, the significance level was set at 0.01.

Results and Discussion:

In tern eggs, the order of concentrations were ΣPFSA (mean: 793 ng/g wet weight (ww); range: 116-4690 ng/g ww) > ΣPFCAs (131; 30.4-506 ng/g ww) ≈ ΣPBDEs (86.7; 32.4-189 ng/g ww) >> ΣNPHFRs (0.67; ND-4.3 ng/g ww) ≈ ΣOPEs (0.46; ND-2.89 ng/g ww). Compared to gull eggs collected from the same area, tern egg exposure contained significantly lower concentrations of ΣPBDE, but with up to 10 times greater mean concentrations of ΣPFSAs and ΣPFCAs (Figure 2). Despite their similar trophic status, these two avian species

showed clearly and significantly (in most of cases) different contamination profiles in terms of 87 monitored CECs. For example, 1) Caspian tern eggs contain significantly less ΣPBDE concentrations compared to herring gull eggs collected from St. Mary's Rivers or Saginaw Bay (Figure 2), and 2) PFAS concentrations in tern eggs were significantly greater compared to herring gull eggs collected from the same area; The reasons for these FR and PFAS differences between herring gull and Caspian tern eggs are very complex, and could be due to several factors, i.e. differing dietary profiles, residency, biotransformation as well as excretion. Caspian terns are known to feed exclusively on fish, whereas herring gulls are more generalist feeders, consuming principally fish, but also feed opportunistically on human food waste, small mammals and insects. Beyond dietary choice difference, annual migratory strategies differ between species, which also affect egg contaminant concentrations.[8] Specifically, adult herring gulls are generally year round residents of the Great Lakes, although birds breeding in the north often winter in the southern Great Lakes. However, Caspian terns are annual migrants to Central America and northern South America. They have different (and often lower) contaminant exposure on the wintering grounds, and they deplete fats (mobilize fat soluble contaminants) during long-distance migration.[9]

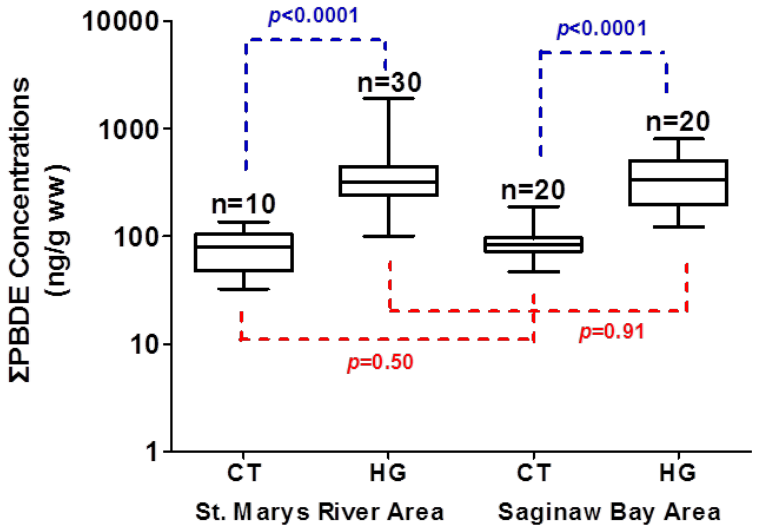

Figure 2. Comparisons of box and whisker of sum polybrominated diphenyl ether concentrations (ΣPBDEs) between Caspian tern (CT) and herring gull (HG) eggs collected from the area of St. Mary´s River (left) and Saginaw Bay (right). For each box, the data points represent the minimum (smallest value), the first quartile, the median, the third quartile and the maximum (largest value) from bottom to top. Blue dotted lines was comparison between tern and gull eggs collected in the area; whereas red dotted lines were comparison between two areas for one species (gull or tern).

Low detection frequencies and contamination levels of OPEs in the Caspian tern eggs were consistent with previous reports on other Laurentian Great Lakes species, i.e. herring gull eggs, Lake Trout (*Salvelinus namaycush*) or Walleye (*Sander vitreus*).[5,6,10]. The *in vitro* biotransformation and kinetics of six OPEs in herring gulls from the Great Lakes using a hepatic microsomal assay demonstrated that administration of five individual OPEs (TNBP, TBOEP, TPHP, TDCIPP and TCIPP) to the *in vitro* assay resulted in rapid depletion.[11]

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