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Accumulation of flame retardants in paired eggs and plasma of bald eagles☆

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ABSTRACT

In this study, we measured the concentrations of 58 flame retardants (and related compounds) in bald eagle (*Haliaeetus leucocephalus*) egg and plasma samples from the Michigan. These analytes include polybrominated diphenyl ethers (PBDEs), novel flame retardants (nFRs), Dechlorane-related compounds (Decs), and organophosphate esters (OPEs). A total of 24 paired eaglet plasma and egg samples were collected from inland (IN, N = 13) and the Great Lakes (GL, N = 11) breeding areas from 2000 to 2012. PBDEs were the most abundant chemical group with a geometric mean of 181 ng/g wet weight (ww) in egg and 5.31 ng/g ww in plasma. Decs were barely found in plasma samples, but they were frequently found in eggs (geometric mean 23.5 ng/g ww). OPE levels were comparable to those of PBDEs in the plasma but lower than those of PBDEs in eggs. Dec and PBDE concentrations were significantly higher in GL than in IN ($p < 0.05$). The ratio of egg to plasma concentrations (lipid normalized) varied with chemicals and correlated with the chemical's octanol-water partition coefficient. The lipid normalized bald eagle egg and plasma concentrations from Lake Superior and Huron were one to three orders of magnitude higher than concentrations measured in composite lake trout (*Salvelinus namaycush*) from the same lake, implying that they biomagnify in the environment.

1. Introduction

As indigenous species and tertiary predator of the aquatic food web, bald eagles (*Haliaeetus leucocephalus*) act as an effective indicator of the contamination and bioaccumulation of persistent environmental organic pollutants in the Great Lakes basin (Bowerman et al., 2000, 2002; Route et al., 2014; Wierda et al., 2016). Legacy chemicals such as organochlorine pesticides (OCPs) including dichlorodiphenyltrichloroethane (DDT) and its metabolites, polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs) and furans (PCDFs) have been recognized as causes of the decline of bald eagle population in the mid-20th century (Bowerman et al., 2002; Elliott et al., 2001/2002). Polybrominated diphenyl ethers (PBDEs), structurally similar to PCBs and the most widely used flame retardants, are also persistent and bioaccumulative, and have been measured in the plasma and liver of bald eagles (Dornbos et al., 2015; Elliott et al., 2009; Route et al., 2014; Venier et al., 2010).

Plasma is generally used to study contaminants' exposure in birds. Plasma in nestlings is an ideal medium to study the spatial and temporal trend of contaminants since the nestlings are sedentary, and they accumulate contaminants directly from prey at the nesting site (Dykstra et al., 2005; Route et al., 2014; Venier et al., 2010). Also, nestlings' blood can be collected over time. In addition to blood, eggs are also used to monitor contaminants' exposure of raptors or other birds, due to the ease of collection (Barón et al., 2015; Chen et al., 2008, 2012b; Donaldson et al., 1999; Elliott et al., 1996). Eggs also provide a measurement of contaminants at the sensitive endpoint for embryo mortality, and lipophilic chemicals are highly accumulative in the lipid of eggs (Strause et al., 2007).

PBDEs are very abundant in eggs of herring gulls from the Great Lakes (Su et al., 2015b) of peregrine falcons from northeastern U.S. states (Chen et al., 2008) and of Caspian Tern eggs in Michigan colonies of the Great Lakes (Su et al., 2017). Novel brominated flame retardants (nFRs) [1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE), di-(2-ethylhexyl)-tetrabromophthalate (BEHTBP) and decabromodiphenylethane (DBDPE)] have been detected in black

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guillemot eggs in Greenland (Vorkamp et al., 2015). As highly bioaccumulative chemicals (Guo et al., 2017b; Shen et al., 2014) dechlorane related compounds (Decs) have been found in egg samples from different birds, such as the peregrine falcon (Guerra et al., 2011), bald eagle (Best et al., 2010), white stork (Muñoz-Arnanz et al., 2010), great black-backed gulls, and herring gulls (Weseloh et al., 2002). In addition to its use as flame retardant, dechlorane was also used as insecticide with the trade name of Mirex during 1959 and 1972 in U.S. (Feo et al., 2012). The concentrations of the less lipophilic organophosphate esters (OPEs), a relatively new group of chemicals used as flame retardants, were also found at relatively high levels in herring gull eggs (Greaves et al., 2014).

In this study, we measured the concentrations of organophosphate esters (OPEs), brominated flame retardants (BFRs) including PBDEs and nFRs, and dechlorane-related compounds (Decs) in paired egg and plasma samples of bald eagle nestlings from Michigan between 2000 and 2012. The objectives of this study were: (*1*) to report the levels of FRs in the bald eagle samples; (*2*) to examine the spatial distribution of these FRs in the Great Lakes basin; (*3*) to establish a relationship between egg and plasma of bald eagles for FRs; and (*4*) to investigate the biomagnification of flame retardants in bald eagles from lake trout (*Salvelinus namaycush*). This is the first study to examine the biomagnification of these compounds in bald eagles and their correlation between bald eagles' eggs and plasma.

2. Materials and methods

2.1. Sampling

Samples were provided from the Michigan Bald Eagle Biosentinel Program archive, which had nestling plasma samples and addled eggs collected during the same visit from 24 bald eagle nests within the state of Michigan from 2000 to 2012 (two egg samples broke during transportation, leaving 22 pairs of egg and plasma). The locations of the sampling sites are shown in Figure S1. Due to past findings of higher organochlorine contaminant levels in bald eagle nestlings located along the Great Lakes' shorelines (Bowerman et al., 2002, 2003), samples were divided into two regions based on breeding areas: nests located more than 8.0 km from the shorelines of the Great Lakes and not along tributaries where anadromous fish were accessible were designated as IN (Inland); nests located less than 8.0 km from the shorelines of the Great Lakes or along tributaries where anadromous fish were accessible were designated as GL (Great Lakes). Plasma samples were never reported in previous peer reviewed publications while the eggs collected in 2000 were used in a study on legacy pollutants (Best et al., 2010).

At time of collection, nestlings were temporarily removed from the nest, and up to 12 mL of whole blood were taken from the brachial vein along with multiple morphometric measurements for age and sex calculations (Bortolotti, 1984a; b; Bowerman et al., 1995). Whole blood was stored on ice for no more than 48 h before it was centrifuged, spun down and plasma was pipetted into separate glass tubes for storage at approximately −20 °C (Simon, 2016). Eggs were taken from the same nest concurrently. Detailed sampling information can be found in the Supporting Information (SI).

2.2. Chemicals and materials

Flame retardant standards were purchased from Wellington Laboratories (Guelph, ON, Canada), AccuStandard (New Haven, CT), Cambridge Isotope Laboratories, Inc. (Tewksbury, MA), Sigma-Aldrich (St. Louis, MO), and Chem Service (West Chester, PA). A detailed list of the targeted chemicals is provided in the SI.

All solvents were HPLC or Optima grade. Granular anhydrous sodium sulfate (Na_2SO_4) was purchased from Fisher Scientific (Pittsburgh, PA). Supelclean™ ENVI™-Florisil® cartridges (500 mg, 6 mL), Supel™ QuE Z-Sep/C18 sorbent (Discovery® DSC-18, 300 mg; Z-Sep, 120 mg), Discovery® DSC-NH_2 cartridges (500 mg, 3 mL), and Florisil sorbent were obtained from Sigma-Aldrich (St. Louis, MO). Florisil sorbent and Na_2SO_4 were baked at 300 °C for >12 h, and then cooled in a desiccator. Florisil sorbent was deactivated with 2.5% water (by weight) for 24 h before use. Bio-Beads SX-3 was purchased from Bio-Rad Laboratories (Hercules, CA) and immersed in hexane/dichloromethane (DCM) mixture (1/1, v/v) for > 24 h before use.

2.3. Extraction and cleanup

The extraction and cleanup of plasma were similar to those previously reported (Liu et al., 2015; Venier et al., 2010). Plasma (2.0–5.4 mL) was weighed, spiked with surrogate standards [BDE-77, BDE-166, carbon labeled BDE-209 ($^{13}C_{12}$-BDE-209) for the BFRs and Decs, and deuterated tris(2-chloroethyl) phosphate (d_{12}-TCEP) and carbon labeled $^{13}C_{18}$-triphenyl phosphate (MTPP) for the OPEs], denatured with 6M HCl and 2-propanol, and liquid-liquid extracted with hexane/methyl *t*-butyl ether (1:1). The lipid content was determined gravimetrically using 10% of the extract. The extract was cleaned on a 2.5% (by weight) water deactivated Florisil column, and three fractions were collected: the first using 35 mL of n-hexane, the second using 35 mL of n-hexane in dichloromethane (1:1 by vol), and the third using 40 mL of acetone in dichloromethane (1:1 by vol). PBDEs, nFRs and Decs eluted in the first and second fractions, and OPEs eluted in the third fraction. Each fraction was then concentrated, solvent exchanged to *n*-hexane, and blown down to ~1 mL with N_2. Each fraction was spiked with known amounts of internal standard compounds (BDE-118, BDE-181 and decabromobiphenyl for the first two fractions and d_{10}-anthracene, d_{12}-benz[*a*]anthracene, and d_{12}-perylene for the third fraction).

The egg extraction procedure was similar to methods previously published (Guo et al., 2017b; Xu et al., 2015). A detailed description can be found in the SI and in Figure S2. Briefly, before extraction, the eggs were thawed, and about 5 g of tissue were mixed with 80 g anhydrous Na_2SO_4 using a mortar and pestle. This homogenized mixture was loaded into a glass Soxhlet extraction thimble, spiked with known amounts of surrogate standards [BDE-166, $^{13}C_{12}$-*syn*-DP, and $^{13}C_{12}$-*anti*-DP for the BFRs and Decs, and d_{12}-TCEP and MTPP for the OPEs], and extracted with 400 mL of *n*-hexane in acetone (1:1 by vol) for 24 h. The lipid content was determined gravimetrically using 10% of the extract. Extracts were then cleaned up on a Florisil® cartridge (preconditioned with dichloromethane and hexane), and two fractions were collected: the first using 12 mL of *n*-hexane in dichloromethane (4:1 by vol), and the second using 10 mL ethyl acetate. Most BFRs [except 2-ethylhexyl 2,3,4,5-tetrabromobenzoate (EHTBB) and bis(2-ethylhexyl)-tetrabromophthalate (BEHTBP)] and the Decs eluted in the first fraction; OPEs, EHTBB, and BEHTBP eluted in the second fraction. The first fraction was further cleaned using a gel permeation chromatography (GPC) column packed with Bio-Beads SX-3. One portion of the GPC column eluent (75–110 mL) was combined with the second fraction of the Florisil column and further cleaned using, first, dispersive solid phase extraction with ~500 mg Z-Sep/C18 mixture and then a DSC-NH_2 cartridge (eluted with 12 mL dichloromethane). The rest of the GPC eluent (110–280 mL) and the DSC-NH_2 cartridge eluent were individually concentrated, solvent exchanged to *n*-hexane, blown down to ~1 mL with N_2, and spiked with known amounts of the internal standards (BDE-118, BDE-181,